Type 2 Diabetes Mellitus and Impaired Glucose Regulation in a multi-ethnic population

Thesis submitted for the degree of Doctor of Philosophy

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by

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Abstract: Background

The incidence of Type 2 Diabetes Mellitus (T2DM) is increasing rapidly, therefore there is a need to detect this disease earlier and more efficiently, and also to identify novel risk factors that may aid both its detection and prevention.

Aims: 1) To discuss the benefits and disadvantages of using HbA1c for diagnosis of T2DM and impaired glucose regulation (IGR).
2) To explore the impact on prevalence of using HbA1c to detect T2DM and IGR in global and local populations.
3) To determine if diagnostic cut-points are equivalent in different ethnic groups
4) To determine the use of the triglyceride-to-HDL ratio and its association with insulin resistance and whether statins and liver enzymes predict T2DM.
5) To investigate if Vitamin D deficiency has a role in the prevention of T2DM by designing a 6 month randomised controlled trial on vitamin D replacement.

Key findings: 1) Using HbA1c for diagnosis has some logistical advantages over glucose testing, but may not detect the same people as having T2DM or IGR.
2) In Leicestershire, using HbA1c will increase numbers of people with T2DM and IGR. On global level, there will be regional variation on the effect on prevalence.
3) HbA1c, fasting and two hour plasma glucose are independently higher in South Asians (SA). Complications of T2DM may begin earlier in SA.
4) The triglyceride-to-HDL ratio associates with insulin resistance in Europeans and SA men but not women. Statin therapy reduces the risk of incident T2DM. Liver enzymes predict T2DM in Europeans but not SA.
5) Vitamin D deficiency may form a target for reducing insulin resistance in SA – the final results of the VITALITY study in 2015 will contribute to evidence base in this area.

Conclusions: In this thesis I have explored new ways of detecting T2DM and IGR by using HbA1c and what impact this may have; also novel risk factors for T2DM have been investigated that may help improve methods of both earlier detection and prevention of T2DM.
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# List of contents

## Abstract

Acknowledgements
List of contents
List of tables
List of figures
List of abbreviations

## Chapter One: Introduction and guide to the thesis

1.1 Overview of the thesis
1.2 Outline of chapters 2 to 8

## Chapter Two: Should HbA1c be used to detect T2DM and IGR?

2.1 Chapter overview
2.2.1 Introduction
2.2.2 Current recommendations for screening tests for T2DM
2.2.3 Glycated Haemoglobin (HbA1c)
2.2.4 Diagnostic recommendations for T2DM with HbA1c
2.2.5 Diagnostic recommendations for IGR based around HbA1c
2.2.6 A new reporting unit for HbA1c
2.2.7 Potential advantages of adding HbA1c to T2DM criteria
2.2.8 Potential limitations of using HbA1c for diagnosis of T2DM
2.2.9 How should additional people detected with T2DM through use of HbA1c be managed and approached?
2.2.10 Potential economic impact of adding HbA1c to T2DM criteria
2.2.11 Future prospects regarding using HbA1c for diagnosis?

2.3 The impact of detecting T2DM and IGR based on HbA1c
2.3.1 How to interpret studies appropriately
2.3.2 Factor regarding glucose studies
2.3.3 Factors regarding HbA1c studies

2.4.1 Studies comparing use of HbA1c and glucose for T2DM
2.4.2 Impact of using HbA1c ≥ 6.5% to detect T2DM
2.4.3 Performance of HbA1c in black and ethnic minority groups
2.4.4 Sensitivity and specificity of HbA1c ≥ 6.5% to detect T2DM
2.4.5 Optimal HbA1c cut-points from ROC curves to detect T2DM
2.4.6 Area under the curve for HbA1c and glucose to detect T2DM
2.4.7 Discordance of tests using Kappa agreement measurements
2.4.8 Prevalence of people with T2DM on glucose but HbA1c < 6.5%
2.4.9 Changes in phenotype and CVD risk of T2DM using HbA1c ≥ 6.5% but not glucose testing?
2.4.10 Long term prediction of macrovascular events using HbA1c
2.4.11 Discussion

2.5.1 Studies comparing use of HbA1c and glucose for IGR
2.5.2 Impact on prevalence of IGR
2.5.3 Optimal cut-point for IGR
2.5.4 Combined use of HbA1c and fasting plasma glucose for IGR
2.5.5 Discordance between diagnostic tests
2.5.6 Changes in phenotype and CVD risk in IGR using HbA1c?
2.5.7 Discussion

2.6.1 Studies analysing progression of HbA1c for incident T2DM
2.6.2 HbA1c to predict incident T2DM
2.6.3 Combined use of HbA1c and fasting plasma glucose
2.6.4 Progression to T2DM using HbA1c in people with IGT
2.6.5 The HbA1c optimal cut-point for detecting incident T2DM
2.6.6 Discussion

2.7 Overall conclusions

Chapter three: the potential impact of using HbA1c to detect T2DM and IGR
3.1 Chapter Overview
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
<td>Introduction</td>
<td>99</td>
</tr>
<tr>
<td>3.3</td>
<td>Research Design and Methods</td>
<td>101</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Design overview, setting, data collection and participants</td>
<td>101</td>
</tr>
<tr>
<td>3.3.2</td>
<td>Screening those at risk (STAR)</td>
<td>101</td>
</tr>
<tr>
<td>3.3.3</td>
<td>ADDITION-Leicester</td>
<td>103</td>
</tr>
<tr>
<td>3.3.4</td>
<td>Laboratory assays</td>
<td>109</td>
</tr>
<tr>
<td>3.4</td>
<td>Statistical Analysis</td>
<td>112</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Analysis of T2DM</td>
<td>112</td>
</tr>
<tr>
<td>3.4.2</td>
<td>Analysis of IGR</td>
<td>113</td>
</tr>
<tr>
<td>3.5</td>
<td>Results</td>
<td>114</td>
</tr>
<tr>
<td>3.5.1</td>
<td>Results for T2DM</td>
<td>115</td>
</tr>
<tr>
<td>3.5.2</td>
<td>Phenotype and Modifiable Risk Factors</td>
<td>116</td>
</tr>
<tr>
<td>3.5.3</td>
<td>Optimal HbA1c cut-points for detecting T2DM on OGTT</td>
<td>117</td>
</tr>
<tr>
<td>3.5.4</td>
<td>Results for IGR</td>
<td>123</td>
</tr>
<tr>
<td>3.5.5</td>
<td>Anthropometrics and clinical characteristics</td>
<td>124</td>
</tr>
<tr>
<td>3.5.6</td>
<td>Determination of optimal HbA1c cut-points for IGR on OGTT</td>
<td>125</td>
</tr>
<tr>
<td>3.5.7</td>
<td>Sub-analysis using ADA definition of IFG</td>
<td>126</td>
</tr>
<tr>
<td>3.6</td>
<td>Discussion</td>
<td>134</td>
</tr>
<tr>
<td>3.6.1</td>
<td>T2DM in LEADER cohort</td>
<td>134</td>
</tr>
<tr>
<td>3.6.2</td>
<td>IGR in the LEADER cohort</td>
<td>138</td>
</tr>
<tr>
<td>3.6.3</td>
<td>Strengths and limitations of the LEADER study</td>
<td>140</td>
</tr>
<tr>
<td>3.7</td>
<td>Conclusion</td>
<td>142</td>
</tr>
</tbody>
</table>

**Chapter 4: Alternate ways to incorporate HbA1c into diagnostic pathways**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Chapter Overview</td>
<td>144</td>
</tr>
<tr>
<td>4.2</td>
<td>Introduction</td>
<td>144</td>
</tr>
<tr>
<td>4.3</td>
<td>Research Design and Methods</td>
<td>146</td>
</tr>
<tr>
<td>4.4</td>
<td>Statistical Methods and data analysis</td>
<td>146</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Cost analysis</td>
<td>147</td>
</tr>
<tr>
<td>4.5</td>
<td>Results</td>
<td>148</td>
</tr>
<tr>
<td>4.5.1</td>
<td>Use of a single HbA1c cut-point to diagnose T2DM</td>
<td>149</td>
</tr>
</tbody>
</table>
4.5.2 Performance of two cut-point strategies to diagnose T2DM 149
4.5.3 Cost analysis 150
4.6 Discussion 156
4.7 Implications for clinicians and policy makers 157
4.8 Conclusion 160

Chapter 5: Are HbA1c diagnostic cut-points appropriate for all people?
5.1 Chapter overview 162
5.2 Introduction 162
5.2.1 Glycaemic markers and ethnic minority groups 162
5.2.2 Glycaemia markers and microalbuminuria 163
5.3 Research Design and Methods 165
5.4 Statistical Analysis 165
5.4.1 The effect of ethnicity on glycaemia 165
5.4.2 Effect of ethnicity on glycaemia and ACR 167
5.5 Results 170
5.5.1 Ethnicity and glycaemia 170
5.5.2 Effect of ethnicity on ACR and glycaemic markers 179
5.6 Discussion 187
5.6.1 Ethnicity and glycaemic markers 187
5.6.2 Ethnicity and microalbuminuria 190
5.6.3 Implications for policy makers and clinicians 193
5.6.4 Conclusion 195

Chapter 6: The Triglyceride-to-HDL cholesterol ratio 196
6.1 Chapter Overview 197
6.2 Introduction 197
6.3 Research Design and Methods 199
6.4 Statistical analysis 200
6.5 Results 202
6.6 Discussion 207
8.3.1 Pancreatic actions 250
8.3.2 Immune actions 251
8.3.3 Genetic risk 252
8.4 South Asians and VDD 252
8.5 Interventional studies of vitamin D replacement 253
8.5.1 Interventional studies of vitamin D replacement in South Asians 255
8.6 The potential advantages gained from the VITALITY study 259
8.6.1 Hypothesis and aims 259
8.7 Rationale and justification of study methods 261
8.7.1 Target population 261
8.7.2 Basis of primary outcome variable 261
8.7.3 Length of the study 262
8.7.4 Baseline levels of insulin resistance 263
8.7.5 Doses of Vitamin D therapies and target Vitamin D levels 264
8.7.6 Safety elements in the VITALITY study 266
8.7.7 Regulatory approvals 268
8.7.8 Maintaining blinding during the randomised controlled trial 268
8.7.9 Investigational medicinal products in the VITALITY study 269
8.8 Outcomes of the VITALITY study 269
8.8.1 Primary outcome 269
8.8.2 Sample size: power required for the primary outcome 270
8.8.3 Secondary outcomes 270
8.9 Methods for the VITALITY study 271
8.9.1 Location of the study 271
8.9.2 Recruitment strategies 271
8.9.3 Study design 275
8.9.4 Randomisation 275
8.9.5 Statistical analysis 275
8.9.6 Eligibility to participate in the VITALITY RCT 277
8.9.7 Participant discontinuation criteria and withholding of IMPs 277
8.10 Participant visits

8.10.1 Screening visit (V \textsuperscript{-2})

8.10.2 Baseline visit (V \textsuperscript{-1})

8.10.3 Visit 1 (V1): Dosing visit – start of the RCT

8.10.4 Visit 2 (V2): 1 week post-dose follow up

8.10.5 Visit 3 (V3): 1.5 month visit

8.10.6 Visit 4 (V4): 2.5 month follow-up

8.10.7 Visit 5 (V5): 3 month follow up: Interim analysis

8.10.8 Visit 6 (V6): 5 month follow-up

8.10.9 Visit 7 (V7): 6 month: final visit

8.10.10 Post study 1 month follow up

8.11 Laboratory assays

8.12.2 Statistical analysis

8.13 Results

8.14 Discussion

8.14.1 Screening Data

8.14.2 Feasibility and reflections of the VITALITY pilot study

8.14.2 Feasibility and implications for future research in this area

Chapter 9: Overall Summary to thesis

9.1 Conclusion to this thesis

Appendix One: Contributions made

Appendix Two: Letters and documents related to the conduct and recruitment to the VITALITY study

Appendix Three: Search strategy for review on HbA1c

Appendix Four: Publications related to work in this thesis

Appendix Five: Awards and media appearances related to this thesis

References
List of tables

Table 2.1 Guide to the key HbA1c values to remember in clinical practice 40
Table 2.2 Summary of studies comparing glucose and HbA1c for T2DM 61
Table 2.3 Summary of studies comparing HbA1c accuracy for detecting IGR 78
Table 2.4 Demographics of selected studies on HbA1c progression to T2DM 90
Table 2.5 Progression of HbA1c to developing incident T2DM in studies 91
Table 3.1 Selected baseline characteristics of the LEADER cohort 115
Table 3.2 Population characteristics according to OGTT and HbA1c results 120
Table 3.3 Analysis of risk factors according to OGTT and HbA1c status 121
Table 3.4 Comparison of diagnostic indices for T2DM at HbA1c ≥ 6.5% 122
Table 3.5 Demographics of IGR on OGTT, HbA1c 6.0-6.4% and 5.7-6.4% 127
Table 3.6 (a) & (b) Selected population & CVD characteristics using IEC & ADA criteria 129
Table 3.7 Distribution of IGR subtypes according to HbA1c cut-points 131
Table 3.8 Optimal HbA1c cut-points derived from ROC curves for IGR 132
Table 4.1 Comparison of diagnostic indices of HbA1c for T2DM 153
Table 4.2 Detection of T2DM and IGR using two HbA1c cut-point thresholds 154
Table 4.3 Comparison of cost estimations for HbA1c cut-point strategies 155
Table 5.1 Baseline characteristics for White Europeans and South Asians 173
Table 5.2 Baseline glycaemic markers stratified by HbA1c categories 174
Table 5.3 The independent effect of variables on HbA1c 175
Table 5.4 Comparison of crude and adjusted differences for HbA1c 176
Table 5.5 Sub-analysis of HbA1c ethnicity differences separated by sex 177
Table 5.6 Sub-analysis of ethnicity differences in fasting plasma glucose 177
Table 5.7 Sub-analysis of ethnicity differences in two-hour plasma glucose 178
Table 5.8 Selected baseline demographics of the ACR cohort analysed 182
Table 5.9 Prevalence of microalbuminuria 183
Table 5.10 A comparison of mean ACR in white European and South Asians 184
Table 5.11 HbA1c independent differences 190
Table 6.1 Selected baseline demographics of cohort investigated 204
List of Figures

Figure 2.1 Diagnostic criteria of T2DM and IGR using glucose and HbA1c  28
Figure 3.0 Study cohorts analysed in this thesis for chapters 3 to 7  102
Figure 3.1 Venn diagram to identify T2DM by using HbA1c or OGTT  118
Figure 3.2 Venn diagram to represent people with T2DM on (a) HbA1c and
OGTT, (b) OGTT but not HbA1c and (c) HbA1c but not OGTT  119
Figure 3.3 A combination of figure 3.1 and 3.2  119
Figure 3.4 Optimal HbA1c cut-points for T2DM  122
Figure 3.5 Prevalence of IGR on OGTT and HbA1c of 6.0 to 6.4%  127
Figure 3.6 Prevalence of IGR on OGTT and HbA1c of 5.7 to 6.4%  128
Figure 3.7 Line graphs of HbA1c sensitivity and specificity for IGR  132
Figure 3.8 Impact on prevalence of T2DM with a high mean cohort HbA1c  137
Figure 3.9 Impact on prevalence of T2DM with a low mean cohort HbA1c  137
Figure 4.1 Strategies for diagnostic accuracy and cost per case of T2DM  152
Figure 5.1. Independent effect of ethnicity on ACR & glycaemia  185
Figure 6.1 ROC curves of triglycride-to-HDL to detect insulin resistance  206
Figure 7.1 Participants used in chapter 7 analyses  221
Figure 8.1 Levels of calcidiol after a dose of 100,000 units Vit D3  265
Figure 8.2 Recruitment for screening for VITALITY study  274
Figure 8.3 Consort schematic for participant flow through the VITALITY  285
Figure 8.4 VITALITY study timelines for progress and delays  306
List of abbreviations used in this thesis

AACE/ACE = American Association of Clinical Endocrinology/ American College of Endocrinology
ABCD = Association of British Clinical Diabetologists
ACR = albumin: creatinine ratio
ADA = American Diabetes Association
ADDITION = Anglo-Danish-Dutch Study of Intensive Treatment in people with Screen Detected Diabetes in Primary Care
ALT = alanine aminotransferase
ARIC = Atherosclerosis Risk in Communities
AUROC = area under the ROC curve
BMI = body mass index
BP = blood pressure
CI = 95% confidence intervals
CTA = Clinical Trial Authorisation
CVD = cardiovascular disease
DBP = Diastolic Blood Pressure
DCCT = Diabetes Control and Complication Trial
DECODE = Diabetes epidemiology: collaborative analysis of diagnostic criteria in Europe
eAG = estimated average glucose
EASD = European Association for the Study of Diabetes
FCG = fasting capillary glucose
FPG = fasting plasma glucose
GGT = gamma-glutaryl transferase
Hb = haemoglobin
HbA1c = glycated Haemoglobin
HOMA-B = Homeostasis model assessment of beta cell function
HOMA-IR = Homeostasis model assessment of insulin resistance
IDF = International Diabetes Federation
IEC = International Expert Committee
IFCC = International Federation of Clinical Chemistry
IFG = Impaired fasting glycaemia
IGT = Impaired glucose tolerance
IGR = Impaired glucose regulation
IMD = Index of Multiple Deprivation
IMP = Investigational Medicinal Products
IPAQ = International Physical Activity Questionnaire
IRAS = Insulin Resistance Atherosclerosis Study
JDS = Japanese Diabetes Society
LEADER = Leicester Ethnic Atherosclerosis and Diabetes Risk study
LFTs = Liver function tests
METs = Metabolic equivalents
MHRA = Medicines and Healthcare products Regulatory Agency
NGSP = National Glycohaemoglobin Standardisation Programme
NICE = National Institute for Health Care Excellence
NHANES = National Health and Nutrition Examination Survey
OGTT = Oral Glucose Tolerance Test
OHA = oral hypoglycaemic agents
OR = odds ratio
QUICKI = quantitative insulin sensitivity check index
RCT = randomised controlled trial.
ROC = Receiver Operating Characteristics
RPG = Random Plasma Glucose
SA = South Asian
SBP = Systolic Blood Pressure
SD = standard deviation
STAR = Screening Those At Risk
T2DM = Type 2 Diabetes Mellitus
UKPDS = United Kingdom Prospective Diabetes Study
VDD = Vitamin D deficiency
VDR = Vitamin D receptor
VITALITY = Can Vitamin D replacement reduce Insulin resistance in South Asians with vitamin D deficiency?
WE = White European
WHO = World Health Organisation
WHS = Women’s Health Study.
25(OH) Vit D level = 25 hydroxy Vitamin D3
Chapter 1

Introduction and overview of the thesis
1.1 Overview of the thesis

Type 2 Diabetes Mellitus (T2DM) has reached epidemic proportions across the world; future projections of this disease suggest by 2030 up to 500 million people may have T2DM (International Diabetes Federation, 2011). One particular ethnic group with a high burden of T2DM and its complications are South Asians (people of Indian, Bangladeshi, Pakistani and Sri Lankan origin), whether they live in South Asia or have migrated to western countries, such as the UK (Gholap et al., 2011). South Asians form an estimated global population of approximately 1.5 billion people, including over 3 million migrants living in the UK and a further three million in the US (India Census, 2011; Pakistan Population Census Organization, 2011; Bangladesh Bureau of Statistics, 2011; UK Ethnicity National Statistics, 2011; US census Bureau, 2010).

There is a need to focus more attention on preventing this disease in high risk groups and also to introduce new measures of detecting T2DM earlier, so that interventions to delay the onset of complications related to T2DM can be implemented earlier. In June 2009, an International Expert Committee advocated use of glycated haemoglobin, HbA1c, at the level of ≥ 6.5% as a diagnostic tool for detecting T2DM (International Expert Committee, 2009). This single position statement changed the nature of screening and diagnosing T2DM. For many previous years it was suggested that HbA1c could one day become a diagnostic tool for T2DM, however this was the first statement advocating its use. Initially, there was some concern about whether making this change was the right step.
forward for detecting this disease (Kilpatrick et al., 2009). Over recent years, there have been a large number of research papers looking at potential changes in prevalence rates of T2DM from using HbA1c and explaining these changes. However there was very little data on what may happen to prevalence rates in the UK. There was also a lack of data on what may happen within South Asians, especially those who migrated to western countries.

This coincided with the start of my research attachment and therefore I was given the opportunity to analyse the impact of diagnostic changes in our local multi-ethnic population in Leicestershire. Within the thesis, chapter’s two to five are dedicated to HbA1c and its role in diagnosing T2DM; an outline of these chapters is described below.

Secondly, as there is a high prevalence and incidence of T2DM, there is a need to identify and investigate novel risk factors associated with developing of T2DM that may help to (a) understand more about the pathogenesis of this disease, (b) suggest new methods to detect people at high risk of developing T2DM and (c) explore new ways to prevent T2DM. Chapters’ six to eight investigate the role of novel risk markers for T2DM.

1.2 Outline of chapters 2 to 8

In Chapter 2, I review the problems that occur with screening for T2DM using glucose based tests and discuss the potential advantages and disadvantages
from using glycated haemoglobin, HbA1c as an additional tool for T2DM. Secondly, I review the recent published research studies about the impact of using HbA1c for T2DM and impaired glucose regulation (IGR) in different populations across world.

In Chapter 3, I investigate the impact of using HbA1c for diagnosis of T2DM and IGR in comparison to an oral glucose tolerance test (OGTT) in our local Leicestershire region using a contemporary dataset of people previously undiagnosed with T2DM, especially focusing on ethnic differences between South Asians and White Europeans.

Due to the some of the concerns of using HbA1c for diagnosis using a single cut-point of 6.5%, in Chapter 4 I explore an alternative method of incorporating HbA1c into a diagnostic pathway, a so called two cut-point ‘rule-in, rule out’ method.

In chapter 5, I discuss whether the same HbA1c cut-point of 6.5% is correct for diagnosing T2DM in all people, using the local South Asian population as an example of this. Here I investigate if HbA1c is independently higher in South Asians compared to White Europeans, which suggest using HbA1c in South Asians could increase prevalence rates of T2DM and IGR. This raises the question of whether there should be ethnic specific cut-points for using HbA1c in South Asians, which we use by looking at differences in microalbuminuria.
In Chapter 6, I investigate a novel lipid ratio which may identify those at highest risk of future glucose intolerance, known as the triglyceride-to-HDL cholesterol ratio. Here I analyse this novel lipid ratio and its relationship with insulin resistance, a risk marker for T2DM.

In Chapter 7, I determine at two novel risk factors which may influence the progression of people with IGR to future T2DM. The first risk factor analysed is use of statin therapy, which traditionally is considered as one of the standard methods of preventing cardiovascular disease (Baigent et al, 2005). However there have been some recent reports about statins increasing risk for T2DM in the general population, but not much is known about whether they influence the progression of IGR to T2DM (Sattar et al., 2011). The second risk factor I investigate is around routinely measured liver enzymes and whether they can be used to assess risk of incident T2DM in people with IGR.

In Chapter 8, I introduce Vitamin D deficiency as a risk factor for T2DM. It is known that people with the lowest vitamin D levels of populations are more likely to get future T2DM (Forouhi et al. 2008; Scragg et al., 2004). However it is unknown whether replacing these low vitamin D levels would reduce this future risk of T2DM. This is a question that I want to answer using our local South Asian population, as they form an ethnic group who present with very low vitamin D levels and a high burden of T2DM. In order to answer this question, I describe the rationale and study methods of a six month double blind randomised controlled trial of high dose vitamin D replacement compared to control dose; the primary aim is to reduce insulin resistance. In order to facilitate this study, I will
also need to find eligible participants with Vitamin D Deficiency and insulin resistance by running a screening programme. I will present the results of the screening data up to September 2013.

Chapter 9 is an overall summary of the thesis.
Chapter 2

Should glycated haemoglobin, HbA1c, be used to detect people with Type 2 Diabetes Mellitus and Impaired Glucose Regulation?

Recent diagnostic recommendations and the potential impact of this change in different populations
2.1 Chapter overview

Chapter 2 of this thesis identifies the current problems that lie within screening for T2DM using glucose based tests and looks the potential advantages and disadvantages from using glycated haemoglobin, HbA1c. Also I review also the recent diagnostic changes and the current published studies that have compared the impact of using glucose tests compared to HbA1c ≥ 6.5%. This chapter is based on two first author published review papers (Mostafa et al., 2010a; Mostafa et al., 2011).

2.2.1 Introduction

Type 2 Diabetes Mellitus (T2DM) is a ubiquitous chronic multi-system disease associated with debilitating micro- and macrovascular complications, which may reduce quality and length of life by 10 to 15 years (Eastman et al., 1997). The financial burden associated with T2DM and its complications is currently reported as £8.8 and £13 billion for direct and indirect costs respectively in the UK and are expected to rise (Hex et al., 2012). Furthermore, rates of T2DM incidence are increasing, which is fuelled by a similar trend of increasing obesity in most areas of the world. In the UK, the prevalence of T2DM was 1.67 million in 2003 and rose to over 3 million by 2012 (International Diabetes Federation, 2005; Diabetes UK, 2012). Similarly, the US prevalence of T2DM of 7.4% is expected to increase
to 8.9% by 2025 (King et al., 1998). These figures are likely to be based on patients diagnosed/registered with T2DM and only estimate those people with undiagnosed T2DM. A global estimate in 2003 suggested that in addition to recognised cases of T2DM, a further 33 to 50% of people have undetected T2DM, which is thought to represent 800,000 people in the UK (World Health Organisation, 2003; Diabetes UK, 2012).

The average period between developing T2DM and its subsequent diagnosis is 7 years (Harris et al., 1992). During this asymptomatic latent phase, patients are may be exposed to chronic hyperglycaemia and therefore risk complications developing, particularly if there is no implementation of lifestyle or therapeutic interventions (Gillies et al., 2008). It is reported that by the time T2DM is eventually diagnosed macrovascular complications may have already developed in up to 20 to 30% of individuals (DECODE study group, 1999). Alternatively, a macrovascular event may be the first presentation of undiagnosed diabetes. Therefore, there is a need to simplify screening tests for T2DM so patients can be identified earlier and more efficiently, which will allow more time to implement strategies to prevent these hyperglycaemic complications. This is one reason why a change in diagnostic criteria has been considered.

The aim of this chapter is to review the following:

(a) The diagnostic/screening tests used for detecting T2DM and impaired glucose regulation (IGR, also known as prediabetes or those at risk of T2DM) prior to 2009 and any potential problems that may exist with use of them.
(b) The advantages and disadvantages of using HbA1c as a diagnostic tool and recommendations for diagnosis in clinical practice.

(c) The newly adopted method for reporting HbA1c in millimoles per mole.

(d) If there are any implications from using of HbA1c for diagnosis in clinical practice.

(e) A review of recent studies analysing potential changes from using HbA1c for diagnosis instead of glucose based tests.
2.2.2 Current recommendations for screening tests for T2DM

The diagnostic criteria for T2DM have changed various times since the first World Health Organisation definition in 1965, reflecting the threshold beyond which the prevalence of microvascular complications increases, particularly diabetes specific retinopathy (World Health Organisation, 1965). Prior to 2011, T2DM was diagnosed using a fasting plasma glucose test (FPG) and/or a two-hour plasma glucose test on an OGTT requiring results values of ≥ 7.0mmol/l and ≥ 11.1mmol/l respectively (World Health Organisation, 1999, and Figure 2.1). As glucose levels may experience daily variability and potential errors in laboratory measurement, all asymptomatic individuals with a positive test result required a repeat confirmatory test. The repeat test should be preferably performed within three days, which prevents individuals from introducing interventions which may lower glucose levels such as increasing physical activity levels or altering dietary patterns, especially lowering carbohydrate load prior to testing. Therefore diagnosis of T2DM required two positive tests in asymptomatic individuals. People with typical symptoms of T2DM (for example polyuria, polydipsia, weight loss, blurred vision or extreme fatigue) require only one test on a single day and this can include a random glucose level ≥ 11.0mmol/l.
Figure 2.1 Diagnostic criteria of T2DM and IGR using glucose testing and the glycated haemoglobin (HbA1c) criteria. Taken from Mostafa et al., 2010.

The onset of most cases of T2DM is usually preceded by a latent phase of glucose intolerance or impaired glucose regulation, IGR. This can also be termed prediabetes, intermediate hyperglycaemia, high risk of T2DM or non-diabetic hyperglycaemia. Prior to 2009, this important metabolic state consisted of impaired fasting glycaemia (IFG) and/or impaired glucose tolerance (IGT);
neither is classified as a disease currently. IFG was first defined when the ADA 1997 diagnostic guidelines placed a heavy emphasis on using a FPG ≥ 7.0mmol/l for T2DM in isolation from the OGTT and introduced the IFG category of 6.1 to 6.9mmol/l, which was further amended to ≥ 5.6 to 6.9mmol/l in 2003, although the WHO did not follow this change (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997 and 2003; World Health Organisation, 2003). The World Health Organisation diagnostic definitions of IFG and IGT are 6.1 to 6.9mmol/l and 2-hour plasma glucose of 7.8 to 11.0mmol/l respectively (WHO, 1999).

FPG has traditionally been considered as the preferred screening tool for detecting T2DM in many countries. It has a higher sensitivity for detecting T2DM and simpler to interpret than either a random plasma glucose (RPG) or glucose urinalysis. However, often a RPG can be used as the first line screening test before a FPG is performed. Furthermore, use of FPG is cheaper and less time consuming than performing an oral glucose tolerance test (OGTT). This is a key reason why the American Diabetes Association (ADA) suggested FPG should be used as the preferred test used for T2DM diagnosis, although an OGTT may be performed when FPG detects IFG (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Performing an OGTT involves a minimum of two hours of patient and staff time, and may pose problems if the patient must take time off from work or has family commitments. However, the OGTT is the only way to measure 2-hour plasma glucose levels and therefore detect people with raised 2-hour plasma glucose levels in the T2DM range or
IGT. Measuring two hour plasma is important as patients may have isolated post prandial hyperglycaemia, therefore may not be detected using a FPG.

There has been much debate about using either FPG or OGTT for diagnosis of T2DM. Both tests require early morning test appointments and therefore limit the scope for opportunistic screening. The two tests require precise patient preparation prior to measurement in order to produce the most accurate results. This includes the absence of introducing potentially glucose lowering interventions for three days prior to testing, such as increasing physical activity levels and lowering the dietary carbohydrate intake load (World Health Organisation, 2003). Furthermore, the patient must attend in an overnight fasting state (a minimum time period of at least eight hours) and avoid smoking on the morning of testing. Patients then self-report whether they followed appropriate preparation advice, which can be viewed as a limitation. Furthermore, in theory patients should not be physically or emotionally stressed as this may alter glucose levels and therefore testing should be delayed until the stress has subsided. Furthermore, the type of glucose load may vary from fizzy drinks (e.g. Lucozade TM) to anhydrous glucose loads, which may affect the rate of consumption from instantaneous to over a few minutes. Additionally, World Health Organisation guidelines suggest plasma glucose samples should be placed on ice immediately (to stop actively living cells within the blood samples utilising glucose supplies) and should be processed within one hour (World Health Organisation, 2003). Both of these conditions appear impractical for routine clinical practice, in particular for community based practice. For many of
these reasons above, the reproducibility of an OGTT result may be relatively low (Ko et al., 1996), as the imprecision co-efficient of variance can vary widely according to appropriate patient preparation and handling of samples.

As its name suggests, FPG accurately detects those with fasting hyperglycaemia, more typical of pancreatic beta cell dysfunction. However it does not assess the degree of post-prandial hyperglycaemia more closely associated with insulin resistance (Davies et al., 2000). A person with post-prandial hyperglycaemia in the diabetic range but FPG in the ‘normal’ defined range will not be detected using FPG. Therefore a simple hypothesis suggests FPG has a bias for identifying those with beta cell dysfunction and may miss more of the insulin resistant group. An individual may only develop both fasting and post prandial hyperglycaemia with progressive glucose intolerance, not necessarily in the early stages of chronic hyperglycaemia (Saad et al., 1998; Del Prato et al., 2003; Ko et al., 1998; Gimeno et al., 1998; Ko et al., 2000). As a result, FPG has a limited sensitivity for detecting 40 to 60% of total T2DM cases from an OGTT (Perry et al., 2001; Tanaka et al., 1998; Barr et al., 2002). A similar principle of limited sensitivity with use of FPG exists for detecting IGR, as those people with isolated IFG and combined IFG and IGT will be detected, but not those with isolated IGT.

In summary, there are certain perceived barriers to detecting T2DM in a timely fashion, including inconvenience of patient fasting and limited sensitivity of tests. This may have led to lower screening rates and under-diagnosis of T2DM and
IGR with glucose based screening tests. Case finding of T2DM needs to become easier for patients and clinicians.

2.2.3 Glycated Haemoglobin (HbA1c)

HbA1c is formed by the non-enzymatic reversible glycation of N-terminal Valine residues of erythrocyte haemoglobin which rises proportionally with increasing blood glucose intolerance. It provides a weighted average of blood glucose for the lifespan of an erythrocyte, typically 2-3 months, with the last month contributing approximately 50% to the final result (Jeffcoate, 2004; Tahara et al., 1993; Goldstein et al., 1993). This explains why a recent change in diet or treatment can alter HbA1c values within 6 weeks.

HbA1c until recently was recommended for monitoring the progress of T2DM, to evaluate the risk of complications and as a guide when to start treatment. However, the role of HbA1c in diagnosis of T2DM has not been so clear and the World Health Organisation has previously rejected this idea in 2006 (World Health Organisation, 2006). Anecdotally, HbA1c has been used in community opportunistic screening for many years, however without the support of guidelines, therefore there was no standardisation of which HbA1c cut-point which detect T2DM and this was left to the discretion of the health care professional (Ealovega et al., 2004). There is accumulating evidence that HbA1c has equally as good if not higher discriminatory ability for detecting T2DM and
cardiovascular disease (CVD) than FPG (Perry et al., 2001; Kim et al., 2008; Droumaguet et al., 2006; Manley et al., 2009; Buell et al. 2007; Rohlfing et al., 2000; Selvin et al., 2010).

2.2.4 Diagnostic recommendations for T2DM based around HbA1c from international organisations and expert panels

In 2008, a US based expert panel reviewed the available evidence and suggested HbA1c should be used for diagnosis of T2DM at the level of ≥ 6.5% (Saudek et al., 2008). This cut-point was based upon three standard deviations (SD) above the mean HbA1c within the National Health and Nutrition Examination Survey III (NHANES) study (5.17%, SD 0.45).

A separate International Expert Committee (IEC) was formed in 2009 from several international organisations including representatives from the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) (International Expert Committee, 2009). This expert panel reviewed current information on HbA1c for diagnosis and concluded a similar recommendation of using an HbA1c ≥ 6.5% to detect T2DM in non-pregnant adults (Figure 2.1). This specific cut-point was selected as it shares the same approximate threshold above which prevalent retinopathy increases with glucose diagnostic cut-points (fasting plasma glucose ≥ 7.0mmol/l and 2-hour plasma glucose levels ≥ 11.1mmol/l), based on three populations and data from the current global DETECT-2 study (International Expert Committee, 2009; Colagiuri
et al., 2010). The IEC stated moderate diabetes specific retinopathy is thought to be rare below an HbA1c of 6.5%.

In 2010, the ADA officially proposed using HbA1c ≥ 6.5% as a diagnostic criteria guideline in their ‘Standards of Medical Care in T2DM’ position statement (American Diabetes Association Position statement, 2010). Furthermore, the HbA1c recommendation was promoted above either FPG or 2-hour plasma glucose, showing a degree of intent from the ADA. Furthermore, a brief joint position statement from the American Association of Clinical Endocrinology/American College of Endocrinology (AACE/ACE) and a separate group, the Endocrine Organisation have also recommended using HbA1c ≥ 6.5% to detect T2DM (American Association of Clinical Endocrinologists / American College of Endocrinologists, 2010; Endocrine society, 2010). Finally, the World Health Organisation released their HbA1c recommendations in 2011 and endorsed HbA1c ≥ 6.5% for detecting T2DM (World Health Organisation, 2011).

In the UK there have been differing recommendations, as Diabetes UK has also suggested using HbA1c ≥ 6.5% as above, however the Association of British Clinical Diabetologists (ABCD), have suggested a different strategy of using two HbA1c cut-points to detect T2DM (John et al; 2012; Kilpatrick et al., 2010). The lower HbA1c cut-point of 5.7% cut-point ‘rules out’ a diagnosis of T2DM and the upper HbA1c of 7.3% cut-point ‘rules in’ T2DM. All individuals with an HbA1c between the two cut-points should undergo a glucose test for confirmation of their glucose status. To my knowledge, this strategy has not been widely adopted to
date. The feasibility of using a two cut-point strategy for diagnosis is not known, but is explored further in Chapter 4.

The various committees/ panels have stated that any asymptomatic people with an HbA1c ≥ 6.5% require a repeat confirmatory test, following a similar format as the current glucose diagnostic criteria. Therefore, T2DM is diagnosed with two HbA1c ≥ 6.5% values in asymptomatic individuals. There is a specific reason for repeat test. Although HbA1c reflects longer term glycaemia, has better reproducibility and less intra-individual variation compared to glucose testing, there may still be some degree of laboratory error in measuring samples, therefore a repeat test would reduce the chances of an inappropriate diagnosis (Ko et al., 1998; Rohlfing et al., 2002).

The expert panels and international committees have given some specific points on the nature of this repeat confirmatory test. The IEC suggest the follow up test should be the same form as the initial test (i.e. two HbA1c tests or two glucose tests) (IEC, 2009). The ADA position statement suggests it is preferable to confirm diagnosis using the same initial test, as there is greater likelihood of concurrence of a positive test result, however two different tests (i.e. an HbA1c and a glucose test) can also be diagnosed as T2DM (ADA Position statement, 2010). Alternatively, the US based expert panel in 2008 have suggested random plasma glucose could form the second test and this may even be performed on the same day as the initial HbA1c, avoiding the requirement of a second day (Saudek et al., 2008).
The time period when a repeat and confirmatory HbA1c should be performed in asymptomatic individuals has not been clarified in most position statements. As mentioned above, HbA1c reflects longer term glycaemia of three months, with the final preceding month providing approximately 50% of the final test result. Therefore a repeat test should ensure any subsequently implemented lifestyle changes do not have a major influence on the repeat result. Diabetes UK has suggested the repeat HbA1c should be any time within two weeks of the initial test, which appears to be a sensible time period (John et al., 2011).

Secondly, all committees agreed using glucose for diagnostic testing is still valid; especially as many developing or remote areas of the world may not have facilities to change to HbA1c. Therefore it is up to the health care professional to determine which diagnostic tool to use. For this and other reasons, there is some debate about using HbA1c for diagnosis of T2DM (Mostafa SA et al., 2010; Kilpatrick et al., 2009). Furthermore, it is generally agreed the new HbA1c diagnostic criterion is not applicable for diagnosing either Type 1 diabetes (as rapid increases in glucose levels will not be reflected so quickly in HbA1c levels) or gestational diabetes (as haemoglobin levels may increase or decrease and not give accurate reflection of glycaemic levels).

### 2.2.5 Diagnostic recommendations for IGR based around HbA1c

In 2009, the International Expert Committee has suggested using HbA1c 6.0 to 6.4% but gave no real explanation for selecting these cut-points (International
Expert Committee, 2009). The ADA in contrast has recommended using a lower cut-point from HbA1c 5.7 to 6.4%, based on a personal communication of a ROC curve analysis of prevalent IFG from the US based NHANES study (ADA Position statement, 2010). This could be seen as a similar move to the ADA reducing the lower limit diagnostic cut-point of IFG from 6.1mmol/l to 5.6mmol/l in 2003 (Expert Committee, 2003). The same HbA1c cut-points are endorsed by the Endocrine Society (Endocrine society, 2010). One reason for this discrepancy in chosen cut-points by these organisations may be due to the lack of evidence for either cut-point (see section 2.3 onwards). Therefore the World Health Organisation and the AACE/ ACE have not recommended using HbA1c to detect IGR in initial position statements, as there is not enough evidence. Instead the AACE/ ACE have suggested an alternative strategy where people with an HbA1c between 5.5 to 6.4% should undergo further glucose testing to define their glucose status, similar to the ABCD (American Association of Clinical Endocrinologists / American College of Endocrinologists, 2010).

The second issue which adds more confusion is the terminology used to describe this ‘IGR’ group. Most people now acknowledge that using dichotomous terms such as prediabetes is misleading as it incorrectly suggests all people will eventually develop T2DM. Instead phrases which reflect a spectrum of risk are preferable. For example, ‘low risk for T2DM’ rather than ‘normal glucose tolerance’ is a better method to confer to patients that everybody is at some risk of future T2DM, even if a small one. Therefore a similar phrase for IGR group should be derived. The IEC and the UK NICE guidance have this termed this
‘high risk for T2DM’, whereas the ADA prefer regarding this as ‘a category of increased risk for T2DM’ (International Expert Committee, 2009; American Diabetes Association Position statement, 2010; NICE, 2012). Either is acceptable and conveys the correct message but global standardization may be required.

2.2.6 A new reporting unit for HbA1c

In the past the lack of standardisation of HbA1c assays has been a major reason why HbA1c has not been recommended as a diagnostic tool (WHO, 2006). However, the National Glycohemoglobin Standardisation Program (NGSP) established in 1996 has been successful in standardising HbA1c assays to produce results equivalent to those of the Diabetes Control and Complication Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) which established the relationships between HbA1c and outcome risks. The International Federation of Clinical Chemistry (IFCC) subsequently developed an accuracy-based definitive reference system for HbA1c that is now recognized as the legitimate anchor for worldwide standardisation. Although the two systems show excellent correlation, the actual number scales for reporting results are different. After much debate over whether to report future HbA1c results in the traditional DCCT/ UKPDS/ NGSP units familiar to clinicians or accuracy-based IFCC units, a worldwide consensus was reached by a number of major international organizations (ADA, EASD, IFCC, IDF Consensus Committee,
The statement recommended the reporting of HbA1c in the DCCT/UKPDS/NGSP units in % and the IFCC units in mmol/mol. Additionally, results could also be reported as average glucose as demonstrated by research exploring the relationship between HbA1c and average glucose and proving that this was feasible (Nathan, 2008).

Various countries are choosing individually how their results will be reported, with some countries choosing not to report the IFCC units (including the US). The US is currently the only country that has recommended the reporting of results as estimated average glucose (eAG) along with HbA1c units. In the UK, dual reporting of NGSP and IFCC units will occur for a certain number of years, after this time only mmol/mol will be used. Originally this switch over was planned for June 2011, however this was extended and no further date has been set to my knowledge. Table 2.1 has a summary of the key IFCC units and the equivalent HbA1c percentage values to remember in clinical practice. In this thesis, the traditional NGSP units are used.
Table 2.1 A guide to the key HbA1c values to remember in clinical practice

<table>
<thead>
<tr>
<th>NGSP/DCCT aligned HbA1c (%)</th>
<th>IFCC HbA1c (mmol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.0</td>
<td>42</td>
</tr>
<tr>
<td>6.5</td>
<td>48</td>
</tr>
<tr>
<td>7.0</td>
<td>53</td>
</tr>
<tr>
<td>7.5</td>
<td>58</td>
</tr>
<tr>
<td>8.0</td>
<td>64</td>
</tr>
<tr>
<td>9.0</td>
<td>75</td>
</tr>
</tbody>
</table>

Key: DCCT = Diabetes Control and Complications Trial, IFCC = International Federation of Clinical Chemistry, NGSP = National Glycohaemoglobin Standardisation Programme.

Converting HbA1c value from the NGSP percentage value to IFCC mmol/mol requires a linear transformation equation possible on computer programmes or a calculator. An easier method in clinical practice is to carry a conversion card with the values already present. For whole numbers only from HbA1c 4.0% to 13.0% a short cut can be employed with the ‘subtract 2, subtract 2 rule.’ When the NGSP value % (x) has 2 subtracted from it, this produces its gives the IFCC first digit in mmol/mol (y). Then if 2 is again subtracted from this value (y), it produces the next digit (z).

Example 1: ‘subtract 2, subtract 2 rule’

HbA1c 9% (x) converts to $9 - 2 = 7$ (y)

Then $7 - 2 = 5$ (z)
So NGSP HbA1c 9% is IFCC HbA1c 75 mmol/mol.

In comparison, the complete equation is:

\[
\text{IFCC HbA1c (mmol/mol)} = (\text{NGSP HbA1c\%} - 2.15) \times 10.929
\]

Example 2: NGSP HbA1c 9.0\% converts to: \((9.0\% - 2.15) \times 10.929\)

\[= \text{IFCC 74.86 mmol/mol}\]
2.2.7 Potential advantages of adding HbA1c to T2DM diagnostic criteria

HbA1c has some important logistical advantages over glucose testing. This includes performing the test in a non-fasting state. Hence screening is not limited to morning appointments and could occur at any time of the working day, irrespective of the patient’s time lapse since their last meal. The test can be performed instantly meaning there is less time required for the patient and health care professionals. Opportunistic screening can take place in people who present to their general practices for other reasons and in particular can target people who tend not to use these services and would decline specific screening appointments. Furthermore, as HbA1c reflects longer-term glycaemia, it is less affected by recent stress and can still give an accurate result. Therefore opportunistic screening can also take place when people present at acute hospital services even if they are physically or emotionally stressed (in the absence of conditions that may alter HbA1c such as acute or chronic haemorrhaging – see below), as part of their routine bloods.

Furthermore, in contrast to FPG, HbA1c incorporates contributions from both fasting and post-prandial glucose, suggesting a better representation of glucose intolerance as a whole (Monnier et al., 2003). In addition, HbA1c has a higher reproducibility rate than glucose testing and has less intra-individual variation on repeated tests (Ko et al., 1998; Rohlfing et al., 2002). FPG variance on a daily basis is reported to be 12 to 15%, creating some potential degree of uncertainty.
for any given value. In contrast, HbA1c variability is less than 2% (Petersen et al., 2005; Ollerton et al., 1999; Sacks et al., 2002). Perhaps one of the most significant advantages of using HbA1c over previous diagnostic tests is its relationship with hyperglycaemic complications, in particular macrovascular disease. Data from several longitudinal studies have shown HbA1c has a continuous relationship with cardiovascular disease and mortality, even below T2DM diagnostic thresholds (Khaw et al., 2002; Khaw et al., 2004; Myint et al., 2007; Haffner et al., 1990). The same relationship is generally reported to exist for 2 hour plasma glucose, but for FPG this generally is reported to exist above the T2DM cut-point of 7.0mmol/l (Qiao et al., 2002). As FPG appears to be used as the main screening tool in clinical practice, then a change to HbA1c may be seen as a better tool to predict macrovascular disease (Selvin et al., 2010).

2.2.8 Potential limitations of using HbA1c for diagnosis of T2DM

Use of HbA1c ≥ 6.5% (48mmol/mol) has some disadvantages compared to an OGTT or FPG. The two sets of tests appear to detect different populations as having T2DM (see section 2.3 onwards), with most studies reporting some discordance between tests, suggesting different people may detected as having T2DM depending on which test is employed (Davidson et al., 1999; Carson et al., 2010; Zhou et al., 2009; Van 't Riet et al., 2010; Mohan et al., 2010). This leads to both false positive and false negatives in diagnosis using HbA1c compared to
glucose testing, i.e. people who now have T2DM on HbA1c criteria but not on glucose criteria (‘additional people detected’ or ‘false positives’) and people who are no longer detected as having T2DM using HbA1c but would have T2DM if glucose testing was used (so called ‘false negatives’).

The other main limitation is if the HbA1c reading does not truly reflect glycaemic control over the previous 2 to 3 months. The presence of haemoglobin variants, including HbS, HbC and HbE traits as well as elevated HbF, can interfere with results from some assay methods. However, many newer methods are not affected by common haemoglobin variants. Furthermore, ion-exchange high performance liquid chromatographic methods can generally identify interferences on chromatograms and an incorrect result is therefore not reported. However, many of these newer methods utilize instrumentation that is not always available in remote or underserved areas of the world. The ADA has recommended use of HbA1c assays for diagnosis of diabetes in those with normal haemoglobin structure and red cell clearance (American Diabetes Association Position statement, 2010).

Falsely raised HbA1c levels are reported when there is increased circulating erythrocyte life span (i.e. decreased red cell clearance) or impaired reticulocyte production. Haemoglobin in these older erythrocytes has longer exposure time to ambient glycaemia, forming higher HbA1c levels. Well known causes include renal failure (uraemia), chronic excess alcohol intake and hypertriglyceridaemia (NGSP, 2013). Falsely decreased HbA1c levels are seen in reduced erythrocyte life span (i.e. increased haemoglobin turnover) or where reticulocytes are
produced and decrease the average erythrocyte age. These younger erythrocytes have less time exposure to ambient glycaemia. Well known causes include acute or chronic blood loss, sickle cell anaemia, thalassaemias, G6PDH deficiency, haemolytic/aplastic anaemia and splenectomy (NGSP, 2013). In cases of monitoring T2DM, where HbA1c is potentially inaccurate, a second line tool, fructosamine, can be used. However fructosamine has not been recommended for diagnosis by international organisations as it has not been measured in any major clinical trial.

Other factors affecting assay measurement such as vitamins C and E, and severe iron deficiency, have been reported to interfere with HbA1c assays (Saudek et al., 2006). Also, age and ethnic disparities in HbA1c values have been reported where most black and minority ethnic groups are shown to have higher HbA1c levels for equivalent glycaemic control (Pani et al., 2008; Herman et al., 2007). For example, Afro-Caribbeans may have up to 0.4 to 0.7% higher HbA1c values compared to white Europeans/ Caucasians for similar glycaemic control (Herman et al., 2007). This value is unknown in South Asians but is investigated in chapter 5. Such ethnic groups are therefore more likely to have an HbA1c ≥ 6.5%; this may suggest whether ethnic specific cut-points are required to diagnose diabetes using HbA1c. However such cut-points have not so far been recommended and are more likely to be related to the onset of microvascular complications.
2.2.9 How should additional people detected with T2DM through use of HbA1c be managed and approached?

Many studies report there will be additional people will be detected as having T2DM (those with HbA1c≥ 6.5%, but not classified with T2DM on glucose testing) (Davidson et al., 1999; Carson et al., 2010; Zhou et al., 2009; Van’t Riet et al., 2010; Mohan et al., 2010). Prospective studies which have clearly described the natural history of people with T2DM diagnosed from glucose testing in terms of progression and risk of complications. However, there is little known about the additional patients diagnosed by using HbA1c ≥ 6.5%. If this group are shown to have a similar natural course of complications, then all these additional patients would require the same structured education on lifestyle intervention and appropriate therapy. However, if these additional patients do not progress to developing complications at a similar rate, then this may indicate an inappropriate use of resources. For example, these people will require counselling / education sessions, annual retinal screening, feet examinations and annual reviews with doctors and nurses. Therefore an increase of numbers of people diagnosed with T2DM has huge implications for service provision by health care organisations. Secondly, interventions which are designed to decrease risk of hyperglycaemic complications used patients with diabetes detected through using FPG or OGTT; it is unclear that the same benefits will be derived if the interventions are used on additional people detected through use of HbA1c.
People with T2DM detected using glucose based diagnostic criteria have been well characterised regarding demographic factors (age, ethnicity and gender) metabolic characteristics (lipids and blood pressure) and phenotypic measures (body mass index, waist circumference and waist: hip ratio). However, this has not been evaluated for additional patients identified through use of HbA1c ≥ 6.5% (48mmol/mol) and this may influence their risk of progression to complications.

2.2.10 Potential economic impact of adding HbA1c to T2DM diagnostic criteria for health care organisations

Considering the logistical advantages of using HbA1c, there is some suggestion that it could become the preferred diagnostic tool for T2DM (Mitka, 2009). It is not known what economic impact this will have. Use of HbA1c is less time consuming, suggesting people from the working population may need to take less time off work. Secondary, an HbA1c test requires fewer resources (oral glucose load and staff time) compared to an OGTT. However, an accurate HbA1c assay is more expensive than glucose testing. For example, within the Leicester region the laboratory costs only for tests in 2010 were: FPG £0.47, OGTT £0.94 and HbA1c £2.66. Despite the exact costs potentially varying from one region to another, the general trend suggests HbA1c is more expensive for screening; however in the long term HbA1c may be more beneficial if more people are screened and detected earlier, allowing more time to implement
interventions which may prevent complications. This assumes that if HbA1c detects slightly different people as having T2DM they will truly benefit from the interventions, as much those people with T2DM detected through using an OGTT.

2.2.11 Future prospects regarding using HbA1c for diagnosis?

Using HbA1c for diagnosis could be simplified once point of care testing (POCT) HbA1c / near-testing machines become more readily available. These portable machines give instant readings using finger prick testing for whole capillary blood. POCT machines are currently used for monitoring levels of established diabetes and are not yet recommended for diagnosis of T2DM as many of them demonstrate suboptimal imprecision and do not appear on proficiency testing surveys. However if these issues are resolved, screening would become easier, as this could be performed in additional health care venues such as pharmacies, in remote areas and in underserved countries which may not have access to expensive laboratory assays.
2.3 The impact of detecting T2DM and IGR based on HbA1c in different populations

In section 2.3, I compare global studies regarding the impact on prevalence of (i) T2DM, (ii) IGR and (iii) incidence of T2DM from using HbA1c compared to glucose methods, as well as how accurate HbA1c is at detecting glucose defined T2DM and IGR.

2.3.1 How to interpret studies appropriately

Before analysing the results from studies, it is important to interpret the findings appropriately. Each study is unique, especially with regards to population demographics and methods employed. Therefore comparison of studies against each other is not necessarily straightforward. Furthermore, some studies report the primary results as the impact on prevalence of changing to HbA1c, while others choose to describe diagnostic indices such as sensitivity and specificity. Investigating the potential changes in prevalence of T2DM or IGR from using HbA1c compared to glucose testing is essentially comparing one diagnostic test to another and could be viewed as analysing using a ratio between the two tests. Therefore in order to explain differences in the magnitude of this ratio, as many different factors explaining the values must be accounted for. The points below may help to explain glucose or HbA1c test results
2.3.2 Factor regarding glucose studies

- Has the study used FPG or OGTT as the glucose diagnostic tool?

FPG is known to under-diagnose T2DM as people with post-prandial hyperglycaemia in the T2DM range will not be detected. This is more likely to be true of T2DM in its early disease stage. Therefore FPG has a reported sensitivity of only 40 to 60% for detecting T2DM (Perry et al., 2011; Tanaka et al., 1998; Barr et al., 2002). Thus studies using FPG as the diagnostic tool would likely have a reduced prevalence of T2DM compared to an OGTT. North American countries continue using this tool is in their region, as the ADA has previously recommended using FPG as the preferred glucose diagnostic tool over an OGTT (Expert Committee, 2003). Regarding the prevalence of IGR, if FPG was solely used then those people with isolated IGT would not be detected.

- Was the diagnosis of T2DM based on one or two glucose tests?

Some epidemiological studies base their T2DM prevalence results on one glucose test which is regarded as acceptable. However, due to the relatively high variability of glucose, those with a test result within the T2DM range require a repeat confirmatory glucose test for final diagnosis of T2DM (World Health Organisation, 2003). Thus, some people with an initial test result within the T2DM range may have a second repeat confirmatory test result in the non T2DM range (either IGR or normal glucose tolerance). Therefore the net effect of performing a repeat test is to reduce the prevalence of T2DM. Repeating the glucose test is
more common in screening studies conducted in clinical practice. Therefore this method point becomes important in context of this review. In contrast, HbA1c readings have far less intra-subject variability on repeating and therefore diagnoses are less likely to change (Rohlfing et al., 2002). Hence using one HbA1c test in an epidemiology study may be considered accepted, although repeating the test is preferred. It should also be noted that some studies adopt a policy of using HbA1c ≥ 7.0% as an endpoint of diagnosing T2DM (Pradhan et al., 2007).

- **Was the study based on routine clinical data or as part of a research study?**

Research studies are more likely to consist of robust methods including random sampling and address recruitment / uptake of testing issues from different age, gender and ethnic groups. Furthermore, research studies are more likely to involve correct participant preparation prior to testing and appropriate handling of glucose samples after venepuncture (World Health Organisation, 2003). The latter two points are important to producing an accurate glucose reading. However research studies may also exclude people who cannot provide appropriate consent, are house bound or suffer from terminal illness/ significant morbidity. In contrast to routine clinical data is more likely to have data on the whole population.

- **Was the diagnosis of T2DM assessed by ‘self-reported’ diagnosis?**
Some studies adopt a policy of assessing the prevalence of T2DM end-points with methods other than blood tests. The most common method is determining a diagnosis of T2DM thorough an interview. These ask participants if they have previously been (a) informed they have a diagnosis T2DM made by a doctor, (b) if they are taking oral hypoglycaemic agents (OHA)/ administering insulin or (c) inspecting the current prescription list. The first point (a) is generally accepted but simultaneously may not always produce accurate results. For instance, ‘has a doctor ever told you have T2DM?’ Answering this question requires some degree of understanding and recall. Statements such as ‘you may have T2DM and need a repeat test’ or ‘you have borderline T2DM (i.e. prediabetes) could be confused with the actual conception that a patient has T2DM. However, it is necessary for cohorts to use this method, especially if large scale screening cannot take place.

Self-report methods usually assess T2DM detected in routine clinical practice and rely directly on the degree of local testing from the general practitioner/family physician. This introduces variation of screening practices for detecting T2DM and variable patient uptake of screening programs. A potential example comes from the Women’s Health Study (WHS), where 20% of people with an HbA1c ≥ 7.0% without T2DM at baseline were subsequently still classified as not having T2DM (determined through self-report) after median follow up of 10.1 years (Pradhan et al., 2007). If formal glucose testing were performed at follow up instead of self-report, it is possible the fore-mentioned group of 20% may have been lower.
- **What are the mean age / age range of the cohort studied?**

T2DM prevalence based on either glucose testing or HbA1c is known to increase with age (Pani et al., 2008; Davidson et al., 2010). IGR would be therefore be expected to show the same trend. Thus a relatively older cohort may in theory have relatively higher rates of HbA1c ≥ 6.5% which is important to consider when comparing one study to another. Some studies have a specific age range as part of the inclusion criteria; therefore those within the age range 25 to 75 years may observe a different prevalence of T2DM and IGR prevalence than those investigating people aged 50 to 80 years old.

- **Did the study focus on a previously undiagnosed population?**

Some studies focus exclusively on undiagnosed populations, while others report results of previously known T2DM and undiagnosed T2DM together which will be higher than only undiagnosed populations. It is important to establish which method the study chose to sample. In the context of this review, it is more useful to consider previously undiagnosed populations.

### 2.3.3 Factors regarding HbA1c studies

- **Was HbA1c measured with a correctly aligned assay machine?**

This is important to ensure accurate HbA1c results are produced. Ion exchange high performance liquid chromatography assays are currently considered the
preferred assays for use. However, these instruments are expensive and may not be available in remote or underserved areas of the world. Point-of-care-testing devices (i.e. near patient testing) are currently not considered to be appropriately aligned with the required standards and therefore may not be precise as they are prone to demonstrating more variability. However, it should be noted that different assay machines in different regions will have some degree of variability, even if correctly aligned, which potentially introduces some degree of region variation depending on which assay method is used. Furthermore, studies with older data beyond the last two-three decades may not have correctly aligned assays as less of these were available, or measure levels of a previous less specific marker, HbA1, rather than HbA1c.

In Japan, HbA1c is generally standardized to the Japanese Diabetes Society (JDS) Committee for the Standardisation of Glycohaemoglobin (Tominaga et al., 2005). An accepted simple and approximate conversion to National Glycohaemoglobin Standardisation Program (NGSP) consists of: JDS + 0.3% (Tominaga et al., 2005). In this thesis, values are reported in accordance with NGSP.

- **What was the ethnic prevalence of the cohort?**

It is reported that non White Europeans have independently higher HbA1c values for equivalent levels of glycaemic control (Herman et al., 2007). Most ethnic groups (e.g. Afro-Caribbean, Asian/ south Asian and Hispanic people) have higher rates of glucose-defined T2DM compared to White European populations,
but one could estimate that using HbA1c for diagnosis may further increase this gap. Furthermore, the effect of migration has now produced many multi-ethnic populations throughout Europe and North America; therefore it is important to consider what proportion of the population is of ethnic minority origin in such studies.

Additionally ethnic prevalence is also relevant in the setting of thalassaemias, haemoglobin (Hb) variants and other genetic haemoglobinopathy disorders. Some haemoglobinopathies may not reflect actual glycaemic control. Thalassaemias and haemoglobin variants in particular are more common in certain ethnic groups. For example, Hb S and C traits are common in Afro-Caribbeans; Hb S in Hispanic, Mediterranean and Middle Eastern populations; Hb D in Indians and Hb E in South East Asians and Indians (National Information Clearinghouse, 2013). Previously, HbA1c assays were not able to adjust for all types of Hb variants; therefore specific assays were theoretically required in different areas where alternate Hb variants existed. Now there are only a few assay methods where there is still interference from Hb S and C traits.

- What is the prevalence of other medical conditions which affect HbA1c values?

Some medical conditions, such as iron deficiency can inappropriately increase HbA1c levels (Kim et al., 2010). Therefore a study with a higher proportion of pre-menopausal females could contain a higher proportion of people with relatively higher HbA1c values due to this effect.
In summary, comparing prevalence studies is not straightforward and requires consideration of the methods employed and population demographics. It is also worth noting whether the study is population-based or high risk as this will influence the prevalence of T2DM.

2.4.1 Studies comparing use of HbA1c and glucose testing for diagnosis of T2DM

For many years, researchers have been investigating the topic of comparing prevalence of T2DM according to glucose testing and corresponding HbA1c values. However since 2008 many studies in this area have been published. An augmented Medline search reviewed 18 studies from 1966 to 1994 on this specific topic (Peters et al., 1996). However many of these studies did not investigate using HbA1c specifically at ≥ 6.5%. This next section of this chapter focuses on the most recent studies reported from different countries from 2007 to 2010, which had a primary aim of addressing the specific questions:

(1) Will use of HbA1c ≥ 6.5% detect the same people as glucose-defined T2DM from use of (a) OGTT or (b) FPG? i.e. how accurate is HbA1c at detecting glucose-defined T2DM on OGTT or FPG?
(2) If different people are detected from use of HbA1c, will they be at the same risk of complications as those detected from glucose criteria?
(3) Will use of HbA1c ≥ 6.5% detect more or less people as having T2DM compared to glucose testing?
(4) Is HbA1c ≥ 6.5% the optimal cut-point to detect undiagnosed T2DM?

As I wanted to answer various questions rather than one single question, this was not performed as formal meta-analyses or systematic reviews. Articles were searched on Medline, Google and Google Scholar. Furthermore, Evidence Services Health Information Resources (via NHS Healthcare Databases) was used to set up searches for new articles from 25/04/2009 onwards on Medline and Embase; the results of new articles were sent to me via weekly email alerts. This search terms were designed to identify titles of articles that contained the following terms:

HbA1c; glycated AND haemoglobin; glycated AND haemoglobin; hemoglobin AND A1c; haemoglobin AND A1c; diabetes; type AND 2 AND diabetes; NIDDM; T2DM; DM; diagnosis; classification; prediabetes, pre-diabetes; impaired AND glucose AND regulation; high AND risk; increased AND risk; non-diabetic AND hyperglycaemia; intermediate AND hyperglycaemia; progression; prediction; long AND term AND follow AND up; future AND risk; subsequent AND risk; incident; developing; development; long AND term AND follow AND up; prognosis; correlation and various combinations of these terms using ‘OR’.

The full search term strategy is listed in Appendix 3.
Some of these terms are for the searches for section 2.5 and 2.6, as the same processes were used to search for papers in these areas. To answer the questions above, 20 studies were reviewed from 2007 to 2010 (listed in Table 2.2). From these studies, six were part of a multi-centre study which was published in a single report, but analysed separately here (Christensen et al., 2010). The studies reviewed in this chapter span from five continents, although showed a strong bias for US and Europe. In addition, there were also two studies from south Asia; however there is very little data on migrant south Asians (this is investigated in chapter 3). Three studies were from the Far-East, one from Africa and Oceania respectively. Two studies focused exclusively on an elderly cohort, while a study of an African population had a mean age of only 37.6 years, suggesting a younger population. Four studies sampled an age range starting from 20 years, while at least three studies focused on middle aged people (40 to either 65 or 75 years). The total number of people within these 20 studies included over 76,000 people. Below, I discuss the results.

2.4.2 Impact of using HbA1c ≥ 6.5% to detect T2DM

Using HbA1c ≥ 6.5% to diagnose T2DM generally favoured a trend of decreasing the prevalence of undiagnosed T2DM compared to glucose defined T2DM (with either use of FPG or OGTT). This trend of HbA1c lowering the prevalence of T2DM was exemplified in ten of the studies reviewed in Table 2.2, with an
absolute reduction of prevalence ranging from 1.3 to 3.5% (Christensen et al., 2010; Mohan et al., 2010; Cowie et al., 2010; Carson et al., 2010; Van 't Riet et al., 2010; Zhou et al., 2010; Davidson et al., 2010; Olson et al., 2010; Araneta et al., 2010; Boronat et al., 2010). This excludes the recent report from the Insulin Resistance Atherosclerosis Study (IRAS) (Lorenzo et al., 2010), which oversampled glucose intolerant categories and therefore cannot be considered population based. Other studies such as NHANES over sampled Afro-Caribbeans and Hispanics specifically to match the distribution of the US population, therefore these studies were considered population based. Only two studies compared the impact in men and women separately (Zhou et al., 2010; Lipska et al., 2010). A Chinese study report no gender differences (Zhou et al., 2010), however US elderly cohort found a higher proportion of women were detected with HbA1c criteria than fasting plasma glucose (Lipska et al., 2010). When FPG was used as the diagnostic tool, in contrast to the OGTT, the absolute differences in T2DM prevalence compared to HbA1c ≥ 6.5% were less pronounced (Lorenzo et al., 2010; Cowie et al., 2010). FPG is known to under diagnose T2DM in comparison to OGTT (Perry et al., 2001; Tanaka et al., 1998; Barr et al., 2002). For example, a sub-sample from NHANES (2003 to 2006) found HbA1c ≥ 6.5% detected 1.6% of this population, FPG 2.5% and OGTT detected 5.1% (Cowie et al., 2010).

Overall, only four out of 22 studies observed an increase in prevalence of T2DM using HbA1c ≥ 6.5% compared to glucose testing (Christensen et al., 2010;
Mohan et al., 2010; Jorgensen et al., 2011; Lipska et al., 2010). Two of these studies used an OGTT to define glucose-based T2DM.
Table 2.2 Summary of recent studies comparing glucose testing and HbA1c for diagnosis of undiagnosed T2DM

<table>
<thead>
<tr>
<th>Study or Country, First Author, Year of publication</th>
<th>Nature of cohort</th>
<th>N</th>
<th>Age range/ Mean age (years)</th>
<th>Mean A1c (%)</th>
<th>DM prevalence (% tool)</th>
<th>A1c ≥ 6.5% Sensitivity/ for DM with A1c ≥ 6.5%</th>
<th>Specificity For DM With A1c ≥ 6.5%</th>
<th>Kappa Agreement Measure</th>
<th>Optimal A1c cut-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHANES 1999-2006, Carson, 2010</td>
<td>Population ‡</td>
<td>6890</td>
<td>≥ 20</td>
<td>-</td>
<td>3.6% FPG</td>
<td>2.3</td>
<td>-</td>
<td>-</td>
<td>0.600</td>
</tr>
<tr>
<td>NHANES 1999-2004, Buell, 2007</td>
<td>Population ‡</td>
<td>4935</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>45% *</td>
<td>98 *</td>
<td>-</td>
</tr>
<tr>
<td>NHANES 2003-2006 Cowie, 2010 #</td>
<td>Population ‡</td>
<td>1502</td>
<td>≥ 20</td>
<td>5.4</td>
<td>5.1 OGTT</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>NHANES 1988-1994, Davidson, 2010</td>
<td>Population ‡</td>
<td>2712</td>
<td>40-74</td>
<td>5.17</td>
<td>8% OGTT</td>
<td>5.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Rancho Bernardo, Kramer, 2010</td>
<td>Older cohort</td>
<td>2107</td>
<td>69.4</td>
<td></td>
<td>-</td>
<td>44%</td>
<td>79</td>
<td>0.119</td>
<td>6.15%</td>
</tr>
<tr>
<td>Health, Ageing study, Lipska, 2010</td>
<td>Older cohort</td>
<td>1865</td>
<td>70-79</td>
<td>5.4</td>
<td>2.7 FPG</td>
<td>3.1</td>
<td>56.9%</td>
<td>98.4%</td>
<td>-</td>
</tr>
<tr>
<td>IRAS, Lorenzo, 2010</td>
<td>Oversampled ‡</td>
<td>855</td>
<td></td>
<td></td>
<td>15.4 OGTT</td>
<td>5.2</td>
<td>30.3 OGTT</td>
<td>55.7 FPG</td>
<td>99.4</td>
</tr>
<tr>
<td>Whitehall, Christensen, 2010 †</td>
<td>Population ‡</td>
<td>4563</td>
<td>60.5</td>
<td>5.2</td>
<td>3.7 OGTT</td>
<td>1.0</td>
<td>25.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HOORN, Van ’t Riet, 2010</td>
<td>Population ‡</td>
<td>2753</td>
<td>40-65</td>
<td>5.5</td>
<td>4.0 OGTT</td>
<td>1.0</td>
<td>24</td>
<td>99</td>
<td>-</td>
</tr>
<tr>
<td>Telde, Boronat, 2010</td>
<td>Population ‡</td>
<td>964</td>
<td>&gt; 30</td>
<td></td>
<td>6.4 OGTT</td>
<td>2.9</td>
<td>38.7</td>
<td>99.6</td>
<td>-</td>
</tr>
<tr>
<td>Inter99, Christensen, 2010; Jorgensen, 2010</td>
<td>Population ‡</td>
<td>5932</td>
<td>46.2</td>
<td>5.8</td>
<td>4.2 OGTT</td>
<td>6.7</td>
<td>42.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Greenland, Christensen, 2010; Jorgensen, 2010</td>
<td>Population ‡</td>
<td>2321</td>
<td>44.1</td>
<td>5.7</td>
<td>7.0 OGTT</td>
<td>3.9</td>
<td>29.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ausdiab, Christensen, 2010 †</td>
<td>Population ‡</td>
<td>7800</td>
<td>50.9</td>
<td>5.1</td>
<td>4.0 OGTT</td>
<td>0.7</td>
<td>17.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hawaii -Native/ Filipino/ Japanese, Araneta et al., 2010</td>
<td>Population ‡</td>
<td>933</td>
<td>54.2</td>
<td></td>
<td>15.5 OGTT</td>
<td>6.5% FPG</td>
<td>8.9</td>
<td>40.0 OGTT</td>
<td>68.9 FPG</td>
</tr>
<tr>
<td>CURES, India (Christensen, 2010; Mohan, 2009) †</td>
<td>Population ‡</td>
<td>2188</td>
<td>≥ 20</td>
<td>5.9</td>
<td>10.2 OGTT</td>
<td>12.9</td>
<td>78.2%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chandigarh, Kumar, 2010</td>
<td>Population ‡</td>
<td>1972</td>
<td>≥ 20</td>
<td>5.6</td>
<td>-</td>
<td>65%</td>
<td>88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>China, Zhou, 2010</td>
<td>Population ‡</td>
<td>2332</td>
<td>34-75</td>
<td></td>
<td>13.4 m OGTT 10.8 f</td>
<td>11.8 m 10.2 f</td>
<td>28 m</td>
<td>21.9 f</td>
<td>-</td>
</tr>
<tr>
<td>China, Bao, 2010</td>
<td>Population ‡</td>
<td>4886</td>
<td>≥ 20</td>
<td>5.6 a</td>
<td>6.2% OGTT</td>
<td>50.5</td>
<td>98.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fungara, Nakagami, 2007 ¥</td>
<td>Population ‡</td>
<td>1799</td>
<td>35-89</td>
<td></td>
<td>-</td>
<td>-</td>
<td>16-20% *</td>
<td>99 *</td>
<td>-</td>
</tr>
<tr>
<td>Kenya, Christensen, 2010 †</td>
<td>Population ‡</td>
<td>296</td>
<td>37.6</td>
<td>5.0</td>
<td>7.0 OGTT</td>
<td>3.9</td>
<td>29.6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The IRAS study over sampled certain numbers of people in different ethnic, age, sex and glucose tolerance groups (therefore likely to be high risk). # Sub-sample only: note 7.8% in this population had previous self report T2DM as well. Optimal cut-points are derived from ROC curve analysis. Key: a= median, A1c = HbA1c, DM = T2DM Mellitus, m = male, f = female, † part of a multi-centre report, * estimated from graphical data provided in report, ‡ confirmed as multi-ethnic population, ¥ Japanese HbA1c values reported in equivalent of NGSP, not JDS.
2.4.3 Performance of HbA1c in black and ethnic minority groups

Studies were generally lacking on Afro-Caribbeans and Hispanic populations, however some US based studies published data for these groups. In the NHANES (1999-2004) the optimal HbA1c cut-point for detecting T2DM was ≥ 5.8% and produced a better sensitivity in African Americans and Hispanic people compared to Non-Hispanic Whites; (sensitivity and specificity: 93% and 86%; 95% and 91%; 84% and 93% respectively) (Buell et al., 2007). Furthermore, the NHANES III 1988 to 1994 extrapolated their results to the US population and predicted the percentage of people aged 40 to 74 years with HbA1c 6.5 to 6.9% was 0.98%, 2.69% and 3.9% in Non-Hispanic whites, Mexican Americans and Non-Hispanic Blacks respectively (Davidson et al., 2010). The same study showed HbA1c levels increase with age and are higher for African-Americans and Hispanic people independent of glycaemia (Davidson et al., 2010). An elderly US based cohort study using HbA1c criteria detected more African-Americans than using glucose based testing (Lipska et al., 2010).

Regarding south Asians, the Chennai Urban Rural Epidemiology Study (CURES) study (n = 2188) showed an increase in diabetes prevalence using HbA1c criteria (Mohan et al., 2010). Interestingly, another UK based study, Whitehall II, separately analysed ethnic minority groups after the main analysis and reported that a small number of south Asians (n=204) had a decrease in T2DM prevalence using HbA1c ≥ 6.5% (Christensen et al., 2010).
The sensitivities of HbA1c ≥ 6.5% to detect glucose-defined T2DM (using an OGTT) in south Asians were reasonably high at 78.2% and 65.0% in the CURES and Chandigarh respectively (Mohan et al., 2010; Kumar et al., 2010). In contrast, Chinese and Japanese studies found sensitivities of HbA1c detecting T2DM on OGTT were both less than 30% (Zhou et al., 2010; Nakagami et al., 2007). Hawaiian Japanese, Filipino and Native Hawaiians found a lower diagnosis of T2DM using HbA1c criteria compared to OGTT diagnosis (Araneta et al., 2010).

Inuit populations were described in two studies (Jorgensen et al., 2011; Christensen et al., 2010). Use of HbA1c ≥ 6.5% detected T2DM in 31.7% in Greenland and 21.3% in Inuit migrants, the highest prevalence of HbA1c T2DM in any population in this review, compared to 11.2% and 9.8% respectively with T2DM using an OGTT (Jorgensen et al., 2011). The same study found the Inuit population had higher HbA1c values than a general Danish population at any given FPG and 2 hour plasma glucose for normal glucose tolerance and impaired glucose regulation.

### 2.4.4 Sensitivity and specificity of HbA1c≥ 6.5% to detect glucose defined T2DM

Overall, the sensitivity of HbA1c ≥ 6.5% to detect T2DM from glucose testing was variable from 17.0 to 78.2%; only five studies produced sensitivity greater than
50% (Kumar et al., 2010; Mohan et al., 2010; Christensen et al., 2010; Bao et al., 2010; Lipska et al., 2010). In contrast, HbA1c ≥ 6.5% produced a high specificity, with seven out of eight studies reporting values > 98.0% (Van ’t Riet et al., 2010; Lorenzo et al., 2010; Buell et al., 2007; Nakagami et al., 2007; Bao et al., 2010; Boronat et al., 2010; Lipska et al., 2010).

2.4.5 Optimal HbA1c cut-points derived from Receiver Operating Characteristics (ROC) curve analysis to detect glucose defined T2DM

A ROC curve to be used to determine how accurate a diagnostic test is at detecting people with the disease of interest (e.g. HbA1c at detecting T2DM). The ROC curve value will account for diagnostic inaccuracies such as false negatives and false positives). The second use of ROC curve analysis is to determine an optimal cut-point of that particular test for the disease, by balancing the highest sensitivity and specificity. i.e. a trade-off between the two diagnostic indices. Overall, these were found to be lower than 6.5% and ranged from 5.6 to 6.3% (Kramer et al., 2010; Mohan et al., 2010; Kumar et al., 2010; Bao et al., 2010, Van ’t Riet et al., 2010; Zhou et al., 2010; Buell et al., 2007; Nakagami et al., 2007; Araneta et al., 2010). Furthermore, five of eight studies reviewed had an optimal HbA1c cut-point of < 5.9% (Van ’t Riet et al., 2010; Zhou et al., 2010; Buell et al., 2007; Nakagami et al., 2007; Araneta et al., 2010).
The sensitivity produced from using optimal HbA1c cut-points varied; the CURES study found their optimal cut-point of HbA1c ≥ 6.1% produced sensitivity and specificity of 88.0% and 87.9% (Mohan et al., 2010). In contrast, Rancho Bernado found their optimal HbA1c cut-point of HbA1c ≥ 6.15% produced both sensitivity and specificity below 65% (Kramer et al., 2010). The optimal cut-points also varied when FPG and 2-hour plasma glucose were considered separately, as demonstrated in the CURES study, producing an HbA1c ≥ 6.4% and 6.1% respectively (Mohan et al., 2009). A multi-ethnic Hawaiian population found an optimal ROC cut-point of HbA1c ≥ 5.8% produced a sensitivity and specificity of 75.9% and 80.0% respectively (Araneta et al., 2010). An elderly US based study found an optimal ROC of HbA1c 6.0% produced sensitivity and specificity of 84.3% and 91.7% (Lipska et al., 2010).

2.4.6 Area under the curve performance for HbA1c and fasting glucose to detect T2DM

Some studies compared the relative ability of combined HbA1c and FPG for detecting undiagnosed T2DM using area under the ROC curve analysis. A value less an 0.7 suggests an inadequate diagnostic performance, 0.7 to 0.8 equates to moderate performance and greater than 0.8 suggests a good test performance. With this is mind, HbA1c generally had a good test performance. The HOORN study reported HbA1c had a lower AUROC than FPG, 0.90 vs. 0.94
(Van 't Riet et al., 2010). Similarly, one Chinese study compared HbA1c to fasting capillary glucose (FCG); AUC was significantly lower in HbA1c than FCG in both men and women (Zhou et al., 2010). This was the only study able to compare FCG and HbA1c. In contrast, a Japanese study found the AUC for undiagnosed T2DM was similar between HbA1c and FPG, 0.856 and 0.902 respectively (Nakagami et al., 2007). A multi-ethnic Hawaiian population found HbA1c found an AUC of 0.68 (Araneta et al., 2010); whilst an elderly US cohort had an AUC of 0.93 (Lipska et al., 2010).

2.4.7 Discordance of diagnostic tests using Kappa agreement measurements

Using HbA1c seems to consistently detect a different population from use of FPG or OGTT, with variable degrees of overlap in people detected by using either test. The kappa agreement measure was less than 0.5 in 3 out 4 studies (Kramer et al., 2010; Zhou et al., 2010), with the remaining study reporting a kappa of 0.6 (Cowie et al., 2010).

2.4.8 Prevalence of people with T2DM on glucose testing but HbA1c <6.5% (‘false negative diagnoses’)
Regarding the percentage of people with false negative diagnosis of T2DM from use of HbA1c, the NHANES III 2005-6/ SIGT study reported 70% (Olson et al., 2010), the Inter-99 found 58% for the same measure. When FPG was used as the diagnostic test in the NHANES 1999-2006, 46.7% of people with T2DM had HbA1c <6.5% (Carson et al., 2010). The HOORN study reported 44% and 22% of people with newly diagnosed OGTT based T2DM had HbA1c <6.0% and <5.7% respectively (Van ’t Riet et al., 2010); while the Rancho Bernado study found one-third of people with T2DM on OGTT had HbA1c<6.0% (Kramer et al., 2010). In contrast, the CURES reported only 7.6% of people with T2DM had HbA1c< 6.0% (Mohan et al., 2010).

2.4.9 Is there a change in phenotype and CVD risk of people classified as having T2DM using HbA1c≥ 6.5% but not glucose testing (‘false positive diagnoses’)?

The Inter-99 study found no significant changes in phenotype and median 10-year ischaemic heart disease risk between people with T2DM from use of an OGTT but HbA1c <6.5% compared to those with HbA1c ≥ 6.5% but a non-diabetic OGTT result (Borg et al., 2010). However the Inter-99 found people with T2DM on OGTT but HbA1c<6.5% had significantly higher levels of hypertension and raised triglycerides than people with HbA1c ≥ 6.5% and non-diabetic OGTTs. The same study demonstrated trends of people with T2DM from both HbA1c and
OGTT criteria having the worst cardiovascular phenotype; this is perhaps the most crucial point as those who are most likely to progress on to complications will be detected using either tool. Furthermore a study of Inuit people in Greenland and Denmark found a similar trend (Jorgensen et al., 2010). There is very little data on what will happen in the UK and this is explored in Chapter 3. The NHANES study 1999-2006 and a Chinese study also measured many CVD risk factors and found no significant differences between additional people detected and those no longer classified as having T2DM using HbA1c (Carson et al., 2010; Zhou et al., 2010). However the latter study may not have had sufficient numbers in the respective sub-groups to detect any significant differences. The NHANES 1999-2006 reported additional people detected from use of HbA1c consisted of more Afro-Caribbeans, who are generally reported to have higher rates of CVD (Carson et al., 2010). The NHANES III + 2005-6, SIGT found a similar result where additional people consisted of more Afro-Caribbeans, while false negative diagnosis consisted of more Non-Hispanic Whites (Olson et al., 2010). A small Spanish study reported that people with HbA1c ≥ 6.5% had a significant less favourable cardiovascular risk profile than individuals with T2DM detected on OGTT – this appears to be the only study which reported this trend from the studies reviewed (Boronat et al., 2010).

2.4.10 Long term prediction of macrovascular events using HbA1c
There is conflicting information on whether FPG, 2-hour plasma glucose or HbA1c is the best predictor of macrovascular complications in previously undiagnosed populations. The answer to this may determine which tool should be used primarily for diagnosis of T2DM. The DECODE study has reported 2-hour plasma glucose is more predictive for CVD than FPG and HbA1c (DECODE study group, 1999), reflecting the continuous relationship between post-prandial hyperglycaemia and cardiovascular disease (CVD).

The Atherosclerosis Risk in Communities (ARIC, n=11,092, follow up 14 years) found baseline HbA1c in people without T2DM was concluded to have good prognostic value for future cardiovascular disease; however FPG was a poor predictor in relative comparison (Selvin et al., 2010). A second ARIC study also found elevated HbA1c ≥ 6.0% was associated with incident heart failure, with multi adjusted hazard ratio HbA1c 6.0-6.4%: 1.41 (95% confidence intervals: 1.10 to 1.80); however there was no association for FPG (Matsushita et al., 2010). In contrast, the Women’s Health Study (WHS, n=26,563, follow up 10.1 years) reported HbA1c was not associated with prognostic value for CVD (Pradhan et al., 2007). A Finnish study (n=593, follow up 9.7 years) found HbA1c predicted CVD only above the cut-point ≥ 6.5% and in women only, whereas 2 hour plasma glucose predicted CVD in the IGT and diabetic range in women only. In contrast FPG did not predict CVD in either men or women (Cederberg et al., 2010). The HOORN and AusDiab study reported 2-hour plasma glucose had a stronger association with CVD or CV mortality than HbA1c (de Vegt et al., 1999; Barr et al., 2009); a third study agreed with this for males only (Qiao et al., 2004). In
contrast, the US Rancho Bernado study reported the HbA1c had better predictive value for CVD in women only (Park et al., 1996). The cross sectional Inter-99 study found HbA1c was a better predictor of 10-year ischaemic heart disease risk ≥ 30% and 40% than either FPG or 2-hour plasma glucose (Borg et al., 2010). A recent systematic review analysed 29 studies and found HbA1c had a somewhat stronger association with coronary heart disease (CHD) than FPG or two hour plasma glucose (Sarwar et al., 2010). The adjusted relative risks were 1.06 (95% confidence intervals 1.00 to 1.12) for every 1mmol/l increase in FPG; 1.05 (1.03 to 1.07) for every 1mmol/l increase in 2-hour PG and 1.20 (1.10 to 1.31) for every 1% increase in HbA1c. This suggested that every 1% increase in a baseline HbA1c value was associated with 20% higher coronary risk, but only 6% and 5% for FPG and 2-hour plasma glucose respectively.

2.4.11 Discussion

The general trend on the prevalence of T2DM suggests using HbA1c ≥ 6.5% will detect less people with T2DM than glucose testing in most, but not all studies – the mean cohort HbA1c plays a key role of determining what proportion of the populations is placed above and below the 6.5% diagnostic threshold. Despite the finding that HbA1c ≥ 6.5% will detect less people with T2DM, it should be noted that as HbA1c can be performed in the non-fasting state in routine appointments, this may increase screening rates and could overall detect more people with T2DM initially. This is especially true when HbA1c is initially
used in clinical practice as there will be a proportion of people who will now be classified as having T2DM with an HbA1c ≥ 6.5% but without T2DM on an OGTT. Furthermore, most studies either reported a single glucose/ HbA1c test, providing a so called epidemiological diagnosis of T2DM, or did not discuss this issue at all (therefore presumed to be from one test). A single glucose test, without a repeat confirmatory test, is likely to detect more people with T2DM than if two tests were used (Ollerton et al., 1999; Sacks et al., 2002).

In addition, the mean cohort HbA1c is also influential in determining the prevalence of T2DM using HbA1c ≥ 6.5%. For example relatively higher mean cohort HbA1c values (e.g > 5.7%) will automatically project a greater proportion of individuals above the 6.5% cut-point than those with a lower mean cohort HbA1c (i.e. <5.3 to 5.4%). This because more of the population is effectively shifted above the HbA1c 6.5% cut-point with a higher mean cohort HbA1c value and less shifted with a lower mean cohort HbA1c value. Therefore the mean cohort HbA1c may also determine whether use of HbA1c to detect T2DM increase prevalence compared to glucose testing (i.e. higher mean cohort HbA1c suggests HbA1c will increase T2DM prevalence). This proposed theory was correct in seven of the eight populations (Christensen et al., 2010; Mohan et al., 2009; Cowie et al., 2010). The only exception was a cohort from Greenland which had high mean HbA1c of 5.7% but still observed a decrease in prevalence using HbA1c compared to glucose testing (Christensen et al., 2010). This Greenland population had a relatively high prevalence of undiagnosed T2DM
(7.0%) using glucose testing, especially given the mean age of 44.1 years. However the population focused on exclusively Inuit people who are considered high risk of T2DM. Therefore, it appears the prevalence of undiagnosed T2DM was so high that it masked over the effects of having a high mean cohort HbA1c.

The specificity of using HbA1c ≥ 6.5% was relatively high, with a lower and more variable sensitivity. The optimal HbA1c cut-point to detect glucose-defined T2DM was lower than 6.5%. This generally agrees with a systematic review finding the most commonly reported HbA1c cut-point was 6.1% (Bennett et al., 2007), although some studies were common to the systematic review and the present study. It should be noted that the differences between HbA1c optimal cut-points may be due to different HbA1c assay methods, not necessarily population differences.

This review also found HbA1c ≥ 6.5% had higher prevalence of T2DM in non–white Europeans (Carson et al., 2010; Olson et al., 2010). This could show the effect of non-white Europeans having higher HbA1c values independent of glycaemic control, which favours / shifts a greater proportion of people above the HbA1c ≥ 6.5% threshold. However this assumption is likely to be influenced by other factors as well, including the assay methods used.

The issue of false positives (additional people detected using HbA1c) and false negatives (people no longer classified as having T2DM using HbA1c) is a
recurrent theme reported in these studies. Within each glucose intolerance classification (i.e. normal glucose tolerance, IGR or T2DM), the HbA1c levels can generally vary from <5.7% to greater than 6.5%, i.e. there is a spectrum of HbA1c values for the various categories.

A potential concern for additional people detected is that within some countries (e.g. US) health insurance may become more expensive, however it may be an overall advantage overall in terms of future management aimed at reducing complications of T2DM. There is also potential concern for people no longer detected as having T2DM using HbA1c, as these people may progress onto developing complications without the opportunity for intervention, especially if classified into low risk groups using HbA1c (e.g. HbA1c <5.7%). However, it should be noted that people without T2DM detected from use of HbA1c initially can still be re-screened in the future at specific intervals, especially if within the high risk range (e.g. HbA1c 5.7 or 6.0 to 6.4%) or if other risk factors are present, such as obesity. This suggests they could still be identified in the future years. This would decrease the chances of developing of complications without any intervention. Secondly, these people can still have interventions initiated to decrease CVD risk if other risk factors are present (e.g. hypertension, hypercholesterolaemia requiring lifestyle and medicinal therapies).

Regarding discordance between diagnostic tests, the reviewed studies suggested Kappa values lower <0.5 were common suggesting weak agreement between HbA1c and glucose testing for T2DM. However, there are different ways of calculating a Kappa measure and not all studies referenced their chosen
method. A final consideration regarding this discordance is that some underserved countries and remote areas may not have access to high quality HbA1c testing, and therefore will continue to use traditional glucose testing. This risks creating a global ‘two-tier’ system where similar people are identified as having T2DM in one region but not the next and also may cause different glycaemic profiles to be interpreted as having ‘T2DM’ in different regions.
2.5.1 Studies comparing use of HbA1c and glucose testing for diagnosis of prevalent impaired glucose regulation (IGR)

It is important to understand the consequences of use of HbA1c for identifying IGR. The main questions to be addressed in the next section of this chapter are:

- What is the impact of using either ADA or IEC recommended HbA1c cut-points on prevalence of IGR?
- What is the optimal cut-point for detecting prevalent IGR?
- How accurate is HbA1c at detecting IGR, or IGT and IFG separately?
- Is a strategy of using a combination HbA1c and FPG together useful at detecting IGR?

The search strategy has been described in section 2.4.1. Below, I discuss the results.

2.5.2 Impact on prevalence of IGR

Table 2.3 has a summary of the studies investigated. The NHANES 2003-2006 sub-samples reported using the recommended IEC criteria of HbA1c 6.0-6.4% decreased the prevalence of IGR to one-tenth of those diagnosed using an OGTT which may be because of a low mean HbA1c of 5.4% (Cowie et al., 2010; personal communication C.C.Cowie). The NHANES III + 2005-6/ SIGT study found 36% had IGR using an OGTT, while 6.2% and 19.5% had HbA1c values between 6.0 to 6.4% and 5.7 to 6.4% respectively (Olson et al., 2010). A Finnish
study reported HbA1c of 5.7 to 6.4% detected 32.8% of their cohort compared to 51.6% with IGR using an OGTT (Cederberg et al., 2010). The IRAS study over selected people with glucose intolerance and defined IGR as having IGT, IFG or HbA1c 5.7 to 6.4%; these three categories detected 69.1%, 59.2% and 23.6% people respectively (Lorenzo et al., 2010). Furthermore, using insulin sensitivity index and first phase insulin secretion, HbA1c was shown to correlate less precisely with insulin resistance and secretion than 2 hour plasma glucose and FPG respectively (Lorenzo et al., 2010).

2.5.3 Optimal cut-point for IGR

This was found to vary between studies. Regarding population based studies, the ADA stated ROC curve analysis found HbA1c ≥ 5.7% was the optimal cut-point for in US individuals with IFG (American Diabetes Association Position statement, 2010). This agreed with a Chinese population based study reporting the same value (Zhou et al., 2009). However a separate Chinese based population reported the optimal cut-point as HbA1c ≥ 5.9%. Within south Asians, the optimal cut-points for IGR were reported as HbA1c≥ 5.6% from the CURES study (Mohan et al., 2009). However less is known about the same optimal cut-point in migrant South Asians but is investigated in chapter 3. The NHANES III + 2005-6/ SIGT report combined three studies and reported this came to between 5.4 to 5.6% (Olson et al., 2010).
2.5.4 Combined use of HbA1c and fasting plasma glucose for detecting IGR

Regarding high risk populations, a Chinese cohort found the optimal cut-point for IGT was HbA1c ≥ 5.6% giving sensitivity and specificity of 66.2% and 51.0% respectively; these values increased to 87.9% and 33.4% with combined use of HbA1c ≥ 5.6% or FPG ≥ 5.6mmol/l (Hu et al., 2010). The AusDiab study investigated a sub-sample of people with at least one risk factor for T2DM (Colagiuri et al., 2010). Using HbA1c ≥ 5.3% (an optimal ROC cut-point for T2DM and IGR together) produced a low sensitivity but good specificity of 42.0% and 88.2% respectively; these values increased to 60.3% and 80.8% with combined use of HbA1c ≥ 5.3% or FPG ≥ 5.5mmol/l. The last two studies show combined use of HbA1c and FPG increases sensitivity for detecting IGR, albeit in a trade-off for decreasing specificity. An Italian study of 1215 people found HbA1c ≥ 5.3% combined with FPG > 6.1mmol/l had sensitivity of 59% and 54.8% in men and women respectively and specificity of 19.3% and 9.3% in women respectively (Mannucci et al., 2003).
### Table 2.3 Summary of recent studies comparing HbA1c accuracy for detecting impaired glucose regulation or impaired glucose tolerance

<table>
<thead>
<tr>
<th>Study, first author, year of publication</th>
<th>Nature of cohort studied</th>
<th>N</th>
<th>Age range</th>
<th>Prevalence (%)</th>
<th>A1c Sensitivity (%)</th>
<th>A1c specificity</th>
<th>Optimal A1c cut-point</th>
</tr>
</thead>
<tbody>
<tr>
<td>China, Zhou, 2009</td>
<td>Population</td>
<td>903</td>
<td>21-79</td>
<td>22.4</td>
<td>A1c≥5.7: 59.4% ‡</td>
<td>A1c≥5.7: 73.9%</td>
<td>5.7%</td>
</tr>
<tr>
<td>China, Hu, 2010</td>
<td>High risk</td>
<td>2298</td>
<td>-</td>
<td>29.3</td>
<td>A1c≥5.6: 66.2% *</td>
<td>A1c≥5.6: 51.0% *</td>
<td>5.6%</td>
</tr>
<tr>
<td>China, Bao, 2010</td>
<td>Population</td>
<td>4886</td>
<td>&gt;20</td>
<td>17.1</td>
<td>A1c≥6.0: 16.7% † ¥</td>
<td>A1c≥6.0: 92.9% † ¥</td>
<td>-</td>
</tr>
<tr>
<td>NHANES 1988-1994, Saydah, 2002</td>
<td>Population, but BMI&gt;24 only</td>
<td>2844</td>
<td>40-74</td>
<td>-</td>
<td>A1c≥6.0: 16.7% † ¥</td>
<td>A1c≥6.0: 92.9% † ¥</td>
<td>-</td>
</tr>
<tr>
<td>NHANES 1999-2006, Mann, 2010</td>
<td>Population</td>
<td>7029</td>
<td>≥ 20</td>
<td>28.2</td>
<td>A1c≥6.0: 9% ‡ ¥</td>
<td>A1c≥6.0: 99% ‡ ¥</td>
<td>5.4% €</td>
</tr>
<tr>
<td>NHANES III + 2005-6, SIGT, Olson, 2010</td>
<td>Population</td>
<td>4643</td>
<td>55, 46, 48</td>
<td>36.0</td>
<td>A1c≥6.0: 13%</td>
<td>A1c≥6.0: &gt;90%</td>
<td>5.3-5.5%</td>
</tr>
<tr>
<td>Health + Ageing Study, Lipska, 2010</td>
<td>Older cohort</td>
<td>1865</td>
<td>70-79</td>
<td>22.1</td>
<td>A1c≥5.7: 47.0</td>
<td>A1c≥5.7: 84.5%</td>
<td>5.6%</td>
</tr>
<tr>
<td>AusDiab, Colagiuri, 2004</td>
<td>At risk</td>
<td>5604</td>
<td>-</td>
<td>-</td>
<td>A1c≥5.3: 42%</td>
<td>A1c≥5.3: 88.2%</td>
<td>-</td>
</tr>
<tr>
<td>Italy, Mannucci, 2003</td>
<td>Population</td>
<td>1215</td>
<td>30-70</td>
<td>10.8 M 4.8 F</td>
<td>A1c&gt;5.5% or FPG&gt;6.1:</td>
<td>A1c&gt;5.5% or FPG&gt;6.1:</td>
<td>-</td>
</tr>
<tr>
<td>CURES, Mohan, 2009</td>
<td>Population</td>
<td>2188</td>
<td>≥ 20</td>
<td>11.8</td>
<td>A1c≥5.7: 65.3%</td>
<td>A1c≥5.6: 62.1% *, 56.5% ‡</td>
<td>5.6%</td>
</tr>
</tbody>
</table>

Data analysed for IGR (i.e. using an OGTT) unless otherwise stated (* IGT only analysed; † combined IGT and IFG together only; ‡ IFG only). IFG refers to WHO 1999 definition unless stated indicated (¥ ADA defined IFG). Key: € = estimated from tabulated data, A1c = HbA1c, F = female, IFG = impaired fasting glucose, IGR = Impaired glucose regulation (defined as IGT and/or IFG); IGT = Impaired glucose tolerance, M = male, OGTT = oral glucose tolerance test, SA = south Asians, WE = white Europeans.
2.5.5 Discordance between diagnostic tests

Most studies report HbA1c was generally a poor tool for detecting IGR (Mohan et al., 2010; Van't Riet et al., 2010; Zhou et al., 2009; Colagiuri et al., 2004), IGT (Peters et al., 1996; Mannucci et al., 2003; Saydah et al., 2002; Salesman et al., 1987) or IFG (Likhari et al., 2008). Regarding discordance between IGR on glucose testing and HbA1c, a Chinese population based study suggested 74.8% and 40.6% of people with glucose defined IGR had HbA1c < 6.0% and < 5.7% respectively, despite HbA1c demonstrating a good AUC of 0.73 (Zhou et al., 2009). The NHANES III + 2005-6/ SIGT study found 89% and 70% of people with IGR had HbA1c < 6.0% and < 5.7% respectively (Olson et al., 2010). The NHANES 1999-2006 proposed their results would theoretically reclassify 37.6 million US adults with IFG to be at low risk with HbA1c criteria and 8.9 million without IFG to have IGR (Mann et al., 2010). Comparing direct use of IEC and ADA criteria for IGR, use of the latter led to less false negative results (people with IGR on OGTT but HbA1c < 5.7%) but more false positive diagnoses (people with HbA1c 5.7 to 6.4% but not IGR on OGTT) (Olson et al., 2010).

2.5.6 Is there a change in phenotype and cardiovascular risk in people classified as having IGR from using HbA1c criteria?
Using ADA criteria of HbA1c 5.7 to 6.4%, the NHANES 1999-2006 reported additional people were more likely to consist of women, non-Hispanic blacks, and possess hypertension, hypercholesterolaemia, chronic kidney disease, microalbuminuria and elevated CRP compared to false negatives (Mann et al., 2010). No differences were found in BMI or waist circumference. Also, people with IGR from both glucose and the respective HbA1c criteria had the worst metabolic profile (Mann et al., 2010). The NHANES III + 2005-6/ SIGT and IRAS studies reported an HbA1c of 5.7 to 6.4% was more likely to consist of non-Hispanic blacks and less non-Hispanic whites (Olson et al., 2010; Lorenzo et al., 2010). An elderly US based cohort reported African-Americans were more likely to be detected with HbA1c 5.7 to 6.4% but Non-Hispanic Whites more likely to be detected using ADA glucose criteria (Lipska et al., 2010).

2.5.7 Discussion

The main limitation was lack of available data in this area; most studies have reported this information through a sub-analysis which primarily focuses on the impact of using HbA1c for prevalent T2DM. Four studies reported a decrease in prevalence of IGR using HbA1c criteria (Cowie et al., 2010; Zhou et al., 2010; Mann et al., 2010). The optimal HbA1c cut-points for IGR from ROC curve analysis were lower than IEC recommended levels of HbA1c 6.0 to 6.4%, generally ranging from 5.6 to 5.8%, with reported sensitivities and specificities
often both below 60%. Therefore this could suggest using ADA cut-points are more appropriate than those recommended from the IEC. Furthermore, populations with a lower mean cohort HbA1c could benefit from using the ADA recommendations, as fewer people would be classified in their range. Clearly more data need to be assessed before more conclusions can be made; this topic is investigated in UK multi-ethnic cohort in Chapter 3. It should be noted that the differences in results and test performance in HbA1c optimal cut-points over different geographic locations and time may be due to different HbA1c assay methods employed, and not necessarily population differences. Furthermore, the degree of discordance between diagnostic tests is potentially larger than for T2DM, suggesting the number of false positives and false negatives diagnoses from using HbA1c criteria will be relatively high. The use of FPG and HbA1c together appears to increase sensitivity for detecting IGR; however this strategy has never been proven as cost effective.

Regarding a potential change in phenotype of people detected as having IGR, studies reported additional people or false positives (people with IGR on HbA1c criteria but not glucose criteria) would consist of more non-Hispanic blacks and less non-Hispanic white Europeans. Furthermore, these additional people were reported to have a worse cardiovascular profile in NHANES. These differences may influence progression rates to T2DM. More research is required.
2.6.1 Studies analysing progression of baseline HbA1c values to developing incident T2DM

The prognostic role of a baseline HbA1c to predict incident T2DM, in those who do not have T2DM at baseline, must be considered as HbA1c has become the preferred diagnostic tool in clinical practice. In contrast to studies investigating the prevalence of T2DM using glucose testing and HbA1c, there are fewer studies for incidence of T2DM using baseline HbA1c values. Therefore formulating conclusions may be harder.

**Aims**

The questions to be assessed are as below:

1) Is there evidence that a baseline HbA1c can predict future T2DM?

2) At what cut-point does a baseline HbA1c begin to predict T2DM?

3) What is the optimal baseline HbA1c cut-point to best predict progression to developing T2DM? Is this cut-point similar in different populations?

4) If people were not diagnosed with T2DM initially, how often should we re-screen these individuals for incident T2DM in (a) the general public and (b) people with IGR?

5) Is there a role for combined use of HbA1c and FPG in predicting progression to incident T2DM?
The search strategy has been described in section 2.4.1. Here, 18 studies were reviewed from diabetes specific and general medicine journals, although not every study had a primary aim of investigating baseline HbA1c progression to developing T2DM (see Table 2.4 and 2.5). Furthermore, some studies focused more on the combined use of HbA1c and FPG, without providing data on HbA1c progression alone. Results from various studies were expressed in a variety of methods including percentage progression to T2DM, ratios (odds, hazard or likelihood) or relative risk. In addition, the ratios were created from models which adjusted for different dependent variables, once again making comparison of studies invariably difficult.

Six studies were based in Japan (Nakagami et al., 2010; Sato et al., 2009; Inoue et al., 2008; Inoue et al., 2007; Takahashi et al., 2006; Takahashi et al., 2010), five within US (although one study focused on Pima Indians only) (Pradhan et al., 2007; Selvin et al., 2010; Edelman et al., 2004; Little et al., 1994; Selvin et al., 2011), five from Europe (Droumaguet et al., 2006; Norberg et al., 2006; Kolberg et al., 2009; Chamnan et al., 2010; Cederberg et al., 2010) and two studies were conducted in China (Ko et al., 1999; Ko et al., 2000). Data was lacking in Hispanic, African and south Asian populations. Regarding the latter population, data from the control arm of Indian T2DM Prevention Program was not reported in detail. The age ranges/means in various studies were approximately similar and appeared appropriate for people at risk of T2DM (Table 2.4). The Kansai Health study focused on men only (Sato et al., 2009), while WHS provided data on females (Pradhan et al., 2007); in contrast the DESIR study which compared...
progression rates for both males and females separately in same cohort (Droumaguet et al., 2006). The length of follow up also varied between studies; the ARIC, WHS and a Finnish study provided long term follow data of approximately 10 years or more (Selvin et al., 2010; Pradhan et al., 2007; Cederberg et al., 2010), while most others (n=13) focused on 3 to 7 years. The results are discussed below.

2.6.2 HbA1c to predict incident T2DM

Two long term studies of greater than 10 years showed baseline HbA1c predicted T2DM beyond HbA1c ≥ 5.5%, Table 2.5. The ARIC study found a multi-adjusted hazard ratio for T2DM of HbA1c 5.0 to 5.5% was 1.86 (1.67 to 2.08), with 21% of people in this range progressing to T2DM (Selvin et al., 2010). The WHS study found the relative risk of developing T2DM showed a rapid increase from 2.9 to 12.1 moving from HbA1c categories of 5.0 - 5.4% to 5.5 - 6.0% (Pradhan et al., 2007). Furthermore both studies found the highest progression rates were above an HbA1c of 6.0%. The ARIC study reported an HbA1c of 6.0 to 6.4% produced a hazard ratio for T2DM of 4.5 (3.9 to 5.1), with 44% progressing to T2DM; WHS reported those with HbA1c 6.0 to 6.4% had a higher relative risk of 29.3. A third long term study, found 32.8% of Finnish people with HbA1c 5.7 to 6.4% developed T2DM after 9.7 years, multi-adjusted crude risk ratio, 2.4 (1.5 to 3.9) (Cederberg et al., 2010).
In shorter studies, a population based VAMC study (n=1197 people without T2DM, follow up 3 years) found people with HbA1c 6.1 to 6.9% had the highest annual incidence of incident T2DM of 7.8% (5.2 to 10.4) compared to those with either HbA1c ≤ 5.5% or HbA1c 5.6 to 6.0%: 0.8% (0.4 to 1.2) and 2.5% (1.6-3.5) respectively (Edelman et al., 2004). However people who were obese and had HbA1c 5.6 to 6.0% had an annual incidence of 4.1%. The authors made two important conclusions in their report; people with HbA1c < 5.5% may not require screening until after 3 years, whereas those with higher HbA1c values will require earlier re-screening, especially if greater than > 6.0% or if the individual was obese (Edelman et al., 2004). To complement this, a large Japanese study found cumulative T2DM incidence rates at 3 years were very low (< 1%) below an HbA1c < 6.0%; however in those with an HbA1c ≥ 6.0% re-screening at one year intervals would be a reasonable strategy (Takahashi et al., 2010). The ARIC study reported that at 10 years after baseline the T2DM incidence was approximately 15% in people with ADA defined IFG compared with 22% in those with an HbA1c 5.7 to 6.4%.

The EPIC-Norfolk study (n=5,735) performed serial HbA1c measurements at baseline and 3 years on people without self-reported T2DM and HbA1c <6.5% (Chamnan et al., 2010). Only 35 (0.6%) people had an HbA1c ≥ 6.5% at the end of 3 years – a relatively low value; in contrast 37 (cumulative incidence 0.6%, 0.4 to 0.9) people had glucose defined T2DM. Incident cases of HbA1c ≥ 6.5% were equally divided between baseline HbA1c categories of <5.5%, 5.5 to 5.9%, 6.0 to 6.4%. However, for a 0.5% incident increase from a baseline HbA1c value, there
was a two-fold risk increase in glucose defined T2DM and/or HbA1c ≥ 6.5% (Chamnan et al., 2010).

Furthermore Japanese studies showed an HbA1c ≥ 5.6% can predict T2DM in the shorter term, similar findings to results from the ARIC and WHS (Nakagami et al., 2010; Inoue et al., 2009; Inoue et al., 2008). A French cohort suggested the same prediction cut-point was from an HbA1c of ≥ 5.7 or 5.8% (Droumaguet et al., 2006). The Inter-99 study found 160 people who developed T2DM within 5 years had a median baseline HbA1c of 6.1% (inter-quartile range: 5.8 to 6.4%) (Kolberg et al., 2009). Using graphical information provided, HbA1c had an AUC for detecting incident T2DM of 0.650.

2.6.3 Combined use of HbA1c and fasting plasma glucose

The ARIC study reported the cumulative incidence of T2DM at ten years was highest with combined HbA1c 5.7 to 6.4% and FPG ≥ 5.6 to 6.9mmol/l (48.8%) compared to either test alone (9.7% for HbA1c 5.7 to 6.4 and 7.2% for FPG ≥ 5.6 to 6.9%) (Selvin et al., 2011). A Chinese high risk population (n=208, mean follow up 1.6 years) found people with HbA1c ≥ 6.1% and normal fasting glucose had a likelihood ratio of 0.90 of developing T2DM and a crude progression to T2DM of 8.7% per year (Ko et al., 2000). When people with HbA1c ≥ 6.1% and WHO defined IFG were assessed the likelihood ratio increased sharply to 9.32, with 44.1% crude progression per year.
A Japanese based cohort (follow up 7 years; 449 people) found those individuals with HbA1c < 6.1% and ≥ 6.1% had progression to T2DM of 2.0% and 18.8% respectively (Inoue et al., 2007). When assessing people with HbA1c ≥ 6.1% and WHO defined IFG, the percentage progressing was higher at 66.7%. A second Japanese study (follow up 5.5 years; n=10,042) reported people with an HbA1c 5.6 to 6.4% and normal fasting glucose level had lower hazard ratio of 7.43 (4.70 to 11.7) than those with HbA1c 5.6 to 6.4 and WHO defined IFG, 38.4 (24.6 to 59.9) (Inoue et al., 2008).

The Japanese Kansai health population study (follow up 4 years, n=6736 men aged 40 to 55 years) found the progression rates to incident T2DM increased from 6.5% to 20.6% in people with HbA1c 5.4 to 5.7% and HbA1c 5.8 to 6.2% respectively (Sato et al., 2009). FPG and HbA1c were both independently associated with developing T2DM; however the combined use of both FPG and HbA1c had a significantly highest AUC. The DESIR study (white Europeans followed up for 6 years) found HbA1c independently predicted future T2DM, especially beyond HbA1c ≥ 5.7 to 5.8%, with over 10% of both men and women developing T2DM at HbA1c ≥ 5.9% (Droumaguet et al., 2006). Furthermore, if people with HbA1c ≥ 5.9% were combined with those who had FPG ≥ 6.1 to 6.9mmol/l, the risk of progression was 50% (odds ratio 7.20).
2.6.4 Progression to T2DM using HbA1c in people with IGT

The value of HbA1c progression in people with IGT has also been reported. A study of 257 predominantly Pima Indians investigated the progression over 3.3 years; 50% of people with HbA1c $\geq 6.03\%$ progressed to T2DM on OGTT, in contrast to 12.1% of those with HbA1c $< 6.03\%$ (Little et al., 1994). If people with IGT were analysed using the same two categories, the progression values were 68.4% and 27.7% respectively. Furthermore, a 1% increase in HbA1c in people with IGT led to an increased odds ratio of 6.76 for developing T2DM on OGTT.

The Japanese Fungata study followed up 1,189 people without T2DM up for 5 years (Nakagami et al., 2010). Baseline HbA1c values predicted future glucose-defined T2DM on OGTT beyond $\geq 5.6\%$ (odds ratio 10.06, 4.44 to 22.79) with 18.7% of people with HbA1c $\geq 5.6\%$ developing T2DM after 5 years, however the majority of these people (15.3%) had IGT at baseline. In contrast, a Chinese based study of people with IGT only found 2-hour plasma glucose, and not HbA1c, was an independent predictor of future T2DM on OGTT after 1.7 years (Ko et al., 1999).

2.6.5 The HbA1c optimal cut-point for detecting incident T2DM

This has been reported in three studies as HbA1c $\geq 5.4\%$ in the Fungata study (sensitivity 86.0%; specificity 61%), HbA1c $\geq 6.1\%$ in DESIR (sensitivity 64%;
specificity 77%) and HbA1c ≥ 5.6% in Kobe study (sensitivity 84.2%; specificity 92.1%) (Nakagami et al., 2010; Droumaguet et al., 2006; Takahashi et al., 2006). Interestingly, the DESIR study also stated the optimal cut-point for FPG still had higher sensitivity, specificity and AUC for predicting T2DM compared to HbA1c (Droumaguet et al., 2006).
Table 2.4 Demographics of selected studies with data available on baseline HbA1c progression to developing T2DM

<table>
<thead>
<tr>
<th>Name, first author, year of publication</th>
<th>Cohort</th>
<th>N</th>
<th>Age (range/ Mean)</th>
<th>F/U years</th>
<th>DM Diagnosis</th>
<th>Incident DM, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIC, Selvin, 2010</td>
<td>Non DM</td>
<td>388</td>
<td>-</td>
<td>14 *</td>
<td>FPG, OHA, SR</td>
<td>20.3</td>
</tr>
<tr>
<td>WHS, Pradhan, 2007</td>
<td>Non DM, F only</td>
<td>26563</td>
<td>&gt;45</td>
<td>10.1 *</td>
<td>SR</td>
<td>4.7</td>
</tr>
<tr>
<td>VAMC, Edelman, 2004</td>
<td>Non DM</td>
<td>1197</td>
<td>45-64</td>
<td>3</td>
<td>FPG, SR, HbA1c&gt;7.0%</td>
<td>6.1</td>
</tr>
<tr>
<td>Pima Indians, Little, 1994</td>
<td>Non DM</td>
<td>257</td>
<td>46.7 †</td>
<td>3.3 †</td>
<td>¥ OGTT</td>
<td>44.0</td>
</tr>
<tr>
<td>Kansai, Sato, 2009</td>
<td>No DM M only</td>
<td>6804</td>
<td>40-55</td>
<td>4</td>
<td>FPG, OHA</td>
<td>9.7</td>
</tr>
<tr>
<td>Japan, Inoue, 2007</td>
<td>Non DM</td>
<td>449</td>
<td>23-65</td>
<td>7</td>
<td>FPG</td>
<td>3.8</td>
</tr>
<tr>
<td>Japan, Inoue, 2008</td>
<td>Non DM</td>
<td>10042</td>
<td>-</td>
<td>5.5 †</td>
<td>FPG, CD</td>
<td>3.7</td>
</tr>
<tr>
<td>Fungata, Nakagami, 2010</td>
<td>Non DM</td>
<td>1189</td>
<td>35-89</td>
<td>5</td>
<td>OGTT</td>
<td>4.8</td>
</tr>
<tr>
<td>Kobe, Takahashi, 2006</td>
<td>Non DM</td>
<td>2659</td>
<td>42.2</td>
<td>4.1 †</td>
<td>OGTT or FPG</td>
<td>1.4</td>
</tr>
<tr>
<td>Japan, Takahashi, 2010</td>
<td>Non-DM</td>
<td>16313</td>
<td>49.7</td>
<td>3.0</td>
<td>A1C ≥ 6.5%, OHA</td>
<td>3.2</td>
</tr>
<tr>
<td>China, Ko et al., 2000</td>
<td>Non DM, high risk</td>
<td>208</td>
<td>35.0 †</td>
<td>1.6 †</td>
<td>OGTT</td>
<td>21.2</td>
</tr>
<tr>
<td>China, Ko et al., 1999</td>
<td>IGT only</td>
<td>123</td>
<td>22-66</td>
<td>1.7 †</td>
<td>OGTT</td>
<td>23.6</td>
</tr>
<tr>
<td>DESIR, Droumaguet, 2006</td>
<td>Non DM</td>
<td>1323 M 1407 F</td>
<td>30-65</td>
<td>6</td>
<td>FPG, OHA, insulin</td>
<td>2.1 F, 4.3 M</td>
</tr>
<tr>
<td>Sweden, Norberg, 2006</td>
<td>Non DM</td>
<td>‡</td>
<td>-</td>
<td>5.4 *</td>
<td>164</td>
<td>-</td>
</tr>
<tr>
<td>Inter99, Kolberg</td>
<td>Non DM</td>
<td>6600</td>
<td>≥ 39</td>
<td>5</td>
<td>160</td>
<td>2.42</td>
</tr>
<tr>
<td>EPIC Norfolk, Chamnan, 2010</td>
<td>Non DM HbA1c&lt;6.4%</td>
<td>5735</td>
<td>40-74</td>
<td>3 †</td>
<td>HbA1c ≥ 6.5%, SR</td>
<td>1.3</td>
</tr>
<tr>
<td>Finland, Cederberg, 2010</td>
<td>Non DM</td>
<td>593</td>
<td>-</td>
<td>9.7 †</td>
<td>OGTT</td>
<td>17.1</td>
</tr>
</tbody>
</table>

Key: CD = Clinical diagnosis, DM = T2DM mellitus, FPG = fasting plasma glucose, Insulin = Insulin therapy use, OHA = Oral hypoglycaemic agent, SR = self report; * median, † mean; ¥ DM diagnosis WHO 1985 with FPG >7.8mmol/l or 2hr 11.1mmol/l; ‡ retrospective study which investigated referred cases of T2DM.
<table>
<thead>
<tr>
<th>Name, first author, year of publication</th>
<th>Progression of HbA1c (reported in various forms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIC, Selvin, 2010</td>
<td>For HbA1c categories: &lt;5.0%, 5.0-5.4%, 5.5-5.9%, 6.0-6.4%, ≥6.5%; a) % progression: 6%, 12%, 21%, 44% and 79%; b) Multi-adjusted HR: 0.52 (0.4-0.69), 1.00 (reference), 1.86 (1.67-2.08), 4.48 (3.92-5.13) and 16.47 (14.22-19.08). Cumulative incidence of T2DM at ten years was highest with combined HbA1c 5.7-6.4% and FPG ≥ 5.6-6.9mmol/l (48.8%) compared to 9.69% and 7.19% for HbA1c 5.7-6.4 and FPG ≥ 5.6-6.9% respectively.</td>
</tr>
<tr>
<td>WHS, Pradhan, 2007</td>
<td>For HbA1c categories: &lt;5.0%, 5.0-5.4%, 5.5-5.9%, 6.0-6.4%, 6.5-6.9% and ≥ 7.0%; multi-variable adjusted RR: 1.0 (reference), 2.9, 12.1, 29.3, 28.2 and 81.2.</td>
</tr>
<tr>
<td>VAMC, Edelman, 2004</td>
<td>For HbA1c categories: ≤ 5.5%, 5.6-6.0%, 6.1-6.9%; a) annual incidence: 0.8%; 2.5%, 7.8%. b) Obese people with HbA1c 5.6-6.0%: 4.1%.</td>
</tr>
<tr>
<td>Pima Indians, Little, 1994</td>
<td>For HbA1c categories &lt;6.03% and ≥ 6.03%, a) % progression: 12.1 and 50%; b) if IGT only: % progression: 27.7% and 68.4%. c) For a 1% increase in HbA1c in people with IGT: OR: 6.76 (1.77-25.8)</td>
</tr>
<tr>
<td>Kansai, Sato, 2009</td>
<td>For HbA1c categories: &lt;5.3%, 5.4-5.7%, 5.8-5.9%, 6.0-6.7%, 6.8% ≥; % Progression: 3%, 6.5%, 20.6%, 41.9% and 69.1%.</td>
</tr>
<tr>
<td>Japan, Inoue, 2007</td>
<td>For HbA1c categories: &lt;6.1% and ≥ 6.1%; a) % progression: 2.0% and 18.8%. b) HbA1c ≥ 6.1% combined with IFG: progression 66.7%, Adjusted OR HbA1c: 3.03 (1.73-5.32)</td>
</tr>
<tr>
<td>Japan, Inoue, 2008</td>
<td>HR: HbA1c &lt;5.5% + ADA IFG: 14.4 (11.9-27.8); HbA1c 5.6-6.4% + FPG &lt;5.6: 7.43 (4.70-11.7) HbA1c 5.6-6.4%+ ADA IFG: 38.4 (24.6-59.9)</td>
</tr>
<tr>
<td>Fungata, Nakagami, 2010</td>
<td>For HbA1c categories: &lt;5.3%, 5.3-5.5%, ≥ 5.6%; a) % Progression: 1.4%; 3.7%, 18.7%. b) OR: 1.0, 2.14 (0.91-5.05), 10.06 (4.44-22.79). c) IGT only % progression: 0.4%, 1.5%, 15.3%.</td>
</tr>
<tr>
<td>Kobe, Takahashi, 2006</td>
<td>HbA1c predicted T2DM: OR: 176.8 (62.2-502.6). Optimal HbA1c cut-point: ≥ 5.6%, HbA1c AUC 0.933 (0.885, 0.981),</td>
</tr>
<tr>
<td>Japan, Takahashi, 2010</td>
<td>Cumulative incidence for T2DM for HbA1c categories &lt;5.5%, 5.0-5.4%, 5.5-5.9%, 6.0-6.4% were 0.05%, 0.05%, 1.2% and 20%.</td>
</tr>
<tr>
<td>China, Ko et al., 2000</td>
<td>a) HbA1c≥6.1% + IFG and NFG: LR: 9.32 and 0.90. Crude progression rate: 44.1% and 8.7% / year. b) HbA1c&lt;6.1% + IFG and NFG: LR: 1.06 and 0.58. Crude progression rate: 17.4 and 8.1% / year.</td>
</tr>
<tr>
<td>Study</td>
<td>Description</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>China, Ko et al., 1999</td>
<td>2 hr plasma glucose predicted progression to T2DM: RR: 2.31 (1.22-4.37). HbA1c not an independent predictor.</td>
</tr>
</tbody>
</table>
| DESIR, Droumague, 2006        | a) For HbA1c categories: 4.5-5.0%, 4.5-5.5%, 4.5-6.0%, 4.5-6.5%: OR: 0.90 (0.5-1.5), 1.5 (0.7-3.4), 5.0 (2.0-12.8), 32.7 (11.5-92.6).  
|                               | b) For a 1% increase in HbA1c combined with FPG categories <5.6, 5.6-6.9, ≥6.10: OR: 0.78 (0.2-3.07), 1.47 (0.36-5.8), 7.20 (3.0-17.0)        |
| Sweden, Norberg, 2006         | OR: HbA1c ≥ 5.7%: men 16.0 (2.23-115.3), women 19.6 (2.52-152.4)                                                                                         |
| Inter99, Kolberg              | People who developed T2DM: median (IQR): HbA1c 6.1% (5.8 – 6.4%)                                                                                   |
| EPIC Norfolk, Chamnan, 2010   | For categories HbA1c <5.0%, 5.0-5.4%, 5.5-5.9%, 6.0-6.4%, Multi-adjusted OR: 1.0, 1.6 (0.7-3.6), 3.3 (1.5-7.4), 15.6 (6.9-35.7), 3 year cumulative incidence (%): 0.5 (0.3-0.9), 0.8 (0.5-1.2), 1.5 (1.0-2.3), 7.0 (4.8-10.1). |
| Finland, Cederberg, 2010      | HbA1c 5.7-6.4%: 32.8% of people developed T2DM. Crude relative risk ratio: unadjusted 2.78 (1.80-4.31); multi-adjusted 2.42 (1.50-3.91)  |

95% confidence intervals are provided for ratios if provided in the study. Japanese and Swedish HbA1c values converted to NGSP values for this table. Key: HR = Hazard ratio, IFG = impaired fasting glucose (WHO criteria 1999 unless stated), Impaired glucose tolerance = IGT, LR = Likelihood ratio, NFG = Normal fasting glucose (WHO 1999 criteria unless stated), OR= odds ratio, RR = relative risk.
2.6.6 Discussion

HbA1c is able to predict glucose-defined T2DM in nearly all studies where this was reported, with only one letter publication suggesting the opposite (Ko et al., 1999). Data has shown T2DM can be predicted starting from approximately HbA1c 5.5 to 5.6% in both long term and short term studies. This would accommodate the ADA HbA1c criteria of ≥ 5.7% for high risk people. However, in some populations with a relatively high mean HbA1c (e.g. 5.7 and above), over 50% of people may be detected as having IGR, therefore this may not feasible. Secondly, shorter term studies additionally show stronger progression rates to incident T2DM starting from HbA1c 5.9 to 6.1% (Edelman et al., 2004; Little et al., 1994; Sato et al., 2009; Inoue et al., 2007; Droumaguet et al., 2006; Ko et al., 2000). This would match results from the T2DM Prevention Program which stated those with HbA1c ≥ 6.0% were more likely to progress to T2DM (unpublished data, Nathan et al., 2007). A recent systematic review found similar results (Zhang et al., 2010) although was not able to include some studies published in 2010 (Chamnan et al., 2010; Cederberg et al., 2010). They found HbA1c values from 5.5 to 6.5% were associated with an increased risk for developing glucose defined T2DM. Furthermore, for HbA1c categories of 5.0 to 5.5%, 5.5 to 6.0% and 6.0 to 6.5% the five year incidence of T2DM were <5 to 9%, 9 to 25% and 25 to 50% respectively (Zhang et al., 2010).

Overall, the HbA1c cut-points reporting relatively higher incidences of T2DM were between HbA1c 5.9 to 6.1%, therefore this may suggest a threshold for the
IGR group as these people can be re-screened more regularly and given intensive lifestyle advice. However, more data are required. In addition, the optimal cut-points derived from ROC curve analysis based on prevalent IGR were generally lower at HbA1c 5.6 to 5.8%. Considering the information available, how often people should be re-screened, based on incidence rates to developing T2DM, is still debatable. Data suggests those with risk factors for T2DM, especially previous IGT, IFG or obesity, should be re-screened earlier, perhaps within one to three years. In contrast, those at low risk and lower HbA1c values could be re-screened less often. Current recommendations from some countries suggest people aged 40 to 75 years old should have a test for T2DM every three years (using traditional glucose tests), while specific high risk groups (e.g. IGT and/ or IFG) could be re-screened every one to two years (NICE 2012; Department of Health, 2009). Whether these principles could be transferred across to HbA1c groups is not yet proven as an effective strategy.

A question I was not able to answer is whether HbA1c or FPG is actually a better tool to predict progression to developing glucose defined T2DM. A meta-analysis of prospective observational studies analysed the progression to T2DM according to various definitions of IGR at baseline. ADA defined IFG, WHO-defined IFG, IGT, combined IGT and IFG, and HbA1c 6.0–6.4% had incident T2DM rates of 35.5 (26.6, 48.0) per 1,000 person-years, 47.4 (37.4, 59.8), 45.5 (37.8, 54.5), 70.4 (53.8, 89.7) and 35.6 (15.1, 83.0) respectively (Morris et al., 2013). This suggests HbA1c 6.0 to 6.4% may slightly lower rates of progression to T2DM. However, studies showed that HbA1c generally predicts T2DM much
stronger if FPG was raised into the WHO defined IFG range. While screening with both HbA1c and FPG remains an option, this strategy has never been proven to be cost effective.
2.7 Overall conclusions to Chapter 2

If the diagnosis of T2DM and IGR shifts away from using traditional glucose testing to an HbA1c, there will be a variable impact on prevalence of these conditions. HbA1c ≥ 6.5% has a good specificity but weaker sensitivity for T2DM; however the optimal cut-point from ROC curve analysis was lower than 6.5%. Using HbA1c as a diagnostic tool gave a better performance in screening for glucose-based T2DM than for glucose based IGR. Furthermore, there is a degree of discordance between people detected from use of glucose testing and HbA1c; however this discordance is larger with IGR than for T2DM. Regarding the change in people detected in T2DM; this may lead to a change in phenotype and cardio-metabolic profile in those classified as having IGR or T2DM.

While the HbA1c diagnostic cut-points for T2DM is agreed between many international organisations (≥ 6.5%), the lower limit cut-point for IGR category needs further review. The ADA recommendations of using a lower cut-point of HbA1c 5.7% generally seem to match the ROC curve analysis of prevalent IGR cut-points (which most commonly reported as between HbA1c 5.6 to 5.8%). In contrast, IEC recommendations of HbA1c 6.0% generally seem to match the best cut-points in terms of progression of baseline HbA1c to incident T2DM in the general population (most commonly reported as 5.9 to 6.1%). Despite these findings, more research is required before HbA1c cut-points for IGR are determined.
The studies reviewed in Chapter 2 may not give a reflection of the potential diagnostic changes in the UK, especially multi-ethnic areas. In Chapter 3, I will re-visit some of the questions asked regarding prevalence of T2DM and IGR from using HbA1c, however using data from a large UK, multi-ethnic, contemporary cohort.
Chapter 3

The potential impact of using glycated haemoglobin, HbA1c, to detect people with Type 2 Diabetes Mellitus and Impaired Glucose Regulation in a Leicestershire multi-ethnic cohort
3.1 Chapter Overview

In Chapter 2, I explored the theme of using HbA1c for detecting T2DM and IGR in different global populations. In Chapter 3, I wish build on the previous information gained by focusing in depth on a Leicestershire multi-ethnic cohort. The work in this chapter is based on two first author papers (Mostafa et al., 2010b; Mostafa et al; 2010c).

3.2 Introduction

There is a need to understand the consequences of using HbA1c for detecting T2DM and IGR in place of an OGTT, in particular for potential changes in the prevalence, phenotype and cardio-metabolic characteristics of those people classified as having T2DM and IGR.

T2DM is an important multi-system disease that requires multi-factorial risk factor intervention in order to reduce the chances of developing complications (Holman et al., 2008). Information on prevalence of T2DM using HbA1c can inform health care providers if the services provided need to expand (e.g. retinal screening), should the UK or a local region switch to using HbA1c for diagnosis. Furthermore, the natural history of people with T2DM detected using an OGTT developing complications has been well described. However, if the people detected as having T2DM using HbA1c have a different cardio-metabolic risk
factor profile, then the risk / rate of developing complications may not be the same.

IGR constitutes an intermediate glycaemic metabolism disorder where blood glucose levels detected on an OGTT range above normal glucose tolerance but below that required to diagnose T2DM (WHO, 1999). The IGR spectrum consists of IGT and/ or IFG which are independent risk factors for developing T2DM (Unwin et al., 2002). IGR usually precedes and is more prevalent than T2DM (Wild et al., 2004). Furthermore, it is well characterised especially with regard to its natural history of progression to T2DM and its associated increased CVD risk (Unwin et al., 2002).

The aims of this chapter were to investigate the effect of using HbA1c≥ 6.5% (48mmol/mol) for diagnosing T2DM compared to the use of the 1999 WHO Criteria, in order to determine the change in prevalence (WHO, 1999) within a multi-ethnic cohort. The same comparison was made for detecting IGR with use of (a) IEC criteria of HbA1c 6.0 to 6.4%, (b) ADA criteria of HbA1c 5.7 to 6.4% (which were both described in chapter 2) compared to (c) IGR detected on an OGTT (International Expert Committee, 2009; American Diabetes Association Position statement, 2010). For IGR, I also examined the same comparison using ADA definition of IFG (5.6 to 6.9mmol/l) (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003). Secondly I examined the change on phenotype, cardio-metabolic characteristics and CVD risk in this cohort from using HbA1c for detecting T2DM or IGR. Thirdly, I wished to choose the optimal cut-points for T2DM and IGR and the corresponding sensitivities and specificities.
3.3 Research Design and Methods

3.3.1 Design overview, setting, data collection and participants

The analysis used in Chapter 3 and 4 consisted of baseline data from the Leicester Ethnic Atherosclerosis and Diabetes Risk (LEADER) study which comprises two cross-sectional screening programmes for T2DM, figure 3.0. Both studies were conducted in Leicestershire, which has a large western multi-ethnic population of over 950,000 people, approximately a one-third of who live in Leicester City. The two screening studies are described below.

3.3.2 Screening those at risk (STAR)

STAR, screening those at risk, was a targeted screening program which invited people with at least one risk factor for T2DM between 2002 and 2004 from 17 randomly selected Leicestershire general practices or through a health campaign (Gray et al., 2010). This risk factors included a documented history of cardiovascular disease (CVD) or peripheral vascular disease, hypertension, current or ex-smokers, dyslipidaemia, IGT and/ or IFG, gestational diabetes or polycystic ovary syndrome in those with a BMI ≥ 25.0kg/m² (south Asians ≥ 23.0kg/m²), first-degree relative with T2DM, BMI ≥ 25kg/m² if leading a self reported sedentary life, otherwise ≥ 30kg/m² (≥ 23 and 27.5 kg/m² respectively in
Figure 3.0 Study cohorts analysed in this thesis for chapters 3 to 7 (LEADER and ADDITION)

**STAR: targeted screening programme**
Leicestershire general practices invited to participate in diabetes screening study or recruitment through a health campaign

- 17 practices accept invitation
- 2,745 people screened forming the **STAR database**

**ADDITION: population based screening programme**
Leicestershire general practices invited to participate in diabetes screening study

- 20 practices accept invitation
- 30,950 invites sent to eligible participants leading to screening of 6,749 people from 2004 to 2009
- 6,749 people screened forming the **ADDITION baseline database**

2 cohorts combined

- Cross-sectional dataset of 6,749 people:
  - Used in chapters 5 and 6
- Insulin sub-sample of 892 people from cross section population:
  - Used in chapter 6
- 1080 (16.1%) people detected with IGR
- Invited for annual re-screening visit
- 912 people entered IGR prospective cohort: **Used in chapter 7**

**Formation of the LEADER cohort:**
cross-section data of 9,494 people

- Used in chapters 3 and 4
south Asians) (Gray et al., 2010). This study screened adults aged 25 to 75 years old, although for white Europeans, only those aged 40 to 75 years were screened. Exclusion criteria consisted of a previous diagnosis of T2DM, the presence of a terminal illness, those housebound preventing attendance, an active psychotic illness preventing informed consent, those pregnant or lactating, or taking part in other clinical trials.

3.3.3 ADDITION-Leicester

The second screening program that formed the LEADER cohort was the ADDITION-Leicester (Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen Detected Diabetes in Primary Care) population based diabetes screening study (trial registration number NCT00318032), figure 3.0. ADDITION was used in combination with STAR (known as the LEADER cohort) for chapters three and four in this thesis; however for chapters five to seven ADDITION was used on its own.

The study contributed to the multi-centre ADDITION-Europe (Griffin et al., 2011), however was also a stand-alone screening study. In this thesis ADDITION refers to ADDITION-Leicester participants only (Webb et al., 2011). ADDITION-
Leicester comprised of a screening phase followed by five year cluster randomised controlled trial for people with early screen detected T2DM to test the intervention that intensive multifactorial risk factor management (using an intermediate care model lead by specialists) leads to less morbidity and mortality than those managed in routine care (Webb et al, 2011). The overall ADDITION study commenced in 2004 and is expected to finish in 2014. My contribution towards this study included leading data collection, nurse support, interpreting test results and physician led annual reviews of participants from April 2009 to August 2013, as well as helping to create and perform checks on the final prospective database for the IGR group (see chapter 7).

The LEADER cohort only includes people from the screening phase of ADDITION-Leicester only. The main difference between ADDITION and STAR is that the former invited people for a test for T2DM irrespective of their risk for T2DM.

Regarding the ADDITION methods, a random sample of participants aged 40 to 75 years old were recruited from 20 Leicestershire community practices and invited to attend a single screening session from 2004 to 2009. Participants were excluded if they had a previous diagnosis of T2DM, terminal illness, psychiatric illness preventing informed consent, pregnancy and lactation. There were no other specific selection criteria.

Both ADDITION and STAR had similar standardised operating procedures for participant appointments and data collection. For both studies, screening was performed at a hospital site, within general practices or on a mobile screening
unit. All individuals underwent an OGTT and HbA1c to identify those with abnormal glucose tolerance. After overnight fast of minimum eight hours, participants underwent an OGTT and had an HbA1c measured. The results of the OGTT were classified using WHO 1999 diagnostic criteria (WHO, 1999). If participants had not fasted for a minimum of eight hours or in case of recent illness, unusual physical activity / dietary pattern (e.g. lowering carbohydrate load) the previous 3 days, the OGTT was postponed. Participants were asked to consume their regular evening meal and snacks (in terms of content and timing) on the evening before testing, except for evening alcohol consumption. On the day of testing participants were asked not to cycle/ run to the test centre or smoke until the test had finished. Asymptomatic individuals who had an OGTT within the diabetes range went on to have a repeat confirmatory OGTT within usually 3-4 days, maximum one week, and asked not to alter their lifestyle between tests. For a diagnosis of T2DM in asymptomatic individuals, one positive result from either FPG (≥ 7.0mmol/l) and/ or 2-hour plasma (≥ 11.1mmol/l) was required on both days for diagnosis of T2DM (WHO, 1999). Only two OGTTs were performed in total, therefore a second test that did not show T2DM would have been classified as either prediabetes or normal glucose tolerance depending on the glucose test results in the second OGTT.

Furthermore, selected T2DM and cardiovascular risk factors were measured. This included fasting samples for urea and electrolytes (including creatinine and potassium), lipids (total-cholesterol, LDL-cholesterol, HDL-cholesterol and
triglycerides) and liver function enzymes (Alanine aminotransferase and gamma-glutaryl transferase). An early morning spot urine sample was for albumin excretion rate measurement and the albumin: creatinine ratio (ACR) subsequently calculated. An ACR ≥ 2.5 mg/mmol in males and 3.5 mg/ mmol in females were taken as defining microalbuminuria. Anthropometric measurements were performed by trained staff following standard operating procedures, with height being measured to the nearest 0.1 cm using a rigid stadiometer. Body weight was measured to the nearest 0.1 kg in light indoor clothing with a Tanita scale (Tanita, Europe). 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. Central obesity was defined as waist circumference as ≥ 80cm in females or ≥ 90cm in south Asian/ Chinese males and ≥ 94cm all other males; waist: hip ratio ≥ 0.85 females and ≥ 0.9 males; and body mass index ≥ 27.5kg/m² in south Asians, all other participants ≥ 30.0kg/m². Brachial blood pressure was measured three times using standardised Omron M7 digital sphygmomanometers (Omron Healthcare, Milton Keynes, UK) with the participant in a seated position. An average of the second and third readings was recorded.

Participants were also asked to complete questionnaires including demographic information on age, sex, and ethnic group. White European and South Asian ethnic groups were defined according to UK national census categories (Office for National Statistics, 2011). In this thesis, other ethnic groups (including mixed racial backgrounds) were omitted from ethnicity analysis due to low numbers.
Socio-economic deprivation was calculated using the Index of Multiple Deprivation (IMD), a postcode based measure of social deprivation which accounts for income, employment, education and living environment (Office for National Statistics, 2007). A higher IMD score suggests higher levels of deprivation. Furthermore, questionnaires had sections on lifestyle habits such as smoking and alcohol intake, as well as family history of T2DM, personal medical history of CVD (defined as myocardial infarction, angina, heart failure, stroke, peripheral vascular disease and arterial revascularisation), and current use of medications. The latter included a specific question on use and type of statin and anti-hypertensive therapies.

Physical activity levels were assessed using the International Physical Activity Questionnaire, an extensively validated measure where levels were computed as metabolic equivalents (METs) per week based on average time spent per week on various leisure-time activities, weighted by their intensity level (Craig et al., 2003). Framingham-10 year cardiovascular disease risk was calculated, adjusted for ethnicity (Brindle et al., 2006). In each study, all participants gave informed consent prior to screening and ethical approval was granted from the local Leicester, Northamptonshire and Rutland ethics committee.

Despite ADDITION-Leicester screening an unselected cohort, two-thirds of people had at least one risk factor for T2DM. Therefore, 75% of the overall LEADER cohort analysed had at least one risk factor for T2DM, suggesting this was predominantly an at risk population. Furthermore, for the LEADER cohort analysed, we focused on adults aged 40 to 75 years only; STAR contributed
2,745 people (71.3% white European, 25.9% south Asian, 2.8% other ethnic groups) and ADDITION contributed 6,749 adults (73.6% white European, 20.9% south Asian, 5.5% other ethnic groups), therefore giving a cohort size of 9494 people. The high number of South Asians (people of Indian, Pakistani and Bangladeshi origin) screened in STAR and ADDITION-Leicester (22.8% of cohort) is proportional to the Leicester city population and consisted of both first and second generation immigrants (Office for National Statistics, 2007).

There are two important sub-samples in ADDITION study which are relevant for later chapters 6 and 7, but are described here. The first sub-cohort is of participants who underwent extra blood samples during baseline screening visits which were stored (n=892). These samples were measured for insulin levels and for purposes of this thesis I will refer to this sub-cohort as the ‘insulin sub-sample’, figure 3.0. The insulin samples were specifically over-selected for measurement from glucose intolerant participants (T2DM or IGR) as these were of interest and achieved better numerical parity with samples from normal glucose regulation participants. Beyond this stratification, all insulin samples were randomly selected and maintained an equivalent proportion of White Europeans and South Asians to the total cohort.

The second sub-cohort from ADDITION-Leicester is a prospective follow-up of people with IGR detected at baseline screening to monitor their progress to T2DM. In this thesis I refer to this group as the ‘IGR sub-cohort’ (figure 3.0). This particular arm of the study was designed to detect novel factors influencing
progression to T2DM and potential ethnic differences in these risk factors. All participants with IGR detected at baseline (n=1080, figure 3.0) were invited to attend annual follow-up OGTT until they developed T2DM or up to October 2012. Participants contributed to person years until their final visit. The family physician of these participants was sent a copy of blood test results, suggesting the participant would benefit from routine lifestyle advice as a management plan. These participants did not undergo any specific intervention. All re-screening appointments consisted of a similar format to those at baseline screening. If participants declined a follow-up invitation, they were still invited for subsequent years. The results of the last re-screening visit were taken as the final result. There were 168 participants who declined invitation for a re-screening test. Two individuals with missing data on glucose outcomes were excluded. Therefore the final IGR sub-cohort consisted of 910 people.

3.3.4 Laboratory assays

All HbA1c and glucose samples were measured in the same laboratory. HbA1c samples were collected in EDTA tubes and measured on the same day the samples were taken using the standardised assay method in alignment with DCCT recommendations on a Biorad Variant HPLC II system (Bio-Rad Laboratories, Hemel Hempstead, UK). The imprecision coefficient of variation of this instrument is 1.88% and 1.06% at HbA1c 5.3% and 9.4% respectively,
therefore, this instrument was standardised to current recommendations for diagnosis of diabetes and is valid for carriers of variant Hb S and C (American Diabetes Association Position statement, 2010). Glucose samples were taken in fluoride oxalate test tubes and placed immediately in a standard portable 4-litre refrigerator (also taken aboard the mobile screening unit) which ran at 4 degrees Celsius. Samples processed within a maximum of two hours, using an Abbott Aeroset clinical chemistry analyser (Abbott laboratories, Maidenhead, UK), which employs the hexokinase enzymatic method. This machinery has an imprecision coefficient of variation of 1.61% at 6.8mmol/l.

Fasting lipid levels and urea and electrolytes were collected in lithium heparin bottles and measured on a Dade Behring Dimension analyser, Newark, USA. HDL cholesterol was measured after isolation of other non HDL apolipoproteins; triglycerides were measured following enzymatic hydrolysis to glycerol. Liver enzyme samples were collected in serum gel tubes and spot urinary ACR samples were collected in plain tubes; both were measured on a Siemens Advia 2400/1800 platforms assay (Siemens, Surrey, UK). Lipid profiles, urea and electrolytes and liver function tests were measured on the same day in the same laboratory with quality control assessments conducted daily.

Fasting insulin levels were collected in lithium heparin tubes and centrifuged immediately, before stored in a -80 degrees Celsius freezer. Insulin samples were analysed on a Perkin Elmer time-resolved fluoro-immuno assay on an Auto DELFIA, which has less than 1% cross-reactivity with C-peptide and proinsulin.
The inter- and intra-coefficient of variations were less than 3.5% for insulin, all lipids and liver enzymes; regarding urinary ACR the same values were up to 5%.
3.4 Statistical Analysis for Chapter 3

3.4.1 Analysis of T2DM

Results were grouped into 5 cohorts labelled A to E: (A) 1999 WHO diagnosis of T2DM on OGTT, (B) HbA1c ≥ 6.5%, so called new diagnosis of T2DM, (C) 1999 WHO diagnosis of T2DM on OGTT and HbA1c < 6.5% (subset of Group (A), also referred to as false negative results), (D) HbA1c ≥ 6.5% with an OGTT in the non-T2DM range (subset of Group (B), also referred to as false positive results or additional people detected) and (E) HbA1c ≥ 6.5% and 1999 WHO diagnosis of T2DM on OGTT. Venn diagrams were produced to provide visual interpretation of the groups. For each group described above, the clinical and metabolic characteristics were described and compared statistically for mutually exclusive groups using Minitab® 14 for chi-squared and unpaired t-tests. The variables included age, ethnicity, sex, waist circumference, waist to hip ratio, body mass index, systolic and diastolic blood pressure, fasting and two-hour plasma glucose, total-, HDL- and LDL-cholesterol, microalbuminuria and Framingham 10 CVD risk. XLSTAT version 2011.5 (Addinsoft, Paris, France) was used to plot receiver operating characteristic (ROC) curves and calculate the area under the ROC curve (AUROC) for HbA1c detecting T2DM. An AUC value of < 0.7 was considered sub-optimal performance of HbA1c. The optimal HbA1c cut-point for T2DM was determined by the best balance between sensitivity and specificity.
and was calculated from line graphs. Confidence intervals were set at 95% (95% CI) and a two-sided p value of <0.05 was considered statistically significant.

3.4.2 Analysis of IGR

In contrast to the analysis for T2DM where only one HbA1c cut-point of 6.5% was employed, the results for IGR were analysed using two sets of HbA1c cut-points. These were HbA1c 6.0 to 6.4% or HbA1c 5.7 to 6.4% reflecting the cut-points suggested by different international organisations. In each case these two cut-points were compared to those with the presence or absence of OGTT detecting IGR (using WHO 1999 criteria). Therefore there were four mutually exclusive groups compared with each set of HbA1c cut-points: (1) HbA1c less than 6.0% and IGR on OGTT; (2) HbA1c less than 6.0% without IGR on OGTT; (3) HbA1c 6.0 to 6.4% with IGR on OGTT and finally (4) HbA1c 6.0 to 6.4% without IGR on OGTT. This was slightly different to the analysis for diabetes as those groups were not mutually exclusive. In the IGR analysis, a similar pattern of grouping was performed using HbA1c 5.7 to 6.4% instead of HbA1c 6.0 to 6.4% and formed groups 5 to 8. The same cardio-metabolic characteristics were analysed for IGR as they were for T2DM. Agreement of the people detected by use of HbA1c and OGTT for IGR was assessed using the Kappa agreement measure. Similarly, the HbA1c AUROC for detecting IGR and optimal cut-points were
derived in similar fashion. Confidence intervals were set at 95% (95% CI) and a two-sided p value of <0.05 was considered statistically significant.
3.5 Results

The initial LEADER population of 9,494 was refined to exclude people less than 40 years (n=600) and those with incomplete OGTT and HbA1c results (n=198). Hence the final cohort included in the study was 8,696. I investigated for causes of a potentially inaccurate HbA1c which were acute blood loss and hyperbilirubinaemia; no cases were reported or found, therefore no exclusions were made. The baseline characteristics of the total cohort are described in Table 3.1. The mean age of the total cohort was 57.3 years (SD 9.7), 52.3% were female and mean HbA1c was 5.7% (0.61). Overall 74.7% of the total cohort was white European and 22.8% south Asian. White Europeans (HbA1c 5.7%, 0.61) had a significantly lower mean HbA1c compared to south Asians (5.9%, 0.61), p<0.0001.

Table 3.1 Selected baseline characteristics of the LEADER cohort used for the analysis in chapter 3 and 4

<table>
<thead>
<tr>
<th>characteristic</th>
<th>Total cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (n)</td>
<td>8696</td>
</tr>
<tr>
<td>Screening OGTT (n)</td>
<td>8696</td>
</tr>
<tr>
<td>Confirmatory OGTT (n)</td>
<td>357 (4.1%)</td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>57.3 (9.7)</td>
</tr>
<tr>
<td>% female</td>
<td>52.3</td>
</tr>
<tr>
<td>Ethnicity % WE</td>
<td>74.7</td>
</tr>
<tr>
<td>% SA</td>
<td>22.8</td>
</tr>
<tr>
<td>Mean HbA1c (%)</td>
<td>5.71 (0.61)</td>
</tr>
</tbody>
</table>
3.5.1 Results for T2DM

Use of an OGTT identified 291 participants with newly diagnosed T2DM (Group A, 3.3%) and there were 502 participants with HbA1c ≥ 6.5% (Group B, 5.8%); both are shown on Figures 3.1 to 3.3 and in Tables 3.2 and 3.3. There were 198 people (Group E, 2.3%) with T2DM detected on OGTT and HbA1c ≥ 6.5%. The Kappa agreement measure for newly diagnosed T2DM on OGTT and HbA1c ≥ 6.5% was 0.477 (0.504 in white Europeans and 0.460 in south Asians). Combining either people with T2DM detected on OGTT or HbA1c ≥ 6.5% together; the prevalence of T2DM was 6.8% (n=595 people).

From introducing HbA1c ≥ 6.5%, there were an additional 304 participants detected with T2DM (Group D, 3.5%), approximately doubling the prevalence. Importantly a larger proportion of south Asians were detected by use of HbA1c ≥ 6.5% compared to White Europeans, a 2.2 vs. 1.4 fold increase in detection rates respectively. Further analysis of the additional 304 people with HbA1c ≥ 6.5% and a non-diabetes OGTT in group D, showed that 172 (56.7%) had IGT and/ or IFG according to 1999 WHO Criteria. If HbA1c ≥ 6.5% was used alone for diagnosis, replacing the OGTT, 93 (1.1%) participants with T2DM detected on OGTT but with HbA1c < 6.5% would not be classified as having T2DM.
3.5.2 Phenotype and Modifiable Risk Factors

The comparison of T2DM detected with OGTT (Group A) and those additional people with HbA1c ≥ 6.5% and a non-diabetes OGTT (Group D) revealed a number of anthropometric differences. The latter had significantly lower mean waist circumference (p=0.025) and waist: hip ratio (p=0.025). Less people were centrally obese when assessed using waist: hip ratio (p=0.020). When the same two groups were compared for modifiable risk factors, Group D had significantly lower systolic and diastolic blood pressure (p < 0.0001 and 0.004 respectively), lower mean triglyceride levels (p < 0.0001), less people had blood pressure > 140 or 85mmHg (p < 0.001), total cholesterol > 5.0mmol/l (0.025) and microalbuminuria (p=0.034) than group B (Table 3.3).

3.5.3 Determination of optimal HbA1c cut-points for detecting T2DM on OGTT

The AUROC for HbA1c detecting T2DM within white Europeans and south Asians were 0.92 (95% CI 0.89 to 0.94) and 0.93 (0.91 to 0.96) respectively. The optimal HbA1c cut-point for detecting T2DM within white Europeans was ≥ 6.1%, producing sensitivity and specificity of 83.0% (76.8 to 91.1) and 87.8% (87.0 to 88.6) respectively. In South Asians, the optimal cut-point was HbA1c ≥ 6.3%
producing sensitivity and specificity of 85.3% (76.6 to 91.1) and 86.5% (84.9 to 88.0), Table 3.4 and figure 3.4. In contrast, HbA1c ≥ 6.5% produced a sensitivity and specificity for T2DM of 62.1% (54.8 to 68.8) and 97.7% (97.3 to 98.1) in white Europeans and 78.9% (69.6 to 86.0) and 92.8% (91.6 to 93.9) in South Asians respectively.
Figure 3.2 Venn diagram to represent the number of people detected with T2DM on (a) HbA1c and OGTT, (b) OGTT but not HbA1c and (c) HbA1c but not OGTT

DM on OGTT & HbA1c≥6.5%

n= 198
(2.3%)

DM on OGTT
n=93
1.2%

n=304
3.5%

HbA1c≥6.5%

Figure 3.3 A combination of both figure 3.1 and 3.2

DM on OGTT & HbA1c≥6.5%

n= 198
(2.3%)

DM on OGTT
n=291
1.1%

n=304
3.5%

HbA1c≥6.5%

n=502
(5.8%)

Total
n= 595
6.8%
### Table 3.2. Population characteristics according to OGTT and HbA1c results

<table>
<thead>
<tr>
<th></th>
<th>Group A: DM on OGTT</th>
<th>Group B: HbA1c ≥ 6.5%</th>
<th>Group C: DM HbA1c &lt;6.5%</th>
<th>Group D: HbA1c ≥6.5% AND DM on OGTT</th>
<th>Group E: HbA1c ≥6.5% AND DM on OGTT</th>
<th>p</th>
<th>p</th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%)</td>
<td>291 (3.3%)</td>
<td>502 (5.8%)</td>
<td>93 (1.1%)</td>
<td>304 (3.5%)</td>
<td>198 (2.3%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>Overall</td>
<td>59.9 (9.3)</td>
<td>58.7 (9.3)</td>
<td>63.9 (8.6)</td>
<td>59.1 (9.5)</td>
<td>63.7 (9.1)</td>
<td>0.248</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>WE</td>
<td>62.5 (8.8)</td>
<td>61.6 (8.9)</td>
<td>65.3 (7.4)</td>
<td>62.3 (8.6)</td>
<td>60.8 (9.1)</td>
<td>0.843</td>
<td>0.001</td>
<td>0.011</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>54.9 (8.5)</td>
<td>54.8 (8.6)</td>
<td>59.0 (10.6)</td>
<td>55.3 (9.1)</td>
<td>53.9 (7.6)</td>
<td>0.689</td>
<td>0.110</td>
<td>0.166</td>
<td>0.061</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>166 (57.0)</td>
<td>285 (56.8)</td>
<td>53 (57.0)</td>
<td>172 (56.6)</td>
<td>113 (57.1)</td>
<td>0.909</td>
<td>0.969</td>
<td>0.944</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>126 (43.0)</td>
<td>217 (43.2)</td>
<td>40 (43.0)</td>
<td>132 (43.4)</td>
<td>85 (42.9)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>WE</td>
<td>182 (63.6)</td>
<td>252 (52.4)</td>
<td>69 (75.8)</td>
<td>139 (48.6)</td>
<td>113 (57.9)</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>95 (33.2)</td>
<td>207 (43.0)</td>
<td>20 (22.0)</td>
<td>132 (46.2)</td>
<td>75 (38.5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>9 (3.1)</td>
<td>22 (4.6)</td>
<td>2 (2.2)</td>
<td>15 (5.2)</td>
<td>7 (3.6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>Overall</td>
<td>103.2 (13.3)</td>
<td>102.1 (13.8)</td>
<td>101.3 (13.3)</td>
<td>100.7 (14.1)</td>
<td>104.1 (13.2)</td>
<td>0.025</td>
<td>0.626</td>
<td>0.680</td>
<td>0.097</td>
</tr>
<tr>
<td></td>
<td>WE</td>
<td>261 (90.3)</td>
<td>423 (87.9)</td>
<td>83 (89.2)</td>
<td>245 (86.0)</td>
<td>178 (90.8)</td>
<td>0.107</td>
<td>0.721</td>
<td>0.417</td>
<td>0.674</td>
</tr>
<tr>
<td>Central Obesity</td>
<td>by Waist Circumference</td>
<td>0.943 (0.08)</td>
<td>0.934 (0.08)</td>
<td>0.945 (0.08)</td>
<td>0.928 (0.09)</td>
<td>0.942 (0.08)</td>
<td>0.025</td>
<td>0.224</td>
<td>0.081</td>
<td>0.809</td>
</tr>
<tr>
<td></td>
<td>Central Obesity by WHR</td>
<td>246 (85.4)</td>
<td>389 (81.0)</td>
<td>79 (84.9)</td>
<td>222 (77.9)</td>
<td>167 (85.6)</td>
<td>0.020</td>
<td>0.373</td>
<td>0.143</td>
<td>0.876</td>
</tr>
<tr>
<td></td>
<td>BMI (kg/m²)</td>
<td>30.8 (5.4)</td>
<td>30.6 (5.8)</td>
<td>29.9 (4.6)</td>
<td>30.1 (5.9)</td>
<td>31.2 (5.7)</td>
<td>0.134</td>
<td>0.239</td>
<td>0.752</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Obesity By BMI</td>
<td>168 (58.7)</td>
<td>280 (58.5)</td>
<td>46 (50.5)</td>
<td>158 (55.6)</td>
<td>122 (62.6)</td>
<td>0.453</td>
<td>0.162</td>
<td>0.397</td>
<td>0.055</td>
</tr>
</tbody>
</table>

Key: BMI = Body Mass Index, BP = Blood Pressure, DM = Newly diagnosed Type 2 Diabetes Mellitus, OGTT = Oral Glucose Tolerance Test, SA = South Asians, SD = Standard Deviation, WE = White Europeans, WHR = Waist Hip Ratio. Continuous Variable expressed as mean (SD); discrete variables as number (%). Groups that are not independent are not compared statistically. Central Obesity defined by (i) Waist Circumference ≥ 80cm in females and ≥ 90cm in SA and Chinese males/ ≥ 94cm all other males, (ii) Waist: Hip Ratio ≥ 0.85 females and ≥ 0.9 males (iii) total body obesity by BMI ≥ 30.0 (SA ≥ 27.5).
Table 3.3 Analysis of modifiable risk factors according to OGTT and HbA1c status

<table>
<thead>
<tr>
<th></th>
<th>Group A: DM on OGTT (n=291)</th>
<th>Group B: HbA1c≥6.5% (n=502)</th>
<th>Group C: DM on OGTT, HbA1c&lt;6.5% (n=93)</th>
<th>Group D: No DM on OGTT AND DM on OGTT (n=304)</th>
<th>Group E: HbA1c≥6.5% AND DM on OGTT (n=198)</th>
<th>p</th>
<th>p</th>
<th>p</th>
<th>p</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>7.9 (2.8)</td>
<td>6.9 (2.4)</td>
<td>6.5 (1.0)</td>
<td>5.8 (0.7)</td>
<td>8.6 (3.1)</td>
<td>&lt;0.0001</td>
<td>0.009</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2-hour plasma glucose (mmol/l)</td>
<td>13.9 (4.4)</td>
<td>10.6 (5.0)</td>
<td>11.9 (2.5)</td>
<td>7.73 (2.4)</td>
<td>14.9 (4.8)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>148.1 (20.0)</td>
<td>142.1 (19.8)</td>
<td>150.4 (21.5)</td>
<td>138.9 (19.6)</td>
<td>147.0 (19.2)</td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>0.200</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>87.3 (11.8)</td>
<td>85.6 (11.2)</td>
<td>87.1 (12.3)</td>
<td>84.5 (10.8)</td>
<td>87.3 (11.6)</td>
<td>0.004</td>
<td>0.283</td>
<td>0.070</td>
<td>0.881</td>
<td>0.008</td>
</tr>
<tr>
<td>Previous Hypertension Diagnosis</td>
<td>141 (48.8)</td>
<td>225 (46.6)</td>
<td>51 (55.4)</td>
<td>135 (47.2)</td>
<td>90 (45.7)</td>
<td>0.703</td>
<td>0.119</td>
<td>0.170</td>
<td>0.122</td>
<td>0.742</td>
</tr>
<tr>
<td>SBP&gt;140 or DBP&gt;85 mmHg</td>
<td>208 (74.6)</td>
<td>320 (67.9)</td>
<td>64 (69.6)</td>
<td>176 (62.0)</td>
<td>144 (77.0)</td>
<td>0.011</td>
<td>0.760</td>
<td>0.188</td>
<td>0.180</td>
<td>0.001</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/l)</td>
<td>5.50 (1.2)</td>
<td>5.38 (1.2)</td>
<td>5.57 (1.2)</td>
<td>5.33 (1.2)</td>
<td>5.46 (1.2)</td>
<td>0.084</td>
<td>0.154</td>
<td>0.083</td>
<td>0.457</td>
<td>0.225</td>
</tr>
<tr>
<td>Total Cholesterol &gt; 5.0mmol/l</td>
<td>195 (65.4)</td>
<td>307 (61.2)</td>
<td>66 (71.7)</td>
<td>178 (58.6)</td>
<td>129 (65.5)</td>
<td>0.025</td>
<td>0.056</td>
<td>0.023</td>
<td>0.290</td>
<td>0.120</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.39 (1.0)</td>
<td>3.38 (0.99)</td>
<td>3.45 (1.0)</td>
<td>3.39 (0.99)</td>
<td>3.36 (1.0)</td>
<td>0.997</td>
<td>0.524</td>
<td>0.603</td>
<td>0.479</td>
<td>0.753</td>
</tr>
<tr>
<td>HDL – Cholesterol (mmol/l)</td>
<td>1.20 (0.33)</td>
<td>1.18 (0.30)</td>
<td>1.28 (0.37)</td>
<td>1.19 (0.30)</td>
<td>1.17 (0.31)</td>
<td>0.792</td>
<td>0.025</td>
<td>0.055</td>
<td>0.014</td>
<td>0.315</td>
</tr>
<tr>
<td>Mean Triglycerides (mmol/l)</td>
<td>2.15 (1.57)</td>
<td>1.89 (1.23)</td>
<td>1.97 (1.45)</td>
<td>1.66 (0.82)</td>
<td>2.24 (1.62)</td>
<td>&lt;0.0001</td>
<td>0.613</td>
<td>0.055</td>
<td>0.163</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>50 (17.4)</td>
<td>69 (13.9)</td>
<td>15 (16.4)</td>
<td>34 (11.3)</td>
<td>35 (17.9)</td>
<td>0.034</td>
<td>0.524</td>
<td>0.194</td>
<td>0.761</td>
<td>0.038</td>
</tr>
<tr>
<td>Mean Framingham 10-year CVD risk</td>
<td>0.22 (0.16)</td>
<td>0.19 (0.15)</td>
<td>0.25 (0.16)</td>
<td>0.19 (0.14)</td>
<td>0.20 (0.15)</td>
<td>0.068</td>
<td>0.009</td>
<td>0.007</td>
<td>0.038</td>
<td>0.523</td>
</tr>
<tr>
<td>Framingham 10-year CVD risk &gt;20%</td>
<td>106 (41.1)</td>
<td>167 (38.2)</td>
<td>45 (52.3)</td>
<td>106 (40.0)</td>
<td>61 (35.5)</td>
<td>0.8</td>
<td>0.015</td>
<td>0.045</td>
<td>0.009</td>
<td>0.341</td>
</tr>
</tbody>
</table>

Continuous variables expressed as mean (SD); discrete variables as number (%). Key: BP = Blood Pressure, DM = Type 2 Diabetes Mellitus, n = number, OGTT = Oral Glucose Tolerance Test, SD= Standard Deviation. Statistical tests based on unpaired t-tests for means and chi-squared for numbers.
Table 3.4. Comparison of diagnostic indices derived from using HbA1c for T2DM at the cut-point of ≥ 6.5% and the optimal cut-point

<table>
<thead>
<tr>
<th>HbA1c cut-point</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 6.1% *</td>
<td>83.0 (76.8-87.7)</td>
<td>87.8 (87.0-88.6)</td>
<td>16.8 (14.3-19.2)</td>
<td>99.4 (99.2-99.6)</td>
</tr>
<tr>
<td>≥ 6.5%</td>
<td>62.1 (54.8-68.8)</td>
<td>97.7 (97.3-98.1)</td>
<td>44.8 (38.7-51.0)</td>
<td>98.9 (98.6-99.1)</td>
</tr>
<tr>
<td>SA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 6.3% *</td>
<td>85.3 (76.6-91.1)</td>
<td>86.5 (84.9-88.0)</td>
<td>24.5 (19.9-29.2)</td>
<td>99.1 (98.7-99.6)</td>
</tr>
<tr>
<td>≥ 6.5%</td>
<td>78.9 (69.6-86.0)</td>
<td>92.8 (91.6-93.9)</td>
<td>36.2 (29.7-42.8)</td>
<td>98.8 (98.3-99.3)</td>
</tr>
</tbody>
</table>

Data expressed as % (95% confidence intervals). Key: NPV = Negative Predictive Value, Positive predictive value, SA = south Asian, WE = white European. * = optimal HbA1c cut-point for T2DM.

Figure 3.4 Optimal HbA1c cut-points for T2DM

Key: SA = South Asian, WE = White European. Sensitivity is represented by colourless boxes, specificity by the black triangles; the optimal cut-point is where the two lines cross.
3.5.4 Results for IGR

From use of an OGTT, there were 1,407 (16.2%) people detected with IGR (66.8% isolated IGT, 17.4% isolated IFG and 15.8% combined IGT/IFG) of whom 61 people had a previous diagnosis of IGR. Using IEC criteria of HbA1c 6.0 to 6.4%, there were more people identified, n=1,610 (18.5%), Table 3.5. This translated into a 1.1 fold increase in prevalence of people classified as having ‘IGR’ (1.1 and 1.5 fold in white Europeans and south Asians respectively, p<0.0001). However, there were only 477 (5.8%) people with both IGR detected on OGTT and HbA1c 6.0 to 6.4%, showing a degree of discordance between the two tests, Table 3.6 and Figure 3.5.

Using ADA recommendations of HbA1c 5.7 to 6.4%, many more people were detected, n=3904 (44.9%), leading to a 2.8 fold increase in numbers (2.8 and 3.0 fold increase in white Europeans and south Asians respectively, p<0.0001). Furthermore, using of ADA recommended criteria; there were 873 (10.7%) people with both IGR on OGTT and HbA1c 5.7 to 6.4%, Table 3.6 and Figure 3.6.

There were 1133 (13.0%) additional people detected using IEC criteria (i.e. individuals without IGR on OGTT but HbA1c 6.0 to 6.4%, group 3). In contrast, if ADA recommended criteria was used, there were additional people identified (i.e. individuals without IGR but HbA1c 5.7 to 6.4%), n=3031 (34.9%), group 7.

Using the IEC criteria for diagnosis, there were 758 (9.3%) people no longer classified as having IGR (i.e. those with IGR on OGTT but HbA1c <6.0%), group 2. Using ADA Criteria there were 362 (4.4%) people no longer classified as having IGR (i.e. individuals with IGR on OGTT but HbA1c < 5.7%).
When IGR detected on OGTT was separated into isolated IFG (n=245), isolated IGT (n=940) and combined IFG/IGT (n=222), the latter had the least people with HbA1c <6.0% (8.1%) but the most people with either HbA1c 6.0 to 6.4% (41.4%) or HbA1c ≥ 6.5% (27.9%), p<0.0001, Table 3.7. In contrast, individuals with isolated IGT had the most people with HbA1c <6.0% (61.5%), but the least with HbA1c 6.0 to 6.4% (30.6%) or HbA1c ≥ 6.5% (7.9%), p<0.0001. Individuals with isolated IGT also had the most people with HbA1c< 5.7% (32.2%) and the least with HbA1c 6.0 to 6.4% (59.9%), p<0.0001.

3.5.5 Anthropometrics and clinical characteristics

Comparing (i) additional people detected from use of IEC criteria to (ii) those with IGR on OGTT but HbA1c< 6.0% (groups 3 and 2 respectively) revealed the former group had (a) lower mean waist circumference and BMI (both p<0.0001), (b) less people obese from use of waist circumference (p<0.0001), (c) lower mean systolic and diastolic blood pressure (both p<0.0001). However, lipids including LDL-cholesterol, HDL-cholesterol and triglycerides were not significantly different; similarly the mean Framingham 10 year cardiovascular disease risk was not significantly different, Table 3.6.

Secondly, comparing (i) additional people detected with ADA criteria to (ii) those with IGR on OGTT but HbA1c <5.7% (group 7 and group 6 respectively), the former had higher obesity from use of waist circumference and BMI (both p<0.0001), lower mean systolic (p<0.0001) and diastolic blood pressure (p=0.004) and less people with microalbuminuria (p=0.02). However these additional people had higher mean LDL-cholesterol (p=0.03);
overall the mean Framingham 10 year CVD risk was not significant between these groups.

Individuals with both IGR on OGTT and HbA1c 6.0-6.4% (group 4) had a relatively large number of significantly adverse cardiovascular risk factors compared to groups 1 to 3 individually. These included higher mean waist circumference, waist: hip ratio, BMI, triglycerides and lower mean HDL. Furthermore, these people in group 4 had higher mean Framingham 10 year CVD risk compared to groups 1 to 3 individually.

3.5.6 Determination of optimal HbA1c cut-points for detecting IGR on OGTT

Using ROC curve analysis, HbA1c had an AUROC for detecting IGR of 0.69 (95% CI 0.68 to 0.71); however in white Europeans and south Asians these were 0.69 (0.67 to 0.71) and 0.72 (0.69 to 0.75) respectively. Regarding detection of just IGT, the HbA1c AUROC in white Europeans and south Asians was 0.67 (0.64 to 0.69) and 0.70 (0.66 to 0.73).

Within white Europeans the sensitivity and specificity from using HbA1c ≥ 6.0% detecting IGR was 39.5% (36.3 to 42.7) and 83.5% (82.5 to 84.5) respectively. The optimal HbA1c cut-point in this ethnic group was HbA1c ≥ 5.8%, producing sensitivity and specificity of 61.5% (58.2 to 64.4) and 67.9% (66.6 to 69.1) respectively (figure 3.7). In contrast, the optimal HbA1c cut-point within south Asians was ≥ 6.0%, sensitivity and specificity, 63.8% (58.6 to 68.7) and 69.4% (67.1 to 71.6) respectively. Using HbA1c ≥ 5.7%, the sensitivity and specificity in white Europeans were 70.5% (67.4 to 73.4%) and 57.9%
(56.6 to 59.2); in south Asians these were 85.6% (81.4 to 88.9) and 41.3 (38.9 to 43.7) respectively. When ROC curve analysis was stratified by age, white Europeans aged 40 to 59 and 60 to 75 years produced different optimal HbA1c cut-points of 5.7% and 5.9% respectively, 3.8. However the same analysis in south Asians produced similar optimal HbA1c cut-points of 6.0% in each age group respectively.

3.5.7 Sub-analysis using ADA definition of IFG

Use of ADA defined IFG criteria (5.6 to 6.9mmol/l) detected more people, n=1685 (19.4%), compared to using HbA1c 6.0 to 6.4%, n=1610 (18.5%), but less than using HbA1c 5.7 to 6.4%, n=3904 (44.9%). There were 575 (6.6%) people with both HbA1c 6.0 to 6.4% and ADA IFG; 862 (9.9%) participants with ADA defined IFG had HbA1c < 6.0%, while 1035 (11.9%) had HbA1c 6.0 to 6.4% without ADA defined IFG. Furthermore, there were 1033 (11.8%) individuals with both HbA1c 5.7 to 6.4% and ADA defined IFG; 404 (4.7%) people with ADA defined IFG had HbA1c < 5.7%, while 2871 (33.0%) had HbA1c 5.7 to 6.4% without ADA defined IFG.
Table 3.5. Baseline demographics of people with IGR on OGTT and HbA1c 6.0 to 6.4% or 5.7 to 6.4%

<table>
<thead>
<tr>
<th></th>
<th>Total cohort</th>
<th>T2DM</th>
<th>Total IGR on OGTT</th>
<th>HbA1c 6.0-6.4%</th>
<th>HbA1c 5.7-6.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence n, % (95% CI)</td>
<td>8696</td>
<td>291</td>
<td>1407</td>
<td>1610</td>
<td>3904</td>
</tr>
<tr>
<td>Screening OGTT (n)</td>
<td>8696</td>
<td>418</td>
<td>1298</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Confirmatory OGTT (n)</td>
<td>357 (4.1%)</td>
<td>228  (2.6%)</td>
<td>109 (1.3%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>57.3 (9.7)</td>
<td>59.9 (9.3)</td>
<td>60.1 (9.4)</td>
<td>59.6 (9.2)</td>
<td>59.0 (9.4)</td>
</tr>
<tr>
<td>% female</td>
<td>52.3</td>
<td>43.0</td>
<td>50.4</td>
<td>51.6</td>
<td>52.6</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% WE</td>
<td>74.7</td>
<td>63.6</td>
<td>70.1</td>
<td>64.2</td>
<td>70.3</td>
</tr>
<tr>
<td>% SA</td>
<td>22.8</td>
<td>33.2</td>
<td>27.2</td>
<td>32.2</td>
<td>27.0</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.71 (0.61)</td>
<td>7.30  (1.76)</td>
<td>5.94 (0.46)</td>
<td>6.14 (0.13)</td>
<td>5.93 (0.20)</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as mean (standard deviation) and discrete variables as number or percentage. Key: IGR = Impaired glucose regulation (defined as prediabetes on WHO 1999 criteria), OGTT = oral glucose tolerance test, SA = south Asian, T2DM = Type 2 Diabetes Mellitus, WE = white European.

Figure 3.5 The prevalence of IGR on OGTT and according to HbA1c Criteria of 6.0 to 6.4%
Figure 3.6 The prevalence of IGR on OGTT and according to HbA1c Criteria of 5.7 to 6.4%
Table 3.6 (a) Selected population & CVD characteristics using IEC criteria

<table>
<thead>
<tr>
<th>Group number</th>
<th>HbA1c &lt; 6.0% (n=5826)</th>
<th>HbA1c 6.0-6.4% (n=758)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence %, 95% CI</td>
<td>71.1, 70.1-72.1</td>
<td>9.3, 8.6-9.9</td>
<td>13.8, 13.1-14.6</td>
<td>5.8, 5.3-6.3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.1 (9.7)</td>
<td>60.0 (9.5)*</td>
<td>59.2 (9.1)*</td>
<td>60.6 (9.2) †*</td>
</tr>
<tr>
<td>Female</td>
<td>3108 (53.4)</td>
<td>392 (51.7)</td>
<td>583 (51.5)</td>
<td>247 (51.8)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>WE 4566 (78.8)</td>
<td>531 (79.1)</td>
<td>721 (64.1)* †</td>
<td>275 (64.6)* †</td>
</tr>
<tr>
<td></td>
<td>SA 1111 (19.2)</td>
<td>123 (18.3)</td>
<td>360 (32.0)</td>
<td>139 (32.6)</td>
</tr>
<tr>
<td>Waist Circumference cm</td>
<td>92.7 (12.7)</td>
<td>97.1 (12.4)*</td>
<td>95.9 (12.8) †</td>
<td>99.7 (11.8)* † ‡</td>
</tr>
<tr>
<td>Central Obesity- WC</td>
<td>4104 (71.2)</td>
<td>562 (84.0)*</td>
<td>901 (80.1)* †</td>
<td>376 (88.3)* † ‡</td>
</tr>
<tr>
<td>Waist: Hip Ratio</td>
<td>0.887 (0.08)</td>
<td>0.910 (0.09)*</td>
<td>0.907 (0.09)*</td>
<td>0.921 (0.08)* †‡</td>
</tr>
<tr>
<td>Central Obesity- WHR</td>
<td>3337 (57.9)</td>
<td>463 (69.1)*</td>
<td>761 (67.7)*</td>
<td>331 (77.9)* †‡</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5 (4.7)</td>
<td>29.0 (5.0)*</td>
<td>28.5 (5.2)* †</td>
<td>30.1 (5.0)* †‡</td>
</tr>
<tr>
<td>Obesity By BMI n (%)</td>
<td>1625 (28.1)</td>
<td>271 (40.7)*</td>
<td>444 (40.0)*</td>
<td>233 (54.7)* †‡</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>4.97 (0.43)</td>
<td>5.49 (0.64)*</td>
<td>5.26 (0.63)* †</td>
<td>5.89 (0.68)* †‡</td>
</tr>
<tr>
<td>2 hour PG (mmol/l)</td>
<td>5.1 (1.37)</td>
<td>8.56 (1.57)*</td>
<td>5.86 (2.02)* †</td>
<td>8.73 (1.80)* †‡</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.47 (0.32)</td>
<td>5.61 (0.27)*</td>
<td>6.12 (0.13)* †</td>
<td>6.12 (0.14)* †‡</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135.5 (19.6)</td>
<td>142.5 (20.2)*</td>
<td>137.7 (19.8)* †</td>
<td>142.4 (20.5)* †‡</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83.7 (10.9)</td>
<td>85.9 (10.8)*</td>
<td>83.7 (10.9)* †</td>
<td>84.9 (11.2)*</td>
</tr>
<tr>
<td>SBP&gt;140 or DBP&gt;85</td>
<td>3095 (53.8)</td>
<td>444 (67.3)*</td>
<td>625 (56.3)* †</td>
<td>283 (67.4)* †‡</td>
</tr>
<tr>
<td>Total cholesterol mmol/l</td>
<td>5.52 (1.04)</td>
<td>5.56 (1.10)</td>
<td>5.58 (1.10)</td>
<td>5.60 (1.05)* †‡</td>
</tr>
<tr>
<td>TC &gt;5.0</td>
<td>4020 (69.4%)</td>
<td>527 (70.5%)</td>
<td>791 (70.0%)</td>
<td>309 (64.9%)* †‡</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.52 (0.89)</td>
<td>3.51 (0.93)</td>
<td>3.57 (0.94)</td>
<td>3.39 (0.93)* †‡</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.39 (0.38)</td>
<td>1.35 (0.39)*</td>
<td>1.35 (0.54)*</td>
<td>1.25 (0.34)* †‡</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.37 (0.87)</td>
<td>1.60 (0.84)*</td>
<td>1.52 (0.91)*</td>
<td>1.70 (0.98)* †‡</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>338 (5.9%)</td>
<td>74 (9.9%)*</td>
<td>90 (8.1%)*</td>
<td>59 (12.5%)* †‡</td>
</tr>
<tr>
<td>FH DM</td>
<td>1204 (20.7%)</td>
<td>159 (21.0%)</td>
<td>227 (20.0%)</td>
<td>110 (23.1%)</td>
</tr>
<tr>
<td>Mean Framingham Risk</td>
<td>13.1 (10.3)</td>
<td>16.8 (11.7)*</td>
<td>17.0 (12.8)*</td>
<td>19.2 (13.8)* †‡</td>
</tr>
<tr>
<td>10 year CVD risk≥20%</td>
<td>1075 (19.5%)</td>
<td>191 (30.9%)*</td>
<td>319 (30.6%)*</td>
<td>146 (37.0%)* †‡</td>
</tr>
</tbody>
</table>

*significant compared to group 1 for groups 2-4, † significant compared to group 2 for groups 3-4, ‡ significant compared to group 3 for group 4. Continuous variables expressed as mean (standard deviation); discrete variables as % (n). Statistical tests are based on unpaired t-tests for means and chi-squared for numbers. Central Obesity defined by (i) Waist Circumference ≥ 80cm in females and ≥ 90cm in SA and Chinese males/ ≥ 94cm all other males, (ii) Waist: Hip Ratio ≥ 0.85 females and ≥ 0.9 males (iii) total body obesity by BMI ≥ 30.0 (SA ≥ 27.5). 10 year cardiovascular risk is calculated using the Framingham model. Key: ADA = American Diabetes Association, BMI = Body Mass Index, BP = Blood Pressure, DM = Diabetes Mellitus, FH = Family History in a 1st degree relative, IGR = Impaired glucose regulation (defined as prediabetes using WHO 1999 criteria on Oral Glucose Tolerance Test), IEC = International Expert Committee, SA = South Asians, SD = Standard Deviation, WC= waist circumference, WHR = Waist: hip ratio, WE = White Europeans, WHR = Waist Hip Ratio.
Table 3.6 (b) Selected population & CVD characteristics using ADA criteria

<table>
<thead>
<tr>
<th>Group number</th>
<th>HbA1c &lt; 5.7%</th>
<th>HbA1c 5.7-6.4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>5) No IGR</td>
<td>6) IGR</td>
<td>7) No IGR</td>
</tr>
<tr>
<td>(n=3928)</td>
<td>(n=362)</td>
<td>(n=3031)</td>
</tr>
<tr>
<td>Prevalence %, 95% CI</td>
<td>47.9, 46.9-49.0</td>
<td>4.4, 4.0-4.9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.2 (9.7)</td>
<td>59.1 (9.4) *</td>
</tr>
<tr>
<td>Female</td>
<td>2094 (53.3)</td>
<td>184 (50.8)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>WE</td>
<td>SA</td>
</tr>
<tr>
<td></td>
<td>3168 (81.1)</td>
<td>660 (16.9)</td>
</tr>
<tr>
<td>Waist Circumference cm</td>
<td>92.3 (12.8)</td>
<td>95.4 (11.3) *</td>
</tr>
<tr>
<td>Central Obesity- WC</td>
<td>2695 (69.5)</td>
<td>258 (81.9)*</td>
</tr>
<tr>
<td>Waist: Hip Ratio</td>
<td>0.885 (0.08)</td>
<td>0.902 (0.08)*</td>
</tr>
<tr>
<td>Central Obesity- WHR</td>
<td>2214 (57.1)</td>
<td>211 (66.8)*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 (4.7)</td>
<td>28.5 (4.7)*</td>
</tr>
<tr>
<td>Obesity By BMI n (%)</td>
<td>1018 (26.1)</td>
<td>112 (35.8)*</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>4.93 (0.4)</td>
<td>5.37 (0.62) *</td>
</tr>
<tr>
<td>2 hour PG (mmol/l)</td>
<td>5.05 (1.3)</td>
<td>8.60 (1.47)*</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.33 (0.28)</td>
<td>5.39 (0.22) *</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>134.4 (19.4)</td>
<td>142.3 (20.4)*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>83.6 (10.9)</td>
<td>85.66 (10.8)*</td>
</tr>
<tr>
<td>SBP&gt;140 or DBP&gt;85</td>
<td>2061 (53.3)</td>
<td>206 (65.8)*</td>
</tr>
<tr>
<td>Total cholesterol mmol/l</td>
<td>5.48 (1.01)</td>
<td>5.47 (1.06)</td>
</tr>
<tr>
<td>TC &gt;5.0</td>
<td>2705 (69.3)</td>
<td>247 (68.9)</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.48 (0.88)</td>
<td>3.41 (0.92) *</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.40 (0.39)</td>
<td>1.37 (0.40)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.35 (0.88)</td>
<td>1.53 (0.83)*</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>220 (5.7)</td>
<td>37 (10.3)*</td>
</tr>
<tr>
<td>FH DM</td>
<td>797 (20.3)</td>
<td>76 (21.0)</td>
</tr>
<tr>
<td>Mean Framingham Risk</td>
<td>12.2 (9.78)</td>
<td>16.0 (11.3)*</td>
</tr>
<tr>
<td>10 year CVD risk≥20%</td>
<td>632 (17.0)</td>
<td>85 (28.4)*</td>
</tr>
</tbody>
</table>

*significant compared to group 5 for groups 6-8, † significant compared to group 6 for groups 7-8, ‡ significant compared to group 7 for group 8. Continuous variables expressed as mean (standard deviation); discrete variables as % (n). Statistical tests are based on unpaired t-tests for means and chi-squared for numbers. Central Obesity defined by (i) Waist Circumference ≥ 80cm in females and ≥ 90cm in SA and Chinese males/ ≥ 94cm all other males, (ii) Waist: Hip Ratio ≥ 0.85 females and ≥ 0.9 males (iii) total body obesity by BMI ≥ 30.0 (SA≥ 27.5). 10 year cardiovascular risk is calculated using the Framingham model. Key: ADA = American Diabetes Association, BMI = Body Mass Index, BP = Blood Pressure, DM = Diabetes Mellitus, FH = Family History in a 1st degree relative, IGR = Impaired glucose regulation (defined as prediabetes using WHO 1999 criteria on Oral Glucose Tolerance Test), IEC = International Expert Committee, SA = South Asians, SD = Standard Deviation, WC= waist circumference, WHR = Waist: hip ratio, WE = White Europeans, WHR = Waist Hip Ratio.
Table 3.7. Distribution of Impaired glucose regulation subtypes according to HbA1c cut-points from the IEC and ADA

<table>
<thead>
<tr>
<th>HbA1c (%)</th>
<th>Isolated IFG (n=245)</th>
<th>Isolated IGT (n=940)</th>
<th>Combined IFG‡ and IGT (n=222)</th>
<th>ADA defined IFG (n=1685)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6.0</td>
<td>112 (45.7%)</td>
<td>578 (61.5%)</td>
<td>68 (30.6)</td>
<td>862 (51.2%)</td>
</tr>
<tr>
<td>6.0-6.4 †</td>
<td>97 (39.6%)</td>
<td>288 (30.6%)</td>
<td>92 (41.4%)</td>
<td>575 (34.1%)</td>
</tr>
<tr>
<td>&lt; 5.7</td>
<td>41 (16.7%)</td>
<td>303 (32.2%)</td>
<td>18 (8.1%)</td>
<td>404 (24.0%)</td>
</tr>
<tr>
<td>5.7-6.4 *</td>
<td>168 (68.6%)</td>
<td>563 (59.9%)</td>
<td>142 (64.0%)</td>
<td>1033 (61.3%)</td>
</tr>
<tr>
<td>≥ 6.5</td>
<td>36 (14.7%)</td>
<td>74 (7.9%)</td>
<td>62 (27.9%)</td>
<td>248 (14.8%)</td>
</tr>
</tbody>
</table>

Percentages refer to each column. IEC and ADA criteria individually produced significant differences, chi-squared p<0.0001; HbA1c <5.7% vs. <6.0% was also significantly different (p=0.011), excluding ADA defined IFG as not mutually exclusive. ‡ = WHO definition of IFG (6.1-6.9mmol/l), # = ADA definition of IFG (5.6-6.9mmol/l), * = IEC recommendations for IGR, † = ADA recommendations for IGR. Key: ADA = American Diabetes Association, International Expert Committee (IEC); IGT = impaired glucose tolerance, IFG = impaired fasting glycaemia.
Figure 3.7 Line graphs to show the relationship between HbA1c level and sensitivity and specificity for detecting Impaired glucose regulation (IGR, defined as prediabetes detected using WHO 1999 criteria) in (a) white Europeans and (b) south Asians.

(a) white Europeans
The dotted line represents the optimal balance/cut-point between sensitivity and specificity (HbA1c ≥ 5.8% for white Europeans and ≥ 6.0% for south Asians).

(b) south Asians
Table 3.8 The optimal HbA1c cut-points derived from using ROC curve analysis for detecting impaired glucose regulation (defined as prediabetes based on WHO 1999 criteria)

<table>
<thead>
<tr>
<th>Ethnic Group:</th>
<th>Stratification variable</th>
<th>Optimal HbA1c cut-point (%)</th>
<th>Sensitivity % (CI)</th>
<th>Specificity % (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>European</td>
<td>40-59</td>
<td>5.7</td>
<td>63.1 (57.7-68.2)</td>
<td>66.9 (65.2-68.6)</td>
</tr>
<tr>
<td></td>
<td>60-75</td>
<td>5.9</td>
<td>53.9 (49.7-58.0)</td>
<td>69.6 (67.7-71.3)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>5.8</td>
<td>60.7 (55.9-65.2)</td>
<td>67.6 (65.8-69.4)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>5.8</td>
<td>62.2 (57.7-66.5)</td>
<td>68.1 (66.4-69.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>≥30.0</td>
<td>5.9</td>
<td>60.6 (55.7-65.3)</td>
<td>69.2 (66.8-71.0)</td>
</tr>
<tr>
<td></td>
<td>≥25.0</td>
<td>5.8</td>
<td>63.6 (60.1-67.0)</td>
<td>66.1 (64.6-67.5)</td>
</tr>
<tr>
<td></td>
<td>&lt;25.0</td>
<td>5.7</td>
<td>59.7 (50.9-67.9)</td>
<td>63.8 (61.4-66.2)</td>
</tr>
<tr>
<td>Glucose</td>
<td>IGT</td>
<td>5.8</td>
<td>58.6 (55.0-62.2)</td>
<td>66.7 (65.4-67.9)</td>
</tr>
<tr>
<td>abnormality</td>
<td>IFG</td>
<td>5.9</td>
<td>67.0 (61.4-72.2)</td>
<td>75.0 (73.9-76.1)</td>
</tr>
<tr>
<td>South Asian</td>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40-59</td>
<td>6.0</td>
<td>60.9 (54.3-67.1)</td>
<td>71.2 (68.6-73.6)</td>
</tr>
<tr>
<td></td>
<td>60-75</td>
<td>6.0</td>
<td>69.2 (60.4-76.7)</td>
<td>62.9 (57.6-67.9)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>6.0</td>
<td>65.4 (58.3-71.9)</td>
<td>67.3 (63.9-70.5)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6.0</td>
<td>61.9 (54.1-69.2)</td>
<td>71.4 (68.3-74.4)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>≥27.5</td>
<td>6.0</td>
<td>66.3 (59.2-72.7)</td>
<td>61.8 (58.0-65.6)</td>
</tr>
<tr>
<td></td>
<td>≥23.0</td>
<td>6.0</td>
<td>64.6 (59.2-69.6)</td>
<td>67.3 (64.8-69.8)</td>
</tr>
<tr>
<td></td>
<td>&lt;23.0</td>
<td>5.8</td>
<td>61.9 (40.8-79.2)</td>
<td>62.3 (56.5-67.8)</td>
</tr>
<tr>
<td>Glucose</td>
<td>IGT</td>
<td>6.0</td>
<td>61.9 (56.1-67.3)</td>
<td>68.0 (65.7-70.2)</td>
</tr>
<tr>
<td>abnormality</td>
<td>IFG</td>
<td>6.1</td>
<td>68.3 (59.5-76.0)</td>
<td>75.3 (73.3-77.3)</td>
</tr>
</tbody>
</table>

The optimal HbA1c cut-point was determined from the best balance between sensitivity and specificity, expressed as % (95% confidence intervals). BMI cut-points are ethnic specific for obesity. Key: BMI = body mass index, IFG = impaired fasting glucose, IGT = impaired glucose tolerance.
3.6 Discussion

3.6.1 T2DM in LEADER cohort

The first point raised from this predominantly at risk cohort is the increase in number of people detected with T2DM if a diagnostic criteria of HbA1c ≥ 6.5% is introduced. The prevalence of T2DM increased from 3.3% detected on OGTT with 1999 WHO criteria to 5.8% with HbA1c ≥ 6.5% criteria. This represented an approximate two fold increase in the prevalence of T2DM, with a 2.2 fold increase in south Asians but a 1.4 fold in white Europeans. These result contrasts with findings from chapter 2 which generally suggested use of HbA1c would decrease the prevalence of T2DM. The increase in prevalence is absolutely dependent on using the HbA1c ≥ 6.5% cut-point and would be different if an alternate HbA1c cut-point were recommended. A recent global multi-centre study observing the impact of using HbA1c ≥ 6.5% for detecting T2DM instead of an OGTT found four populations with a decrease in prevalence and two populations with an increase (Christensen et al., 2010). The latter two populations had a relatively higher mean HbA1c, similar to that within this cohort (5.71%, SD 0.61), which generally favours a greater proportion of the population with HbA1c above the cut-point of ≥ 6.5% (Figure 3.8 and 3.9). Furthermore, the cohort had a large proportion of non-white European people, who are reported to have higher HbA1c values for equivalent glycaemic control (Herman et al., 2007). HbA1c values are also known to
independently increase with age; the mean cohort age of 57.3 years (SD 9.3) suggested this could be another influence in some people (Pani et al., 2008).

A further key point explaining the results lies within this study design. Asymptomatic people with an OGTT within the diabetes range required a repeat confirmatory OGTT for T2DM diagnosis, as per preferable WHO guidelines (WHO, 1999). However, due to low reproducibility of the OGTT the final T2DM population was reduced. If I only chose to analyse the results of the initial screening OGTTs (excluding all confirmatory OGTTs), the 'T2DM' prevalence would be higher at 4.4% (n=416) which reduced the magnitude of observed differences in prevalence between OGTT and HbA1c tests. Furthermore, the additional people detected with HbA1c $\geq$ 6.5% but a non-diabetes OGTT is then reduced to 3.3% (n=285).

Within the cohort, I found that 30% of those with T2DM on OGTT had HbA1c < 6.5%. If only the screening OGTT were analysed, this value is higher at 48%. As the use of recommended HbA1c criteria misses a proportion of people with a positive OGTT, it remains uncertain whether HbA1c $\geq$ 6.5% is the correct cut-point for detecting T2DM. The fate of such individuals’ glycaemic status may depend on whether there HbA1c value is within the suggested high risk range of $\geq$ 6.0 to 6.4%. However, I found 10.3% of all positive OGTTs had HbA1c< 6.0% in the cohort, which was lower than some reported studies and can again be explained in part by the relatively high mean population HbA1c. This appears to be a potential limitation of using HbA1c $\geq$ 6.5% to detect T2DM.
A comparison of anthropometry and modifiable risk factors in those with T2DM detected on OGTT (group A) and additional people with HbA1c ≥ 6.5% (group D) revealed the latter were slimmer, had lower blood pressure and triglycerides. This suggests the additional people, the majority of whom had IGT/IFG on OGTT, are detected at a stage where cardiovascular risk factors may be less adverse, another advantage of using HbA1c. The counter argument is that these people are a lower risk group for CVD, therefore this could indicate inappropriate use of resources, as well as inappropriate labelling of someone as having T2DM.

It is not known whether glucose testing or HbA1C is the most accurate method for diagnosing T2DM as there is no long term follow-up study looking at specificity and sensitivity for the development of microvascular disease. This is also the only way to determine the best cut-points for both glucose and HbA1c. However such a study may be considered unethical to perform, unless lifestyle advice and basic pharmacological therapies were offered (e.g. Metformin). Therefore a true result may be hard to derive.
Figure 3.8. A theoretical model of the impact on the prevalence of HbA1c

T2DM with a relatively high mean cohort HbA1c

Population with a relatively high mean cohort HbA1c (e.g. 5.7%), which shifts more people across the 6.5% line.

Figure 3.9. A theoretical model of the impact on the prevalence of HbA1c

T2DM with a relatively low mean cohort HbA1c

Population with a relatively low mean cohort HbA1c (e.g. 5.3%), which shifts less people across the 6.5% line.
3.6.2 IGR in the LEADER cohort

The results of this population suggests using IEC and ADA criteria to detect people with ‘IGR’ increases prevalence by 1.1 and 2.8 fold compared to WHO Criteria respectively. Similar results were found using ADA defined IFG instead of WHO defined IGR. These observed increases are a direct function of using the recommended HbA1c cut-points and would be different if other HbA1c cut-points were used. Furthermore, the cohort favours towards a high risk population and alternate results may be found with different populations.

These results are in contrast to a recent study of NHANES 2005-2006 which found using HbA1c 6.0 to 6.4% decreased the prevalence of IGR to one-tenth of using an OGTT (Cowie et al., 2010). This study had a lower mean cohort HbA1c of 5.41% (SD 0.5) compared to LEADER 5.71% (SD 0.61), which shifts less people above the HbA1c 6.0% cut-point. (Cowie et al., 2010; personal communication C.C. Cowie).

Analysing the agreement between diagnostic tests, there is a relatively large degree of discordance between OGTT and HbA1c results with either set of cut-points. This suggests using either set of recommended HbA1c cut-points introduces a change in those people classified as having IGR. There are three key points resulting from this. Firstly, there is a sub-cohort who is no longer classified as having IGR with HbA1c values below <6.0% or <5.7%. Secondly, using either IEC or ADA criteria the additional people detected consisted of significantly more south Asians than white Europeans. This largely resulted as
south Asians had a higher mean HbA1c. Thirdly, the additional people detected had some less adverse CV risk factors compared to individuals who were no longer classified as having IGR using HbA1c. These included lower mean systolic/diastolic blood pressure for criteria, lower mean waist circumference and BMI using IEC criteria, and less people with microalbuminuria using ADA criteria. However individuals with IGR on OGTT and HbA1c 6.0 to 6.4% (or HbA1c 5.7 to 6.4%) appeared to have the most adverse CV profile/ phenotype and higher 10-year Framingham CVD risk overall.

The natural history of progression from IGT and/ or IFG to diabetes and CVD risk is well reported (Unwin et al., 2002), less is known with use of HbA1c. Furthermore, successful intervention trials reducing the risk of diabetes have been based primarily around those with IGT, therefore requiring the continuation of an OGTT to obtain a 2-hour plasma glucose value (Tuomilehto et al., 2002; Knowler et al., 2001). The data showed over 60% of people with isolated IGT had HbA1c < 6.0%, classifying them as low risk for diabetes using IEC criteria, and only 30.6% with ADA criteria. In contrast, those with combined IGT/ IFG, who are known to have the high progression rate to developing diabetes (Unwin et al., 2002), had over 25% with HbA1c ≥ 6.5% but another 30.6% with HbA1c < 6.0%. HbA1c had a suboptimal AUC (< 0.7) suggesting it is a weak tool for detecting IGR, particularly in white Europeans. Within white Europeans the optimal cut-point was HbA1c≥ 5.8%, suggesting using 5.8 to 6.4% for IGR which correctly identified 53.3% with IGR on OGTT. However using this less specific but more inclusive range increases the number of additional people detected (18.1% with
HbA1c 5.8-6.4% compared to 8.3% with HbA1c 6.0-6.4%). In contrast, within south Asians the optimal cut-point was HbA1c ≥ 6.0%, suggesting using the IEC recommended 6.0 to 6.4%, however this only identified 40.9% of people with IGR on OGTT. Therefore, the findings suggest a strategy utilising different HbA1c cut-points according to ethnic group, although this is a more complex approach. Furthermore, stratification of the data showed optimal HbA1c cut-points varied by BMI and glycaemic disorder and within white Europeans by age. This suggests another problem of using one HbA1c cut-point for all people.

3.6.3 Strengths and limitations of the LEADER study

A major advantage of the study was all participants underwent a full OGTT which was repeated if within the diabetes range, giving better prevalence data of T2DM. This would also allow detection of both IGT and IFG. The large sample size within the cohort increased the ability of the study to detect clinically important effects with only a small proportion missing data excluded, reducing the effects of bias. The multi-ethnic cohort has representation of black and minority ethnic groups, therefore the data can apply to much of northern Europe. The results of the data could apply to multi-ethnic, predominantly at risk areas of the UK; however due to many regional variations, including laboratory methods for HbA1c and ethnic minority prevalence, I cannot make this conclusion.
The study was limited as I did not have repeat HbA1c data to test for repeatability and laboratory error in those people with HbA1c ≥ 6.5%. Also, the HbA1c analyser was able to detect Hb S and C, therefore such values were not reported in the present study, but in theory no other Hb variants were excluded or people with iron deficiency anaemia. Haemoglobinopathies may increase or decrease HbA1c as they represent factors affecting HbA1c other than glycaemia. Iron and vitamin B12 deficiency, hyperbilirubinaemia, carbamylated haemoglobin, and splenectomy are other examples of conditions which can increase HbA1c. In contrast, administration of erythropoietin, chronic liver disease and hypertriglyceridaemia can decrease HbA1c (WHO, 2011). Regarding OGTT limitations, the plasma glucose samples were kept at 4 degrees Celsius for up to 2 hours which could have reduced glucose levels. Furthermore, only participants with a screening OGTT in the diabetes range went on to have a confirmatory OGTT, with the remaining population undergoing one OGTT. Repeat confirmatory OGTTs were generally performed within 3 to 4 days of the screening OGTT; however this could have been up to one week, which gave a limited time for people to introduce interventions to decrease glucose levels. On an individual basis there is likely to be much more variation of OGTT results compared with HbA1c. Finally, plasma glucose samples were taken from participants who self-reported they followed the recommended preparation advice.
3.7 Conclusion to Chapter 3

In summary, in this UK multi-ethnic cohort, use of HbA1c increased the prevalence of both T2DM and IGR in comparison to using an OGTT, with some changes in metabolic characteristics of people classified as having T2DM or IGR. This leads to questions on whether there are other ways of incorporating HbA1c in a diagnostic pathway particularly for T2DM. I will explore a potential method using two HbA1c cut-points in Chapter 4.
Chapter 4

Are there other ways to incorporate HbA1c into a diagnostic pathway for type 2 diabetes and impaired glucose regulation?

Diagnostic performance of using one or two HbA1c cut-point strategies
4.1 Chapter Overview

In Chapters 2 and 3, I have discussed some of the problems of using HbA1c for diagnosis of T2DM, especially regarding the discordance in the people detected as having T2DM compared to an OGTT. In Chapter 4, I investigate an alternate strategy of incorporating HbA1c for diagnosis of T2DM. This information is based on first author published paper (Mostafa et al., 2012a).

4.2 Introduction

There are recommendations to use HbA1c ≥ 6.5% (48mmol/mol) as a diagnostic tool to detect T2DM from various international organisations (WHO, 2011; International Expert Committee, 2009). This cut-point represents the approximate level above which prevalent retinopathy begins to increase, in a similar fashion to fasting plasma glucose ≥ 7.0mmol/l and two-hour plasma glucose ≥ 11.1mmol/l (WHO, 2011; International Expert Committee, 2009). However, there are concerns about using HbA1c for diagnosis or at the selected threshold (Mostafa et al., 2010; Kilpatrick et al., 2009). An HbA1c of ≥ 6.5% identifies a different population from those detected with T2DM by using a traditional oral glucose tolerance test (OGTT) or fasting plasma glucose (FPG) (Van 't Riet et al., 2010; Carson et al., 2010; Mostafa et al., 2010). The specificity of using HbA1c ≥ 6.5% to detect T2DM on an OGTT is generally high (> 88%) however the sensitivity varies between 17.0 to 72.8%, which may reflect different testing methods for
diagnosis, the age and ethnicity of populations studied, as well as HbA1c assays employed (Mostafa et al., 2011). Furthermore, the HbA1c cut-points above which prevalent retinopathy increases can vary from HbA1c ≥ 5.5% (37mmol/mol) to ≥ 6.5 to 6.9% (48 to 52mmol/mol) (Cheng et al., 2009; Sabanayagam et al., 2009; Colagiuri et al., 2010). Various studies have investigated or proposed using different HbA1c diagnostic thresholds for T2DM, including ≥ 6.0% (42mmol/mol) or ≥ 7.0% (53mmol/mol, (Davidson et al., 2010; Saudek et al., 2008; Zhou et al., 2009).

An alternative strategy has been suggested which uses two HbA1c cut-points to either exclude (‘rule out’) or diagnose (‘rule in’) the disease (Lu et al., 2010). The first threshold of HbA1c ≤ 5.5% would ‘rule out’ T2DM, while the second cut-point of ≥ 7.0% would ‘rule in’ T2DM, based on 2.5th and 97.5th percentiles for non-T2DM and T2DM populations respectively. It is suggested the remaining individuals (5.6 to 6.9%, 38 to 52mmol/mol) have ‘intermediate HbA1c’ and will consist of a mixture of people with either normal or abnormal glucose tolerance. Alternate definitions of intermediate HbA1c have been suggested by the American Association of Clinical Endocrinologists/ American College of Endocrinology (AACE/ACE) of 5.5 to 6.4% (37 to 46mmol/mol) and the Association of British Clinical Diabetologists (ABCD) of 5.8 to 7.2% (40 to 55mmol/mol) (AACE/ ACE position statement 2010; Kilpatrick et al., 2010). These organisations recommend further glucose testing to establish a diagnosis of T2DM for anyone with intermediate HbA1c.
The aim of this study was to establish how a two threshold strategy for detection or exclusion of T2DM performed compared with using a single HbA1c cut-point in a multi-ethnic cohort. Furthermore I investigated cost per single case of T2DM detected according to each strategy.

4.3 Research Design and Methods

The analysis within chapter 4 uses the same LEADER cohort and study methods that are described in detail in Chapter 3.

4.4 Statistical Methods and data analysis

I tested various strategies for detection of T2DM compared to the standard diagnosis of WHO 1999 criteria diagnosis using an oral glucose tolerance test (WHO 1999), Figure 4.1. XLSTAT version 2011.5 (Addinsoft, Paris, France) was used to compare diagnostic indices with 95% confidence intervals (CI) at various HbA1c cut-points. The indices were sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). For a one cut-point strategy, I analysed performance of HbA1c ≥ 6.5% based on recent recommendations (WHO, 2011; International Expert Committee, 2009). Furthermore, Receiver Operating Characteristic (ROC) curves were plotted and used to determine the
optimal HbA1c cut-point defined as best balance between sensitivity and specificity. An area under the ROC curve (AUROC) was also calculated.

Regarding the two threshold strategy I investigated five different models where individuals underwent an HbA1c measurement at stage 1 using different ‘rule-in’ and ‘rule-out’ cut-points. Any individual placed in-between the two cut-points (intermediate HbA1c) was considered to require a subsequent glucose test for diagnosis at stage 2, which was an OGTT in this study. Models 1, 2 and 3 used ‘rule-out’ cut-points of HbA1c ≤ 5.5%, ≤ 5.4% and ≤5.7% respectively reflecting recommendations from an Australian study, AACE/ACE and the ABCD respectively (Lu et al., 2010; AACE/ ACE position statement 2010; Kilpatrick et al., 2010). Models 1 and 2 used HbA1c ≥ 7.0% as the ‘rule in’ cut-point, whereas Model 3 used HbA1c ≥ 7.3% based on the same recommendations. Model 4 selected HbA1c cut-points based 2.5th and 97.5th HbA1c percentiles for people with and without T2DM respectively derived from the LEADER cohort. These cut-points were HbA1c 5.6 and 6.4% for white Europeans and 5.9 and 6.8% for South Asians respectively. Model 5 determined the broadest range of a two HbA1c cut-point strategy allowing high diagnostic accuracy but simultaneously kept the number of people requiring a second test as low as possible.

4.4.1 Cost analysis.

Cost per case was defined as the total cost of screening the cohort using a particular strategy divided by the number of T2DM cases detected from that
strategy. The cost per case of T2DM was estimated for a single cut-point strategy of HbA1c ≥ 6.5% compared to a two cut-point strategy from the best model described above (balancing diagnostic ability and numbers requiring a second test). Furthermore, costs were re-evaluated with prior application of a validated non-invasive self-assessment risk score at stage 1, with score ≥ 14 proceeding to an HbA1c measurement at stage 2, before an OGTT for those with ‘intermediate HbA1c’ at stage 3 (Gray et al., 2010). All strategies assume 60% uptake at stage 1 based on previous literature (Goyder et al., 2008). Costs of strategies were derived from different sources. Screening laboratory blood tests were based on local prices and are reasonably representative of the UK (£0.47 per glucose sample and £2.66 per HbA1c sample) (quote from Leicester Pathology Service, 2011). Health care professional’s time for performing screening tests were approximated at £18 per hour and assumed 10 minutes per single blood test and 30 minutes per oral glucose tolerance test (Curtis, 2009). Administrative costs were estimated at £5.32 for blood tests and £2.71 for a risk score (Department of health, 2008).

4.5 Results

The initial LEADER population of 9,494 was refined to exclude people less than 40 years (n=600) and those with incomplete OGTT and HbA1c results (n=198). Hence the final cohort included in the study was 8,696 people.
4.5.1 Use of a single HbA1c cut-point to diagnose T2DM

Using either HbA1c ≥ 6.0%, 6.5% or 7.0% produced significantly lower sensitivity in white Europeans compared to south Asians, for HbA1c ≥ 6.5% these were 62.1% and 78.9% respectively (all diagnostic indices and 95% CI in Table 4.1). The AUROC for HbA1c detecting T2DM within white Europeans and south Asians were 0.92 (95% CI 0.89 to 0.94) and 0.93 (0.91 to 0.96) respectively. The optimal ROC curve derived HbA1c cut-point for detecting T2DM within white Europeans was ≥ 6.1% (sensitivity 83.0%/specificity 87.8%) and in South Asians HbA1c ≥ 6.3% (sensitivity 87.9%/specificity 85.5%).

Regarding the actual numbers of people detected, use of an OGTT detected 291 (3.3%) people with T2DM. There were 198 people (2.3%) who had T2DM on both on OGTT and an HbA1c ≥ 6.5%. In contrast, the number of people with T2DM on OGTT and were also detected with T2DM on two cut-points strategies models 1 to 5 were 287 (3.3), 283 (3.2%), 274 (3.1), 268 (3.0%) and 270 (3.1%) respectively.

4.5.2 Performance of two cut-point strategies to diagnose T2DM

There were five models were investigated in Table 4.2; associated diagnostic indices and 95% CI for HbA1c cut-points are in Table 4.1. Within Model 1, using
thresholds of HbA1c ≤ 5.5% and ≥ 7.0% produced high sensitivity, specificity and NPV greater than 98.0% in both ethnic groups (but moderate PPV of 76.0% in white Europeans and 68.1% in south Asians). However, a potential disadvantage was that there were 5115 (58.8%) people with intermediate HbA1c of 5.6 to 6.9%, who would require a second test. A similar problem was found with Models 2 and 3 where the intermediate HbA1c group also consisted of many individuals using 5.5 to 6.9% (n=6133, 70.5%) and 5.8 to 7.2% (n=3447, 39.6%) respectively. Within Model 5, use of HbA1c ≤ 5.8% and ≥ 6.8% (40 and 51mmol/mol) for ‘rule in’ and ‘rule out’ respectively produced an intermediate HbA1c group of 2504 (28.8%) people. Using these narrower thresholds maintained a similar high sensitivity, specificity and negative predictive value of greater than 91.0% in both ethnic groups (with a moderate PPV of 69.8% in white Europeans and 53.6% in south Asians). Therefore Model 5 was considered the most efficient overall two-cut point strategy, as it balanced highest diagnostic performance with the lowest numbers requiring a second test in a trade-off between these two factors.

4.5.3 Cost analysis

Using a two cut-point strategy of HbA1c ≤ 5.8% and ≥ 6.8% (Model 5) in White Europeans, a lower cost per case for T2DM of £38.53 (95% CI 1.89 to 86.81) was produced compared to using HbA1c ≥ 6.5% (Table 4.3). However the same
two cut-point strategy was more expensive in South Asians by £84.50 (69.72 to 100.92) per case of T2DM. When a self-assessment risk score $\geq$ 14 was applied at stage 1, the same two cut-point strategy demonstrated a cost reduction in White Europeans of £63.33 (23.33 to 113.26) in comparison to HbA1c $\geq$ 6.5%. In comparison to standard diagnostic tests, when OGTT was used to diagnose T2DM using WHO 1999 criteria, the cost per case for T2DM was £547.26 (547.26 to 569.47) in white Europeans and £326.89 (326.89 to 333.90) in South Asians. If fasting plasma glucose $\geq$ 7.0mmol/l only was used to diagnose T2DM, the cost per case for T2DM was £498.89 (450.98 to 562.92) in white Europeans and £365.05 (306.66 to 451.53) in South Asians.
Figure 4.1 Flow diagram describing various strategies tested for diagnostic accuracy and cost per case of diabetes in different ethnic groups (taken from Mostafa et al., 2012)

Key: DM = diabetes, IGR = impaired glucose regulation, OGTT = Oral glucose tolerance test, ROC = Receiver Operating Characteristics. Notes: (1) For two HbA1c cut-point strategies, 5 models were tested: the best overall model was underwent cost estimation vs. one-cut point of HbA1c ≥ 6.5% with combination of a self-assessment risk score. For DM and IGR, HbA1c ≥ 6.0% was used for one-cut point analysis. (2) Individuals in-between the ‘rule-out, rule-in’ cut points were labelled ‘impaired hba1c’ and underwent an OGTT for diagnosis.
Table 4.1 Comparison of diagnostic indices of HbA1c for screen detected undiagnosed T2DM according to 1999 WHO diagnostic criteria.

<table>
<thead>
<tr>
<th>HbA1c</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>PPV %</th>
<th>NPV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5.4%</td>
<td>98.9 (95.8-99.9)</td>
<td>24.2 (23.1-25.3)</td>
<td>3.7 (3.2-4.2)</td>
<td>99.9 (99.7-100.0)</td>
</tr>
<tr>
<td>≥ 5.5%</td>
<td>98.4 (95.0-99.6)</td>
<td>33.7 (32.5-34.9)</td>
<td>4.2 (3.6-4.8)</td>
<td>99.9 (99.7-100.0)</td>
</tr>
<tr>
<td>≥ 5.7%</td>
<td>93.4 (88.7-96.3)</td>
<td>55.4 (54.2-56.6)</td>
<td>5.8 (5.0-6.4)</td>
<td>99.6 (99.5-99.9)</td>
</tr>
<tr>
<td>≥ 5.8%</td>
<td>91.8 (86.7-95.0)</td>
<td>65.5 (64.3-66.6)</td>
<td>7.3 (6.2-8.3)</td>
<td>99.6 (99.4-99.8)</td>
</tr>
<tr>
<td>≥ 6.0%</td>
<td>86.8 (81.0-91.0)</td>
<td>82.3 (81.3-83.2)</td>
<td>12.7 (10.8-14.5)</td>
<td>99.5 (99.3-99.7)</td>
</tr>
<tr>
<td>≥ 6.1%</td>
<td>83.0 (76.8-87.7)</td>
<td>87.8 (87.0-88.6)</td>
<td>16.8 (14.3-19.2)</td>
<td>99.4 (99.2-99.6)</td>
</tr>
<tr>
<td>≥ 6.4%</td>
<td>67.6 (60.5-74.0)</td>
<td>96.7 (96.2-97.1)</td>
<td>37.4 (32.2-42.6)</td>
<td>99.0 (98.8-99.3)</td>
</tr>
<tr>
<td>≥ 6.5%</td>
<td>62.1 (54.8-68.8)</td>
<td>97.7 (97.3-98.1)</td>
<td>44.8 (38.7-51.0)</td>
<td>98.9 (98.6-99.1)</td>
</tr>
<tr>
<td>≥ 6.8%</td>
<td>49.5 (42.3-56.6)</td>
<td>99.4 (99.1-99.5)</td>
<td>69.8 (61.8-77.7)</td>
<td>98.5 (98.2-98.8)</td>
</tr>
<tr>
<td>≥ 7.0%</td>
<td>41.8 (34.8-49.0)</td>
<td>99.6 (99.4-99.7)</td>
<td>76.0 (67.6-84.4)</td>
<td>98.3 (98.0-98.6)</td>
</tr>
<tr>
<td>≥ 7.3%</td>
<td>32.4 (26.0-39.5)</td>
<td>99.8 (99.7-99.9)</td>
<td>85.5 (77.1-93.4)</td>
<td>98.0 (97.7-98.3)</td>
</tr>
<tr>
<td>SA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 5.4%</td>
<td>98.9 (93.6-100)</td>
<td>15.0 (13.4-16.7)</td>
<td>5.7 (4.5-6.8)</td>
<td>99.6 (98.9-100)</td>
</tr>
<tr>
<td>≥ 5.5%</td>
<td>98.9 (93.6-100)</td>
<td>21.4 (19.5-23.3)</td>
<td>6.1 (4.9-7.3)</td>
<td>99.7 (99.3-100)</td>
</tr>
<tr>
<td>≥ 5.7%</td>
<td>98.9 (93.6-100)</td>
<td>38.4 (36.2-40.6)</td>
<td>7.6 (6.2-8.9)</td>
<td>99.8 (99.4-100)</td>
</tr>
<tr>
<td>≥ 5.8%</td>
<td>97.9 (92.1-99.8)</td>
<td>48.5 (46.2-50.8)</td>
<td>8.9 (7.2-10.6)</td>
<td>99.8 (99.5-100)</td>
</tr>
<tr>
<td>≥ 6.0%</td>
<td>94.7 (87.9-98.0)</td>
<td>66.6 (64.4-68.7)</td>
<td>12.7 (10.3-15.2)</td>
<td>99.6 (99.2-99.9)</td>
</tr>
<tr>
<td>≥ 6.3%</td>
<td>85.3 (76.6-91.1)</td>
<td>86.5 (84.9-88.0)</td>
<td>24.5 (19.9-29.2)</td>
<td>99.1 (98.7-99.6)</td>
</tr>
<tr>
<td>≥ 6.5%</td>
<td>78.9 (69.6-86.0)</td>
<td>92.8 (91.6-93.9)</td>
<td>36.2 (29.7-42.8)</td>
<td>98.8 (98.3-99.3)</td>
</tr>
<tr>
<td>≥ 6.8%</td>
<td>63.2 (53.1-72.2)</td>
<td>97.2 (96.3-97.8)</td>
<td>53.6 (44.3-62.8)</td>
<td>98.1 (97.5-98.7)</td>
</tr>
<tr>
<td>≥ 7.0%</td>
<td>51.6 (41.7-61.4)</td>
<td>98.8 (98.1-99.2)</td>
<td>68.1 (57.3-78.8)</td>
<td>97.5 (96.8-98.2)</td>
</tr>
<tr>
<td>≥ 7.3%</td>
<td>36.8 (27.8-46.9)</td>
<td>99.7 (99.3-99.9)</td>
<td>87.5 (75.8-96.0)</td>
<td>96.8 (95.7-97.4)</td>
</tr>
</tbody>
</table>

Data expressed as % (95% confidence intervals). The selected cut-points relate to HbA1c strategies in the various models throughout this study. Key: NPV = Negative Predictive Value, Positive predictive value, SA = south Asian, WE = white European.
Table 4.2 Detection of T2DM and IGR using various two HbA1c cut-point thresholds

<table>
<thead>
<tr>
<th>Model</th>
<th>HbA1c</th>
<th>% of cohort</th>
<th>% of Total T2DM WE</th>
<th>% of Total T2DM SA</th>
<th>% of Total IGR WE</th>
<th>% of Total IGR SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≥ 7.0%</td>
<td>2.1</td>
<td>41.8</td>
<td>26.9</td>
<td>1.4</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>5.5 to 6.9%</td>
<td>68.5</td>
<td>56.6</td>
<td>47.4</td>
<td>83.1</td>
<td>88.8</td>
</tr>
<tr>
<td></td>
<td>&lt;5.5%</td>
<td>29.5</td>
<td>1.6</td>
<td>1.1</td>
<td>15.5</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>≥ 7.0%</td>
<td>2.1</td>
<td>41.8</td>
<td>26.9</td>
<td>1.4</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>5.6 to 6.9%</td>
<td>58.8</td>
<td>54.4</td>
<td>47.4</td>
<td>76.5</td>
<td>86.6</td>
</tr>
<tr>
<td></td>
<td>&lt;5.6%</td>
<td>39.1</td>
<td>3.8</td>
<td>1.1</td>
<td>22.1</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>≥ 7.3%</td>
<td>1.3</td>
<td>32.4</td>
<td>36.8</td>
<td>0.4</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>5.8 to 7.2%</td>
<td>39.6</td>
<td>59.3</td>
<td>61.1</td>
<td>61.0</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>&lt;5.8%</td>
<td>59.0</td>
<td>8.3</td>
<td>2.1</td>
<td>38.6</td>
<td>19.1</td>
</tr>
<tr>
<td>4</td>
<td>WE ≥ 6.4%</td>
<td>5.2</td>
<td>67.4</td>
<td>-</td>
<td>11.3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5.6 to 6.3%</td>
<td>51.4</td>
<td>28.7</td>
<td>-</td>
<td>66.6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&lt;5.6</td>
<td>43.4</td>
<td>3.9</td>
<td>-</td>
<td>22.1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>SA ≥ 6.8%</td>
<td>5.8</td>
<td>-</td>
<td>63.2</td>
<td>-</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>5.9 to 6.7%</td>
<td>39.0</td>
<td>-</td>
<td>34.7</td>
<td>-</td>
<td>62.1</td>
</tr>
<tr>
<td></td>
<td>&lt;5.9%</td>
<td>55.2</td>
<td>-</td>
<td>2.1</td>
<td>-</td>
<td>26.7</td>
</tr>
<tr>
<td>5</td>
<td>≥ 6.8%</td>
<td>3.0</td>
<td>49.5</td>
<td>63.2</td>
<td>2.6</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>5.9 to 6.7%</td>
<td>28.8</td>
<td>40.6</td>
<td>34.7</td>
<td>48.6</td>
<td>62.1</td>
</tr>
<tr>
<td></td>
<td>&lt;5.9%</td>
<td>68.2</td>
<td>9.9</td>
<td>2.1</td>
<td>48.8</td>
<td>26.7</td>
</tr>
</tbody>
</table>

In the HbA1c column, the top number represents rule in threshold, the middle numbers the intermediate range and the bottom number the rule out threshold. Key: WE: White European; SA: South Asian.
Table 4.3 Comparison of cost estimations for one and two HbA1c cut-point strategies

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Test at each stage</th>
<th>Total Cost (£)</th>
<th>Cost per case, £ (95% CI)</th>
<th>Difference in cost per case of two-cut point strategy minus one cut-point, £ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
<td>Stage 3</td>
<td></td>
</tr>
<tr>
<td>HbA1c test at stage 1 for T2DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One cut-point</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE (n=6345)</td>
<td>HbA1c</td>
<td>-</td>
<td>-</td>
<td>69,668.10</td>
</tr>
<tr>
<td>SA (n=1940)</td>
<td></td>
<td></td>
<td></td>
<td>21,301.20</td>
</tr>
<tr>
<td>Two cut-point</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE (n=6345)</td>
<td>HbA1c</td>
<td>OGTT</td>
<td>-</td>
<td>96,525.70</td>
</tr>
<tr>
<td>SA (n=1940)</td>
<td></td>
<td></td>
<td></td>
<td>34,287.46</td>
</tr>
</tbody>
</table>

Application of Self-assessment risk score at stage 1 for detection of T2DM

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Test at each stage</th>
<th>Total Cost (£)</th>
<th>Cost per case, £ (95% CI)</th>
<th>Difference in cost per case of two-cut point strategy minus one cut-point, £ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
<td>Stage 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One cut-point</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE (n=6284)</td>
<td>Risk score</td>
<td>HbA1c</td>
<td>-</td>
<td>58,830.50</td>
</tr>
<tr>
<td>SA (n=1931)</td>
<td></td>
<td></td>
<td></td>
<td>21,428.51</td>
</tr>
<tr>
<td>Two cut-point</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE (n=6284)</td>
<td>Risk Score</td>
<td>HbA1c</td>
<td>OGTT</td>
<td>79,675.66</td>
</tr>
<tr>
<td>SA (n=1931)</td>
<td></td>
<td></td>
<td></td>
<td>32,659.87</td>
</tr>
</tbody>
</table>

Cost per case for T2DM and IGR together using self-assessment risk score at stage 1

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Test at each stage</th>
<th>Total Cost (£)</th>
<th>Cost per case, £ (95% CI)</th>
<th>Difference in cost per case of two-cut point strategy minus one cut-point, £ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 1</td>
<td>Stage 2</td>
<td>Stage 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One cut-point</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE (n=6284)</td>
<td>Risk score</td>
<td>HbA1c</td>
<td>-</td>
<td>58,830.50</td>
</tr>
<tr>
<td>SA (n=1931)</td>
<td></td>
<td></td>
<td></td>
<td>21,428.51</td>
</tr>
<tr>
<td>Two cut-point</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE (n=6284)</td>
<td>Risk Score</td>
<td>HbA1c</td>
<td>OGTT</td>
<td>79,751.96</td>
</tr>
<tr>
<td>SA (n=1931)</td>
<td></td>
<td></td>
<td></td>
<td>32,659.87</td>
</tr>
</tbody>
</table>

The one cut-point strategy uses HbA1c ≥ 6.5% for T2DM and HbA1c ≥ 6.0% for IGR. Key: CI: confidence interval; IGR: impaired glucose regulation; WE: White European; SA: South Asian; OGTT: oral glucose tolerance test.
4.6 Discussion

The main findings of this study demonstrated the improved sensitivity of detecting T2DM from using a two cut-point strategy compared to a single cut-point of HbA1c ≥ 6.5%. However the main disadvantage was the high number of people requiring a subsequent test, over 50% in some models tested, suggesting the clinical utility of using a two cut-point strategy was low, in this population at least. To reduce the number of people in the intermediate HbA1c group undergoing a second test, I found using narrower HbA1c cut-points of ≤ 5.8% and ≥ 6.8% (Model 5) still detected 28.8% of the cohort as requiring a second test. However, this two cut-point strategy produced a lower cost per case of T2DM than using HbA1c ≥ 6.5% in white Europeans.

Previous studies have analysed the diagnostic performance of a single HbA1c cut-point to detect T2DM, but only isolated studies have investigated using a two cut-point strategy (Lu et al, 2010; Manley et al., 2010). The present study is the first to compare performance of various recommended two HbA1c cut-point strategies against each other and to include a cost per case analysis. Other strengths of this study included the ability to test each strategy in a large cohort, with high numbers of different ethnic groups reflecting much of the UK and northern Europe. Regarding limitations, the various HbA1c cut-points and strategies were analysed for detection of T2DM from a standard definition on an OGTT. Secondly, although the cost estimations produced a lower cost per case of T2DM using the two cut-point strategies in white Europeans, the 95%
confidence intervals were large in most cases. Thirdly, haemoglobinopathies may increase or decrease HbA1c as they represent factors affecting HbA1c other than glycaemia. There are some suggestions of normal haematological variation in HbA1c and in theory; these may impact more on using a single HbA1c cut-point than a two cut-point strategy, as the latter introduces glucose tests for people in the intermediate range (Cohen et al., 2007).

The ethnic differences in detection rates of T2DM using HbA1c were higher in South Asians than white Europeans. A potential explanation for this may be because South Asians have higher levels of insulin resistance (McKeigue et al., 1991). As HbA1c incorporates a 24-hour glucose profile for the previous two to three months, it will be able to account for more of the insulin resistance.

Secondly, the standard diagnostic tool was an OGTT including two-hour plasma glucose which is more closely related to insulin resistance. Theoretically if FPG had been the standard diagnostic tool only, then results may have been different.

**4.7 Implications for clinicians and policy makers**

HbA1c is likely to become the preferred diagnostic tool to detect T2DM in clinical practice. However using a single cut-point of HbA1c ≥ 6.5% lacked some degree of sensitivity (62.1%) for detecting T2DM in white Europeans suggesting that some caution is required. Despite an overall low clinical utility in the present study, the two cut-point strategy increased sensitivity to 91.8% using a ‘rule out’
cut-point of HbA1c ≤ 5.8% (Model 5). Therefore, despite a higher total cost of the
two cut-point strategy than HbA1c ≥ 6.5%, the extra number of T2DM cases
detected reduced the overall cost per case by £38.53. In contrast, there was no
associated cost benefit within South Asians as the total number of extra T2DM
cases detected from using the two cut-point strategy compared to HbA1c ≥ 6.5%
was relatively smaller (sensitivity 78.9% vs. 97.9% respectively). Also, a two cut-
point strategy makes a somewhat assumption that glucose defined T2DM is a
‘gold standard’ diagnosis, whereas this may not necessarily be true.
This study found the most efficient ‘rule out, rule in’ strategy (i.e. a trade-off
between high diagnostic performance and low numbers requiring a second test)
used narrower HbA1c cut-points of ≤ 5.8% and ≥ 6.8% than those recommended
from international organisations. These selected cut-points reduced the size of
the intermediate HbA1c group and remained within the range that predicts the
onset of retinopathy (Cheng et al., 2009; Sabanayagam et al., 2009; Colagiuri et
al., 2010). However whether using narrower or broader HbA1c cut-points should
be employed is likely to vary between populations. To understand how many
people require a subsequent test, the key influence is to observe the distribution
of HbA1c values throughout a given population (i.e. the mean cohort HbA1c and
its standard deviation) which determines the number of people shifted into the
intermediate HbA1c group. A simple hypothesis would suggest populations with a
higher mean cohort HbA1c (for example ≥ 5.7%) require narrower intermediate
HbA1c cut-points to reduce the size of the group, such as those suggested in
Model 5. In contrast, populations with lower mean cohort HbA1c (for example ≤
5.3%) can use broader intermediate HbA1c cut-points, such as 5.6 to 6.9%, as less people are shifted into this range. The cohort had a relatively high mean cohort HbA1c (5.71%, SD 0.62), which resulted in a large spread of HbA1c values throughout the 5.6 to 6.9% intermediate HbA1c range which reduces potential clinical application. However other populations may not experience this problem as other population based cohorts show mean HbA1c ranging from 5.1% to 5.9% (Christensen et al., 2010). Therefore, separate regions or populations may have to define their own optimal two cut-points strategy and determine any clinical utility.

Other organisations have suggested alternate methods of incorporating HbA1c into diagnosis of T2DM. In the US, the Veterans Affairs-Department of Defence have suggested a single HbA1c cut-point for T2DM diagnosis which is higher than other organisations, at HbA1c ≥ 7.0% or both an HbA1c ≥ 6.5% together with FPG ≥ 7.0mmol/l (Pogach et al., 2011). They also recommend IGR as HbA1c 5.7 to 6.4% and FPG between 5.6 to 6.9mmol/l (Pogach et al., 2011). The WHO have suggested an HbA1c ≥ 6.5% should be used for diagnosis of T2DM, however a value less than 6.5% does not exclude T2DM diagnosed using glucose tests, which is also endorsed by UK expert groups (John et al., 2012). Future research should focus on the value of using a two HbA1c cut-point strategy for diagnosis of T2DM and IGR and other suggested strategies.
4.8 Conclusion to Chapter 4

A two cut-point strategy for detecting T2DM is an interesting strategy that does appear to have some advantages but lacks evidence base and may not be feasible to implement. Whether this is a better strategy than using a single cut-point of HbA1c ≥ 6.5% needs further analysis. Staying with this theme, Chapter 5 investigates whether a single HbA1c cut-point is correct for all ethnic groups, in particular South Asians.
Chapter 5

Are recommended HbA1c diagnostic cut-points appropriate for all people?

The independent effect of ethnicity on glycaemia and microalbuminuria in South Asians and White Europeans
5.1 Chapter overview

In chapters 1 to 4, I have reviewed the use of HbA1c as a diagnostic tool for T2DM and IGR using the recommended cut-points from international organisations and subsequently tested these cut-points in white Europeans and South Asians in the LEADER cohort. However, there is assumption made that same cut-point of an HbA1c ≥ 6.5% for T2DM (and HbA1c of 6.0 to 6.4% or 5.7 to 6.4% for IGR) is the equivalent in all people and ethnic groups. A feature of results from chapter 3 was the different HbA1c cut-points derived from ROC curve analysis for T2DM (and IGR) in South Asians compared to White Europeans. This suggests that the same cut-point may not be suitable for all people. However this needs further exploration in a more robust manner which I shall do in Chapter 5. Secondly, this difference may not be unique to HbA1c as the same principle may exist in FPG and two hour plasma glucose. This chapter is based on one published paper (Mostafa et al., 2012b).

5.2 Introduction

5.2.1 Glycaemic markers and ethnic minority groups

Glycated haemoglobin, HbA1c, is now recommended as a diagnostic tool for detecting T2DM, alongside fasting and 2-hour plasma glucose, as well remaining as the standard test for monitoring disease progression (WHO, 2011;
International Expert Committee, 2009). Previous studies have demonstrated HbA1c values are higher in some black and minority ethnic groups compared to White Europeans independent of glycaemic control or factors that differ between ethnic groups (Selvin et al., 2011; Herman et al., 2007; Herman et al., 2009, Ziemer et al., 2010; Saaddine et al., 2002). However this comparison has not been performed among South Asians and White Europeans. Small studies suggest South Asians possess higher HbA1c values, without adjusting for factors affecting glycaemia (Burden et al, 2009; Likhari et al., 2010). In addition, this independent effect of ethnicity in South Asians and White Europeans may exist for other markers of glycaemia as well, including FPG and 2-hour plasma glucose.

There is a consistently reported high prevalence of T2DM and IGR in South Asians, which develops earlier compared to White Europeans and leads on to higher rates of complications including CVD (McKeigue et al., 1991; McKeigue et al., 1993; Gholap et al., 2011). The data presented in Chapter 3 suggest using HbA1c as the preferred diagnostic tool further increases the prevalence of T2DM and IGR in South Asians compared to using an oral glucose tolerance test. Potential explanations for this include higher HbA1c values in South Asians or a better sensitivity of HbA1c to detect insulin resistance as it represents a 24 hour glucose profile of the last three months (i.e. combining both fasting and prandial hyperglycaemia).

5.2.2 Glycaemia markers and microalbuminuria
If independent differences in ethnicity exist for HbA1c, FPG or two hour plasma glucose, this may suggest a need for ethnic specific cut-points. However, this can only be truly suggested if there are ethnicity differences in the rates of hyperglycaemic complications before the diagnosis of T2DM, particularly microvascular complications (WHO, 2011). This is because the diagnostic cut-points for T2DM are traditionally based on microvascular complications, particularly diabetes-specifically prevalent or incident retinopathy. However international organisations also recognise the onset of microalbuminuria as an appropriate tool to consider defining T2DM, as microvascular complications often run in parallel (WHO, 2011).

South Asians are generally shown to have higher levels of microalbuminuria compared to white Europeans in most studies, although the UKPDS suggested there were no significant differences in people with recently diagnosed T2DM (UK Prospective Diabetes Study XII, 1994; Mather et al., 1998; Dixon et al., 2006; Chandie et al., 2006; Fischbacher et al., 2003). However it is not well reported whether South Asians have higher levels of microalbuminuria before the diagnosis of T2DM or whether urinary albumin: creatinine ratio (ACR) levels are independently higher in South Asians after adjusting for appropriate confounding risk factors across a range of levels of glycaemic markers. This would establish if complications of T2DM were occurring before the diagnosis of this disease. A previous of a high risk cohort suggested the prevalence of microalbuminuria was 8.9% (95% CI 6.9 to 11.4) and 20.0% (12.4 to 30.3) in people with IGR and
T2DM respectively (Crasto et al., 2009). However less is known about ethnic differences for the same comparison in population based data. Although a systematic review conducted previously in our department suggested there is no difference in the overall prevalence of microalbuminuria between White Europeans and South Asians with T2DM (Crasto, 2011).

The aim of this study was to evaluate the independent effect of ethnicity on glycaemic markers in South Asians and White Europeans and to quantify the magnitude of any differences. Secondly, I investigated the independent effect of ethnicity on the relationship between glycaemic markers and the ACR in South Asian and White European men and women.

5.3 Research Design and Methods

The analysis within chapter 5 uses cross-sectional data from the ADDITION cohort which is described in detail in Chapter 3.

5.4 Statistical Analysis

5.4.1 The effect of ethnicity on glycaemia
SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. From the cohort of 6749, exclusions were made for those without an HbA1c measurement, OGTT result, those who did not disclose their ethnic group or were not White European or South Asian (n=709). Therefore there were 6040 people (4688 White Europeans and 1352 South Asians) aged 40 to 75 years used in this part of the analysis. Selected baseline characteristics were stratified by ethnic group and then by HbA1c categories of <5.5%, 5.5 to 5.9%, 6.0 to 6.4% and ≥ 6.5%. Clinical characteristics were tested for significant differences between categories using chi-squared for discrete variables, unpaired t-tests and Mann-Whitney for normally and non-normally distributed continuous variables respectively. Fasting insulin and fasting glucose was used to derive homeostasis model assessment insulin resistance, HOMA1-IR: glucose (mmol/l) x insulin (µIU/ml) / 22.5, and beta cell function, HOMA-B: 20 x insulin (µIU/ml) / glucose (mmol/l) – 3.5 (Matthews et al., 1985). Insulin and the two HOMA indexes were not normally distributed and therefore logarithmically transformed. Multiple regression analysis was used to determine all significant associations (using the enter method) of HbA1c in the total cohort. The variables tested were age, sex, ethnic group (White European vs. South Asian), body mass index, waist circumference, FPG, 2-hour plasma glucose, LDL- and HDL-cholesterol, triglycerides, potassium, systolic and diastolic blood pressure, creatinine, ACR, fasting insulin, HOMA1-IR and HOMA-B. The latter three variables were only tested on insulin sub-sample cohort described in Chapter 3.
Secondly, analysis of covariance modelling was used to calculate the mean difference of HbA1c between South Asians and White Europeans using stepwise models. Model 1 compared unadjusted HbA1c values. Model 2 calculated HbA1c levels adjusted for traditional risk factors including age, sex, BMI, waist circumference, systolic and diastolic blood pressure, LDL- and HDL-cholesterol, triglycerides, creatinine, ACR, FPG and 2-hour plasma glucose. Model 3 consisted of model 2 with the addition of adjusting for fasting insulin. As HOMA1-IR and HOMA-B were calculated using FPG and insulin they were not included in model 3. Multiple regression and analysis of covariance models combined continuous and categorical variables together; however this is consistent with similar studies (Herman et al., 2007; Herman et al., 2009; Ziemer et al., 2010). As there was a possibility of co-linearity between FPG, 2-hour plasma glucose and HbA1c in models, separate linear regression and analysis of covariance models were run without FPG and 2-hour plasma glucose (model 4). Models were briefly repeated using FPG and 2-hour plasma glucose as the dependent variables for models 1 and 3 only. Adjustments were made for multiple comparisons using Bonferroni corrections.

5.4.2 Effect of ethnicity on the relationship between glycaemia and ACR

The ACR analysis was carried out by Dr. Danielle Morris (University of Leicester). Stata version 12.1 (StataCorp, Texas, US) was used to perform statistical
analysis. From the initial cohort of 6749 people, those without an ACR measurement were excluded (n=117), as were people who did not declare their ethnic group or were not classified as White European or South Asian (n=377). The final sample consisted for the ACR analysis consisted of 5924 people. Baseline demographics of the population were reported stratified by ethnic group and sex. Means and standard deviations were reported for normally distributed variables. Discrete variables were analysed using chi-squared tests. Secondly, the prevalence of microalbuminuria (defined as an ACR ≥ 2.5 mg/mmol in males and 3.5 mg/mmol in females) was compared between white Europeans and South Asians stratified by OGTT result, first in the total cohort and then after removing those participants taking anti-hypertensive medications (defined as ACE-inhibitors, angiotensin receptor blockers, alpha blockers, beta-blockers, calcium channel blockers or diuretics). Thirdly, using linear regression models the mean ACR was compared between white European and South Asian men and women respectively in an unadjusted model and then adjusted for age, FPG, 2-hour plasma glucose, HbA1c, systolic and diastolic blood pressure, anti-hypertensive medications, creatinine, body mass index, and smoking status. Finally, we investigated the independent effect of ethnicity on the relationship between ACR and glycaemic variables including FPG, two hour plasma glucose and HbA1c. The analysis was performed in White European and South Asian men and women separately. For each variable of interest, a fractional polynomial model was fitted with the natural logarithm of ACR as the outcome, ethnicity the other variable of interest and their interaction included as covariates. Unless it
was the variable of interest, all models were adjusted for FPG, 2 hour plasma glucose, HbA1c, systolic and diastolic blood pressure, body mass index, creatinine, age, smoking status (never, ex- or current smoker), and prescribed anti-hypertensive medication (yes or no). Line graphs were plotted based on these models with back transformed geometric mean ACR on the y axis and variable of interest on the x-axis. A two sided p-value of <0.05 was considered statistically significant throughout this chapter.
5.5 Results

5.5.1 Ethnicity and glycaemia

Baseline demographics of selected characteristics from the 6040 people are in Table 5.1 and 5.2. South Asians were younger, with a lower body mass index, waist circumference, systolic blood pressure, creatinine, LDL- and HDL-cholesterol compared to White Europeans. However South Asians had a higher fasting and 2-hour plasma glucose, insulin, HOMA1-IR and HOMA-B, and a higher prevalence of T2DM and IGR than White Europeans. Within HbA1c categories of HbA1c< 5.5% and 5.5 to 5.9%, South Asians had higher 2-hour plasma glucose, fasting insulin and HOMA-IR than White Europeans.

The significant baseline variable associations of HbA1c using multiple linear regression analysis are listed in Table 5.3. These included ethnicity, FPG, 2-hour plasma glucose, and HOMA-B (all p<0.001), age and sex, (both p<0.01), insulin and potassium (both p<0.05). The variables tested produced an adjusted R squared of 0.773. When the linear regression model was repeated without fasting and two plasma glucose, the significant associations with HbA1c were insulin, HOMA-IR, HOMA-B (all p<0.001), ethnicity, sex, age, triglycerides (all p<0.01), potassium, waist circumference and BMI (all p<0.05).

The mean crude HbA1c in the White European and South Asian total population was 5.65% (standard error 0.01) and 5.81% (0.01) respectively, resulting in a
mean difference of 0.22% (95% confidence levels 0.18 to 0.25%, p<0.001), Table 5.4. After adjustment, the mean HbA1c remained higher in South Asians, with a significant mean difference of 0.20% (0.10 to 0.30, p<0.001). Stratification by OGTT results (normal glucose tolerance compared to combined IGR and T2DM) demonstrated similar findings. South Asians with normal glucose tolerance had higher mean adjusted HbA1c difference of 0.27% (0.12 to 0.42%) p<0.01, compared to White Europeans. Similarly, South Asians with diabetes or impaired glucose regulation had a higher adjusted mean HbA1c difference of 0.16% (0.03 to 0.32%) p<0.05. The same results in each ethnic group were stratified by sex and demonstrated similar differences of approximately 0.2% higher HbA1c values in South Asian men and women compared to European men and women for unadjusted and adjusted results, Table 5.5.

When FPG was used as the dependent variable, mean crude values were 5.18mmol/l (0.01) and 5.27mmol/l (0.03) in White Europeans and South Asians respectively, producing a mean difference of 0.09mmol/l (0.03 to 0.14, p<0.01), Table 5.6. After adjustment for risk factors, the corresponding values were 5.15mmol/l (0.01) and 5.30mmol/l (0.03), a mean difference of 0.15mmol/l (0.09 to 0.21, p<0.001) higher in South Asians.

Finally, using 2-hour plasma glucose as the dependent variable, the mean crude values were 5.89mmol/l (0.08) and 6.46mmol/l (0.07) in White Europeans and South Asians respectively, producing a mean difference of 0.58mmol/l (0.43 to 0.73, p<0.001), Table 5.7. After adjustment for risk factors, the corresponding
values were 5.82mmol/l (0.04) and 6.57mmol/l (0.07), a mean difference higher in South Asians of 0.75mmol/l (0.59 to 0.92, p<0.001).
Table 5.1 Baseline characteristics for White Europeans and South Asians used in the analysis from the ADDITION-Leicester study

<table>
<thead>
<tr>
<th></th>
<th>White European</th>
<th>South Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>4688</td>
<td>1352</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58.6 (9.5)</td>
<td>53.0 (8.7) *</td>
</tr>
<tr>
<td>Males, n (%)</td>
<td>2209 (47.1)</td>
<td>664 (49.1)</td>
</tr>
<tr>
<td>Deprivation level</td>
<td>13.5 (13.2 to 13.8)</td>
<td>19.5 (18.6 to 20.0)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 (0.1)</td>
<td>1.61 (0.1) *</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.0 (16.0)</td>
<td>72.1 (14.2) *</td>
</tr>
<tr>
<td>Body Mass Index (m/kg²)</td>
<td>28.3 (5.0)</td>
<td>27.5 (4.9) *</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>94.7 (13.5)</td>
<td>92.6 (11.6) *</td>
</tr>
<tr>
<td>Waist: hip ratio</td>
<td>0.89 (0.09)</td>
<td>0.90 (0.08) †</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.2 (0.9)</td>
<td>5.3 (0.8) †</td>
</tr>
<tr>
<td>2 hour plasma glucose (mmol/l)</td>
<td>5.9 (2.4)</td>
<td>6.4 (2.7) *</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.65 (0.6)</td>
<td>5.87 (0.6) *</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>128 (2.7)</td>
<td>68 (5.1) *</td>
</tr>
<tr>
<td>Diabetes and IGR, n (%)</td>
<td>758 (16.2)</td>
<td>298 (22.1) *</td>
</tr>
<tr>
<td>Normal glucose tolerance, n (%)</td>
<td>3908 (83.8)</td>
<td>1048 (77.9) *</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>139.0 (19.4)</td>
<td>134.8 (19.2) *</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>85.7 (10.5)</td>
<td>85.6 (10.8)</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.6 (0.9)</td>
<td>3.3 (0.8) *</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.4 (0.4)</td>
<td>1.3 (0.3) *</td>
</tr>
<tr>
<td>Triglycerides (mmol/l) ‡</td>
<td>1.2 (0.8)</td>
<td>1.2 (0.9)</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.3 (0.4)</td>
<td>4.3 (0.4)</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>87.2 (15.6)</td>
<td>83.4 (17.8) *</td>
</tr>
<tr>
<td>Albumin: creatinine ratio ‡</td>
<td>0.7 (0.6)</td>
<td>0.7 (0.7)</td>
</tr>
<tr>
<td>Fasting Insulin (μIU/ml) §</td>
<td>7.6 (7.2 to 8.0)</td>
<td>8.9 (8.2 to 9.6) †</td>
</tr>
<tr>
<td>HOMA1-IR §</td>
<td>2.1 (2.0 to 2.3)</td>
<td>2.4 (2.1 to 2.7) †</td>
</tr>
<tr>
<td>HOMA-B (%) §</td>
<td>77.2 (71.8 to 83.0)</td>
<td>90.5 (82.8 to 99.0) †</td>
</tr>
</tbody>
</table>

Continuous variables presented as mean (standard deviation), except ‡ median (IQR) and § geometric mean (95% confidence intervals) after initial log transformation (for sub-sample only). Key: B = beta cell function, BP = blood pressure, HOMA = Homeostatic model assessment, IGR = impaired glucose regulation and IR = Insulin resistance. Significance between ethnic groups: * p<0.001, † p<0.05.
Table 5.2 Baseline glycaemic markers stratified by HbA1c categories

<table>
<thead>
<tr>
<th>Variables</th>
<th>HbA1c &lt; 5.5%</th>
<th>HbA1c 5.5 to 5.9%</th>
<th>HbA1c 6.0 to 6.4%</th>
<th>HbA1c ≥ 6.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WE</td>
<td>SA</td>
<td>WE</td>
<td>SA</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>4.9 (0.4)</td>
<td>4.9 (0.5)</td>
<td>5.1 (0.5)</td>
<td>5.1 (0.4)</td>
</tr>
<tr>
<td>2hr pg (mmol/l)</td>
<td>5.3 (1.5)</td>
<td>5.9 (1.5)*</td>
<td>5.6 (1.8)</td>
<td>6.0 (1.8)*</td>
</tr>
<tr>
<td>Fasting Insulin‡ (µIU/mL)</td>
<td>5.9</td>
<td>7.43</td>
<td>7.1</td>
<td>8.5</td>
</tr>
<tr>
<td>HOMA1-IR‡</td>
<td>(5.4-6.5)</td>
<td>(6.2-9.0)†</td>
<td>(6.6-7.5)</td>
<td>(7.6-9.5)†</td>
</tr>
<tr>
<td>HOMA-B (%)‡</td>
<td>1.2</td>
<td>1.9</td>
<td>1.6</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>(1.1-1.5)</td>
<td>(1.4-2.6)†</td>
<td>(1.5-1.8)</td>
<td>(1.9-2.6)†</td>
</tr>
<tr>
<td></td>
<td>79.6</td>
<td>109.1</td>
<td>82.2</td>
<td>118.6</td>
</tr>
<tr>
<td></td>
<td>(68.7-92.5)</td>
<td>(82.8-123.5)</td>
<td>(75.0-90.4)</td>
<td>(100.0-140.6)†</td>
</tr>
</tbody>
</table>

Variables adjusted for age, sex and body mass index and presented as mean (Standard deviation), except ‡ geometric mean (95% CI) after log transformation (for the sub-sample only). White European (WE) vs. South Asian (SA): * p<0.001, † P<0.05.
Table 5.3 The independent effect of variables on HbA1c derived from multiple linear regression modelling

<table>
<thead>
<tr>
<th>Variable</th>
<th>B-coefficient</th>
<th>SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.006</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Deprivation level</td>
<td>0.002</td>
<td>0.002</td>
<td>0.347</td>
</tr>
<tr>
<td>Sex (male vs. female)</td>
<td>0.205</td>
<td>0.065</td>
<td>0.002</td>
</tr>
<tr>
<td>Ethnicity (WE vs. SA)</td>
<td>0.189</td>
<td>0.051</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.014</td>
<td>0.009</td>
<td>0.107</td>
</tr>
<tr>
<td>Waist Circumference</td>
<td>0.005</td>
<td>0.003</td>
<td>0.163</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.424</td>
<td>0.031</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2hr plasma glucose</td>
<td>0.040</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>-0.022</td>
<td>0.021</td>
<td>0.312</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.031</td>
<td>0.065</td>
<td>0.635</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.053</td>
<td>0.030</td>
<td>0.0775</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.002</td>
<td>0.001</td>
<td>0.187</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>-0.005</td>
<td>0.003</td>
<td>0.052</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.097</td>
<td>0.046</td>
<td>0.034</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.002</td>
<td>0.002</td>
<td>0.316</td>
</tr>
<tr>
<td>ACR</td>
<td>0.002</td>
<td>0.002</td>
<td>0.028</td>
</tr>
<tr>
<td>Insulin *</td>
<td>0.055</td>
<td>0.022</td>
<td>&lt;0.014</td>
</tr>
<tr>
<td>HOMA1-IR *</td>
<td>0.127</td>
<td>0.068</td>
<td>0.043</td>
</tr>
<tr>
<td>HOMA-B *</td>
<td>0.003</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The adjusted R square was 0.773 (F=86.1, p<0.001)

* Sub-sample only. Key: ACR = albumin creatinine ratio, BMI = body mass index, BP = blood pressure, SA = South Asian, WE = White European
### Table 5.4. A comparison of crude and adjusted differences for HbA1c in White Europeans and South Asians

<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>Normal OGTT</th>
<th>T2DM + IGR on OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbA1c (%)</td>
<td>Mean difference</td>
<td>HbA1c (%)</td>
</tr>
<tr>
<td>Model 1</td>
<td>WE</td>
<td>5.65 (0.01)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>5.87 (0.01)</td>
<td>(0.18 to 0.25) *</td>
</tr>
<tr>
<td>Model 2</td>
<td>WE</td>
<td>5.65 (0.01)</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>5.82 (0.03)</td>
<td>(0.14 to 0.20) *</td>
</tr>
<tr>
<td>Model 3 §</td>
<td>WE</td>
<td>5.92 (0.02)</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>6.10 (0.32)</td>
<td>(0.11 to 0.27) *</td>
</tr>
<tr>
<td>Model 4</td>
<td>WE</td>
<td>5.63 (0.01)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td>5.88 (0.02)</td>
<td>(0.21-0.30) *</td>
</tr>
</tbody>
</table>

HbA1c presented as mean (standard error); the mean difference (95% confidence intervals). Model 1 is unadjusted. Model 2 is adjusted for age, sex, deprivation level, systolic and diastolic blood pressure, creatinine, albumin–creatinine ratio, BMI, waist circumference, triglycerides, HDL-cholesterol, LDL-cholesterol, potassium, FPG and 2-hour plasma glucose. Model 3 is adjusted for model 2 plus fasting insulin. Model 4 is adjusted for model 2 without fasting and 2-hour plasma glucose. Key: IGR = Impaired glucose regulation, OGTT = oral glucose tolerance test, SA = South Asian, T2DM = Type 2 Diabetes Mellitus, WE = White European. § = Sub-sample population only. Significant differences between ethnic groups: * p< 0.001, † p< 0.01, ‡ p< 0.05.
Table 5.5 Sub-analysis of HbA1c ethnicity differences separated by sex

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HbA1c (%) Mean difference</td>
<td>HbA1c (%) Mean difference</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE</td>
<td>5.66 (0.02) 0.23</td>
<td>5.65 (0.01) 0.20</td>
</tr>
<tr>
<td>SA</td>
<td>5.88 (0.03) (0.17-0.29) *</td>
<td>5.85 (0.02) (0.16 -0.25) *</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE</td>
<td>5.66 (0.01) 0.15</td>
<td>5.64 (0.01) 0.18</td>
</tr>
<tr>
<td>SA</td>
<td>5.81 (0.2) (0.10 – 0.198) *</td>
<td>5.82 (0.02) (0.14-0.22) *</td>
</tr>
</tbody>
</table>

HbA1c presented as mean (standard error); the mean difference (95% confidence intervals). Model 1 is unadjusted. Model 2 is adjusted for age, sex, deprivation level, systolic and diastolic blood pressure, creatinine, albumin–creatinine ratio, BMI, waist circumference, triglycerides, HDL-cholesterol, LDL-cholesterol, potassium, FPG and 2-hour plasma glucose. Significant differences between ethnic groups: * p< 0.001, † p< 0.01, ‡ p< 0.05.

Table 5.6 Sub-analysis of ethnicity differences in fasting plasma glucose

<table>
<thead>
<tr>
<th>Total population</th>
<th>Normal OGTT</th>
<th>T2DM + IGR on OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FPG (mmol/l) Mean difference</td>
<td>FPG (mmol/l) Mean difference</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE</td>
<td>5.18 (0.01) 0.09</td>
<td>5.00 (0.01) 0.02</td>
</tr>
<tr>
<td>SA</td>
<td>5.27 (0.03) (0.03-0.14) †</td>
<td>5.02 (0.01) (0.01-0.05)</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE</td>
<td>5.15 (0.01) 0.15</td>
<td>4.99 (0.01) 0.05</td>
</tr>
<tr>
<td>SA</td>
<td>5.30 (0.03) (0.09-0.21) *</td>
<td>5.05 (0.02) (0.02-0.09) †</td>
</tr>
</tbody>
</table>

FPG presented as mean (standard error); the mean difference (95% confidence intervals). Model 1 is unadjusted. Model 2 is adjusted for age, sex, deprivation level, systolic and diastolic blood pressure, creatinine, albumin–creatinine ratio, BMI, waist circumference, triglycerides, HDL-cholesterol, LDL-cholesterol, potassium, HbA1c and 2-hour plasma glucose. Significant differences between ethnic groups: * p< 0.001, † p< 0.01, ‡ p< 0.05.
# Table 5.7 Sub-analysis of ethnicity differences in two-hour plasma glucose

<table>
<thead>
<tr>
<th></th>
<th>Total population</th>
<th>Normal OGTT</th>
<th>T2DM + IGR on OGTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2hrPG (mmol/l)</td>
<td>2hrPG (mmol/l)</td>
<td>2hrPG (mmol/l)</td>
</tr>
<tr>
<td></td>
<td>Mean difference</td>
<td>Mean difference</td>
<td>Mean difference</td>
</tr>
<tr>
<td>Model 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE</td>
<td>0.58</td>
<td>0.26</td>
<td>0.60</td>
</tr>
<tr>
<td>SA</td>
<td>0.43-0.73 *</td>
<td>0.17-0.34 *</td>
<td>(0.16-1.04) †</td>
</tr>
<tr>
<td>Model 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WE</td>
<td>0.75</td>
<td>0.36</td>
<td>0.63</td>
</tr>
<tr>
<td>SA</td>
<td>0.59-0.92 *</td>
<td>0.26-0.46 *</td>
<td>(0.13-1.13) ‡</td>
</tr>
</tbody>
</table>

Two hour plasma glucose presented as mean (standard error); the mean difference (95% confidence intervals). Model 1 is unadjusted. Model 2 is adjusted for age, sex, deprivation level, systolic and diastolic blood pressure, creatinine, albumin–creatinine ratio, BMI, waist circumference, triglycerides, HDL-cholesterol, LDL-cholesterol, potassium, FPG and HbA1c. Significant differences between ethnic groups: * p< 0.001, † p< 0.01, ‡ p< 0.05.
5.5.2 Effect of ethnicity on ACR and glycaemic markers

From a total of 5924 participants, 2174 (36.7%) and 653 (11.0%) were white European and South Asian men respectively, while there were 2429 (41.0%) and 668 (11.3%) white European and South Asian women respectively. The baseline demographics of the cohort are described in Table 5.8. Regarding both men and women, South Asians were younger than white Europeans (p<0.001), had lower levels of systolic blood pressure (p<0.001), creatinine (p<0.001), were less likely to be a current or ex-smoker (p<0.001), but had higher levels of HbA1c (p<0.001) and two hour plasma glucose (p<0.001). Additionally, South Asian males had lower levels of body mass index compared to White European males (p<0.001); whereas South Asian females were less likely to be prescribed an anti-hypertensive compared to White European females (p=0.005).

The prevalence of microalbuminuria was significantly higher in South Asians than white Europeans when analysing the total cohort (9.6 vs. 7.4%, p=0.008) and those with an OGTT result in the normal defined range (8.3 vs. 6.4%, p=0.032), Table 5.9. When the cohort was reduced to those people not prescribed anti-hypertensive medications (n=4546), South Asians once again had higher levels of microalbuminuria in the total cohort (8.0 vs. 6.3%, p=0.046), however regarding people with T2DM, the opposite result was found as microalbuminuria was higher in White Europeans (24.7 vs. 9.5%, p=0.047).

The unadjusted mean ACR level was significantly higher in South Asian women compared to White European women, 2.48 mg/mmol (SD 11.37) and 1.64
mg/mmol (9.71), giving a mean difference of 1.08 mg/mmol (95% CI 1.01 to 1.16, p=0.029), Table 5.10. The same comparison was not significant in males, 1.92 mg/mmol (6.91) and 1.48 mg/mmol (5.94), with a mean difference of 1.07 mg/mmol (0.99 to 1.16). However for after adjusting for HbA1c, FPG, 2 hour plasma glucose, systolic and diastolic blood pressure, anti-hypertensive medication, age, creatinine, BMI and smoking status, the mean ACR differences were now significantly higher in South Asian men, 1.18 mg/mmol (1.09 to 1.27, p=0.031), but no longer significant in women, 1.15 (1.06 to 1.24, p=0.515).

The independent relationship between ACR and glycaemic variables was plotted in line graphs in Figure 5.1 (a) to (c) for men and in (d) to (f) for women. All line graphs were adjusted for body mass index, HbA1c, FPG, age, creatinine, diastolic and systolic blood pressure, anti-hypertensive medication, 2 hour plasma glucose and smoking status. In all glycaemic variables (FPG, two hour plasma glucose and HbA1c) the slope of the line/ trajectory representing the ACR levels in South Asians was steeper than that of White Europeans for both men and women. Furthermore, these lines/ trajectories were more divergent with increasing levels of the glycaemic variable of interest, suggesting the magnitude of the differences in ACR values tended to increase as the glycaemic marker increased. Thirdly, the lines/ trajectories tended to follow linear trend increases for two hour plasma glucose in men and women and fasting plasma glucose in women. The other demonstrated lines/ trajectories that tended to follow a quadratic shape curve for HbA1c.
On inspecting these curves, the magnitude of differences in ACR between the two ethnic groups varied according to the glycaemic marker. In both men and women, the ACR differences were less than 0.1 mg/mmol higher in South Asians before the diagnostic cut-points for T2DM and IGR for HbA1c, fasting and 2 hour plasma glucose levels. The ACR difference of less than 0.1 mg/mmol remained with increasing levels of 2-hour plasma glucose in both men and women, and for fasting plasma glucose in women only. In contrast, ACR differences increased in South Asian men to approximately 0.5 to 0.6 mg/mmol higher than White European men beyond a FPG and HbA1c levels of 12.0mmol/l and 12.0% respectively. Additionally, in South Asian women ACR differences increased to 0.15 to 0.20 mg/mmol higher than White European women above an HbA1c of 13.0%.
Table 5.8 Selected baseline demographics of the ACR cohort analysed stratified by ethnic group and sex

<table>
<thead>
<tr>
<th></th>
<th>All (n=5924)</th>
<th>White European (n=2174)</th>
<th>Men South Asian (n=653)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>White European (n=2429)</th>
<th>Women South Asian (n=668)</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>57.3 (9.6)</td>
<td>58.7 (9.4)</td>
<td>53.8 (9.1)</td>
<td>&lt;0.001</td>
<td>58.5 (9.5)</td>
<td>52.2 (8.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28.1 (4.9)</td>
<td>28.2 (4.1)</td>
<td>26.6 (4.1)</td>
<td>&lt;0.001</td>
<td>28.4 (5.6)</td>
<td>28.5 (5.4)</td>
<td>0.581</td>
</tr>
<tr>
<td>FPG (mmol/l)</td>
<td>5.2 (0.9)</td>
<td>5.3 (1.1)</td>
<td>5.4 (0.9)</td>
<td>0.071</td>
<td>5.1 (0.8)</td>
<td>5.1 (0.8)</td>
<td>0.021</td>
</tr>
<tr>
<td>2 hour PG (mmol/l)</td>
<td>6.0 (2.4)</td>
<td>5.8 (2.6)</td>
<td>6.5 (2.8)</td>
<td>&lt;0.001</td>
<td>5.9 (2.1)</td>
<td>6.4 (2.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.7 (0.6)</td>
<td>5.7 (0.7)</td>
<td>5.9 (0.7)</td>
<td>&lt;0.001</td>
<td>5.6 (0.5)</td>
<td>5.9 (0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>138.1(19.4)</td>
<td>141.8(17.9)</td>
<td>138.7(18.0)</td>
<td>&lt;0.001</td>
<td>136.5 (20.2)</td>
<td>131.3 (19.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>85.7 (10.5)</td>
<td>87.1 (10.1)</td>
<td>86.8(10.6)</td>
<td>0.466</td>
<td>84.4 (10.6)</td>
<td>84.5(10.8)</td>
<td>0.836</td>
</tr>
<tr>
<td>BP medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not prescribed</td>
<td>4546 [76.7]</td>
<td>1641 [75.5]</td>
<td>499 [76.4]</td>
<td>0.238</td>
<td>1860 [76.6]</td>
<td>546 [81.7]</td>
<td>0.005</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>86.3 (16.2)</td>
<td>96.0 (15.3)</td>
<td>93.0(18.3)</td>
<td>&lt;0.001</td>
<td>79.3 (11.0)</td>
<td>74.1 (11.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>3361 [57.1]</td>
<td>879 [40.8]</td>
<td>443 [68.4]</td>
<td>1377 [57.1]</td>
<td>662 [99.3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>1682 [28.6]</td>
<td>894 [41.5]</td>
<td>103 [15.9]</td>
<td>683 [28.3]</td>
<td>2 [0.3]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>839 [14.3]</td>
<td>381 [17.7]</td>
<td>102 [15.7]</td>
<td>&lt;0.001</td>
<td>353 [14.6]</td>
<td>3 [0.5]</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean (standard deviation) or number [percentage]. <sup>a</sup>P-values test for a difference between ethnic groups and were estimated using t-tests, except for 'female' which was compared using a chi-squared test. BP medication defined as ACE-inhibitors, angiotensin receptor blockers, alpha blockers, beta-blockers, calcium channel blockers or diuretics. Key: BMI = body mass index, BP = blood pressure, FPG = fasting plasma glucose, PG = plasma glucose.
Table 5.9 Prevalence of microalbuminuria according to people taking and not taking anti-hypertensive medications

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th>White European</th>
<th>South Asian</th>
<th>All</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total cohort (n= 5924)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGT</td>
<td>6.4</td>
<td>8.3</td>
<td>6.8</td>
<td>0.032</td>
</tr>
<tr>
<td>IGR</td>
<td>10.8</td>
<td>14.6</td>
<td>11.8</td>
<td>0.124</td>
</tr>
<tr>
<td>T2DM</td>
<td>21.0</td>
<td>11.8</td>
<td>17.7</td>
<td>0.110</td>
</tr>
<tr>
<td>All</td>
<td>7.4</td>
<td>9.6</td>
<td>7.9</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Not on anti-hypertensive medications (n=4546)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NGT</td>
<td>5.7</td>
<td>7.1</td>
<td>6.0</td>
<td>0.118</td>
</tr>
<tr>
<td>IGR</td>
<td>7.8</td>
<td>12.4</td>
<td>9.2</td>
<td>0.097</td>
</tr>
<tr>
<td>T2DM</td>
<td>24.7</td>
<td>9.5</td>
<td>19.1</td>
<td>0.047</td>
</tr>
<tr>
<td>All</td>
<td>6.3</td>
<td>8.0</td>
<td>6.7</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Data are percentages. P-values show the comparison between White Europeans and South Asians and were estimated using chi-squared tests. Key: IGR = impaired glucose regulation, NGT = normal glucose tolerance, T2DM = type 2 diabetes mellitus.
Table 5.10 A comparison of mean albumin: creatinine ratio (ACR) values in white European and South Asian men and women

<table>
<thead>
<tr>
<th></th>
<th>Ethnicity</th>
<th>Unadjusted&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adjusted&lt;sup&gt;a, b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean difference (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Sex</td>
<td>White</td>
<td>South Asian</td>
<td></td>
</tr>
<tr>
<td></td>
<td>European</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mg/mmol)</td>
<td></td>
<td>1.48 (5.94)</td>
<td>1.07 (0.99, 1.16)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>1.64 (9.71)</td>
<td>1.08 (1.01, 1.16)</td>
</tr>
</tbody>
</table>

<sup>a</sup> From linear regression models using log transformed ACR, thus mean differences are back transformed.  
<sup>b</sup> Adjusted for body mass index, HbA1c, FPG, age, creatinine, diastolic and systolic blood pressure, anti-hypertensive medication, 2 hour plasma glucose and smoking status.
Figure 5.1. The independent effect of ethnicity between White European and South Asian men in the relationship between urinary ACR and glycaemic variables (note ACR measured in mg/mmol).

(a) fasting plasma glucose in men

(b) 2 hour plasma glucose in men

(c) HbA1c in men
(d) fasting plasma glucose in women

(e) Two hour plasma glucose in women

(f) HbA1c in women
5.6 Discussion

5.6.1 Ethnicity and glycaemic markers

The main findings of the first section of chapter 5 suggest HbA1c values were independently higher in South Asians than White Europeans, even in analysis stratified by glucose intolerance status. Previous research has demonstrated HbA1c levels are independently higher in African Americans by 0.2 to 0.4%, Hispanic populations by 0.1 to 0.3% and South East Asians/ East Asians by 0.2 to 0.3% (Selvin et al., 2011; Herman et al., 2007; Herman et al., 2009, Ziemer et al., 2010; Saaddine et al., 2002; Table 5.11). The present analysis is the first to demonstrate this effect persisted in South Asians after adjusting for factors that may affect glycaemia or differed between the ethnic groups. The crude and adjusted HbA1c difference was approximately 0.2% higher in South Asians in all models tested. Furthermore, the same findings of independently higher values were observed in both FPG and two-hour plasma glucose with in South Asians of 0.15mmol/l and 0.75mmol/l respectively in adjusted analysis. This suggests all three glycaemic tests used in the diagnosis of T2DM are independently higher in South Asians, which may partly explain the higher prevalence of T2DM in this ethnic group, although other reasons are likely to exist.

There were many strengths of this analysis, including some improvements on previous reports (Selvin et al, 2011; Herman et al., 2007; Herman et al., 2009, Ziemer et al., 2010; Saaddine et al., 2002; Burden et al, 2009; Likhari et al.,
The cohort size included large numbers of both White Europeans and South Asians allowing detection of any clinically significant differences. A minority of South Asians may have been included in a similar analysis of the Diabetes Prevention Program, however due to low numbers; these participants were amalgamated with other Asian ethnic groups (Herman et al., 2007). A recent US based T2DM study suggested South Asians should be analysed separate from other Asian ethnic groups, to reduce potential masking of true population findings (Gupta et al., 2011). Secondly, in ADDITION-Leicester all participants underwent robust measurements of risk factors which included non-traditional risk factors such as fasting insulin.

The T2DM risk factors included in the multiple regression analysis explained 63.9% of the variation in HbA1c, which is relatively higher than other studies (Herman et al., 2007; Yudkin et al., 1990). However, there are other unmeasured factors influencing HbA1c which we cannot account for. Fasting and two hour plasma glucose levels may not give a robust representation of 24-hour glucose profile, a problem recognised in similar studies (Herman et al., 2007; Ziemer et al., 2010). While dietary intake may differ between ethnic groups, South Asians are not reported to have a more ‘unhealthy’ diet. Another example is iron deficiency anaemia (Kim et al., 2010). Therefore our finding that sex independently predicts HbA1c should be interpreted with caution. Other studies which accounted for either haematocrit or haemoglobin provide contradictory reports of an independent effect of sex on HbA1c (Herman et al., 2007; Ziemer et al., 2010). Within the present study, the higher HbA1c levels in South Asians of
0.2% were consistent when results in each ethnic group were separated in to males and females.

Ethnic variation in HbA1c levels could be attributed predominantly to biological variation in haemoglobin glycation and differential red cell survival. However African-Americans, who also possess higher HbA1c levels than White Europeans, simultaneously have more adverse profiles of glycaemic markers unaffected by haematological factors, including fructosamine, glycated albumin and 1,5 anhydroglucitol (Selvin et al., 2011). This suggests haematological factors do not explain HbA1c differences. The ADDITION-Leicester study was not designed for screening of haemoglobin or haemoglobinopathies, which are more prevalent in ethnic minority groups. However, our HbA1c analyser reported cases of potential haemoglobinopathies and such results were excluded from the baseline data. This included Haemoglobin S and C, but not haemoglobin E. Furthermore, there is some evidence that HbA1c is associated with certain genetic loci which may influence glycaemic control, suggesting a common genetic risk may explain the HbA1c differences in the present study (Snieder et al., 2001; Simonis-Bik et al., 2008; Cohen et al., 2006). However to our knowledge this evidence has not been evaluated specifically in South Asians compared to White Europeans.
**Table 5.11** HbA1c independent differences between ethnic groups and White Europeans

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>NGT</th>
<th>IGR or IGT only</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td>+0.13 to 0.2%</td>
<td>+0.26 to 0.4%</td>
<td>+ 0.3 to 0.56%</td>
</tr>
<tr>
<td>South East Asian/ Chinese</td>
<td>*</td>
<td>+ 0.22%</td>
<td>+ 0.32%</td>
</tr>
<tr>
<td>Hispanic (US)</td>
<td>*</td>
<td>+ 0.15%</td>
<td>+ 0.27%</td>
</tr>
<tr>
<td>South Asian</td>
<td>+ 0.2%</td>
<td>+ 0.2%</td>
<td>+ 0.2%</td>
</tr>
</tbody>
</table>

Data represents HbA1c independent differences, by adjusting for either fasting plasma glucose, mean plasma glucose, glucose area under the curve and/or two-hour plasma glucose, as well as various other risk factors which may include age, gender, one or more anthropometric measures, blood pressure, lipids, social deprivation, highest education level achieved, marital status and insulin levels where possible. Key: NGT = normal glucose tolerance, IGR = impaired glucose regulation, T2DM = Type 2 Diabetes Mellitus. * = no data available. Data taken from Herman et al., 2007; Herman et al., 2009; Ziemer et al., 2010; Selvin et al., 2011. South Asian data is taken from this chapter.

### 5.6.2 Ethnicity and microalbuminuria

The above information raises the question of whether ethnic specific diagnostic cut-points are required for T2DM (and IGR) in South Asians; however there should be evidence that complications of hyperglycaemia occur before the diagnosis of T2DM. The second part of the chapter 5 analysis suggests South
Asians have significantly higher levels of microalbuminuria than White Europeans overall, although the opposite trend was present in people with T2DM. South Asians were experiencing higher levels of microalbuminuria before the diagnostic cut-points for T2DM and less after the diagnosis of T2DM. Secondly, South Asian males had an independently higher mean ACR level of 1.2mg/mmol compared to White European males, however this was shown in line graphs (Figure 5.1) to be explained mainly by those with poorer glycaemic control for FPG and HbA1c. Before the diagnosis of T2DM, the same line graphs demonstrated there were no real differences in ACR between ethnic groups for both men and women. Overall, this suggests though the prevalence of microalbuminuria was significantly higher in South Asians before the diagnosis of T2DM, this only represented a small proportion of the total South Asian cohort without T2DM (less than one-quarter), therefore there could still be an overall finding that there was no ACR differences between ethnic groups before the diagnosis of T2DM.

The main limitations of the ACR analysis included microalbuminuria being defined using one spot ACR measurement which was not repeated within three to six months, as this was not the focus of the ADDITION study. Therefore we cannot confirm this is persistent microalbuminuria. Secondly, microalbuminuria is also a complication of raised blood pressure, as well as hyperglycaemia, and therefore the data presented on the microalbuminuria prevalence may be influenced by the effect of blood pressure. This is one potential disadvantage from using microalbuminuria in contrast to retinopathy where certain pathological lesions can be confirmed as diabetes specific without the need for invasive
procedures. However, the analysis of ACR differences included adjustment for systolic and diastolic blood pressure, as well as the presence of anti-hypertensive medications. Thirdly, the overall analysis was cross-sectional in design and investigated a point prevalence, rather than incidence, of microalbuminuria. However an important recent multi-centre studies using diabetes specific retinopathy to determine T2DM diagnostic cut-points were also cross-sectional (Colagiuri et al., 2010). Finally, lines graphs representing ACR differences did not include 95% confidence intervals, therefore I was not able to inspect for significant differences between ethnic groups, although this would have not impacted on the main results from the line graphs.

The strengths of this analysis included having ACR measurements for nearly 6000 individuals whilst undergoing an OGTT, ensuring there was a sample size to detect significant differences between ethnic groups. Additionally, ACR samples were taken at early morning visits, which is the preferred testing time for more accurate results. Similarly, as part of the advice for preparing for T2DM screening, participants were asked not to introduce lifestyle interventions to reduce glucose levels for three days prior to testing. Therefore it is less likely that participants performed any heavy exercise 24 hours before undertaking ACR measurements using SOPs. Finally, participants also underwent various risk factor measurements, meaning there was a robust group of confounder risk factors available when adjusting the ACR results, which is an improvement on some South Asian reports in this area (Fischbacher et al., 2003; Mather et al., 1998). This included blood pressure and medications (including ACE-inhibitors
angiotensin receptors blockers). In addition, two further important confounders included in the adjusted analysis were creatinine and body mass index. ACR measurements are thought to be less accurate in people with too little or too much muscle mass due to the variation in creatinine produced by the muscle (K/DOQI, 2002; Basi et al., 2008).

5.6.3 Implications for policy makers and clinicians

International organisations have recommended using ethnic specific cut-points for South Asians in relation to BMI, waist circumference and metabolic syndrome (WHO expert consultation, 2004; Snehalatha et al., 2003). The recommendations came as a response to the high rates of T2DM and its complications within this group. However there is no suggestion of ethnic specific cut-points for diagnosis of T2DM or IGR using any glycaemic measure (WHO, 2011). I have demonstrated that all three glycaemic measures used in the diagnosis of T2DM are independently higher in South Asians; furthermore despite this finding the prevalence of microalbuminuria is higher in South Asians before the diagnosis of T2DM. The data suggest that South Asians may require ethnic specific cut-points for diagnosis of T2DM where the cut-points would need to be reduced in comparison to White Europeans. However this would increase the prevalence of T2DM further in this ethnic group and secondly, it is not clear by what magnitude the cut-points would need reducing. In addition, these suggestions are based on
microalbuminuria and not diabetes specific retinopathy. Additionally, having a strategy of different diagnostic cut-points for various ethnic groups is a more complicated strategy than having one unifying cut-point. In contrast the main advantages from setting a lower T2DM diagnostic cut-point in South Asians is that it may help detect complications earlier and therefore implement strategies to preventing them from worsening or alternatively, offer opportunities to implement treatment strategies to delay progression to developing these complications. Overall more research is required to determine if ethnic specific cut-points are beneficial in South Asians for the three markers of glycaemia by assessing if using these cut-points, followed by appropriate risk factor intervention, reduces the incidence of hyperglycaemic complications in well-designed prospective outcome studies.

Secondly, it is reported that a greater proportion of South Asians with established T2DM do not achieve glycaemic guideline targets in comparison to White Europeans (Millet et al, 2009). As our study demonstrates independently higher HbA1c, fasting and 2-hour plasma glucose levels in South Asians, this result may be partially explained by factors not related to glycaemia. Therefore there are potential limitations in using HbA1c, fasting or 2-hour plasma glucose to measure quality of care or assess ethnic health disparities between these groups. Finally, microalbuminuria is also an important target for reducing total mortality in those with T2DM through intensive multi-risk factor management (Gaede et al., 2008). It is not known if the same result is applicable to people who develop microalbuminuria before the diagnosis of T2DM, however if this were shown, it
may suggest microalbuminuria should be screened for even in high risk populations (e.g. South Asians) even if T2DM is not present.

5.6.4 Conclusion to chapter 5

In this chapter I have demonstrated ‘one size does not necessarily fit all’ as South Asians had higher levels of HbA1c, fasting and 2-hour plasma glucose than White Europeans independent of factors affecting glycaemic control, and also microalbuminuria is more common in South Asians before the diagnosis of T2DM. Future research is required to determine if using lower ethnic diagnostic specific cut-points for T2DM in South Asians is beneficial or feasible. The first five chapters of this thesis have had a large focus on HbA1c in relation to T2DM. From Chapter 6 onwards, I will investigate alternate and novel risk markers for T2DM, starting with the triglyceride-to-HDL cholesterol ratio.
Chapter 6

Novel risk factors for Type 2 Diabetes: the Triglyceride-to-HDL cholesterol ratio and its association with insulin resistance
6.1 Chapter Overview

Chapters two to five focused on HbA1c and diagnostic changes associated with its use. From chapter six onwards, I will move away from HbA1c for diagnosis and focus on novel risk factors for T2DM; as there is a need to find simple ways to identify those at highest risk of future glucose intolerance and determine if they are more accurate than traditional methods. Here, I Investigate a novel lipid ratio formed by combining triglycerides and HDL cholesterol values. This work is based on a first author published paper (Mostafa et al., 2012c).

6.2 Introduction

The triglyceride-to-HDL cholesterol ratio has been investigated recently for various potential clinical uses in adult and paediatric populations (Di Bonito et al., 2012; He et al., 2012; Cordero et al., 2008; Musso et al., 2011; Zoppini et al., 2011; Gaziano et al., 1997; Giannini et al., 2011; Bittner et al., 2009; Li et al., 2008; Quijada et al., 2008; McLaughlin et al., 2003; Fan et al., 2007). Previous research has demonstrated its positive associations with adverse cardio-metabolic risk factor profiles, metabolic syndrome and prediction of incident T2DM or its complications (Di Bonito et al., 2012; He et al., 2012; Cordero et al., 2008; Musso et al., 2011; Zoppini et al., 2011; Gaziano et al., 1997; Giannini et al., 2011; Bittner et al., 2009; Li et al., 2008; Quijada et al., 2008; McLaughlin et
al., 2003; Fan et al., 2007). This may occur as the triglyceride-to-HDL cholesterol ratio demonstrates an association with insulin resistance (Giannini et al., 2011; Li et al., 2008; Quijada et al., 2008; McLaughlin et al., 2003; Fan et al., 2007). Therefore, it may form a convenient method of estimating levels of insulin resistance in comparison to time consuming glucose clamp techniques, and therefore aid detection of people at risk of developing future glucose intolerance.

Previous research has shown that the association between the lipid ratio and insulin resistance is not consistent in African-Americans, particularly women (Giannini et al., 2011; Sumner et al., 2005; Kim-Dorner et al., 2010; Bovet et al., 2006; Sumner et al., 2010). A similar non-significant association has been reported in a small study of South Asians but that study did not assess findings by sex (Gasevic et al., 2012). I wished to assess if the potential problem in African-American women extended to South Asian women or if the triglyceride-to-HDL cholesterol ratio was consistent in both sexes.

South Asians form over one-fifth of the world’s population, including over 4 million migrants living in both the US and UK. There are consistent reports of high levels of T2DM, IGR and CVD in South Asians, which is attributed predominantly to increased levels of insulin resistance (Mostafa et al., 2010; Mostafa et al., 2010; Hall et al., 2008; McKeigue et al., 1993). Therefore the triglyceride-to-HDL cholesterol ratio could form a valuable clinical tool in this ethnic group.

The aim of this study was to investigate the association between triglyceride-to-HDL cholesterol ratio and markers of insulin resistance in a western multi-ethnic
cohort of White European and South Asian men and women. Secondly, I wished to compare performance of the triglyceride-to-HDL ratio for detecting insulin resistance compared to using triglyceride alone.

6.3 Research Design and Methods

The analysis within chapter 6 uses the ‘insulin sub-sample’ from the ADDITION cross-sectional cohort; the study methods and laboratory assays are described in detail in Chapter 3. From the 892 participants in the insulin sub-sample, participants on lipid lowering therapy (n=157) or oral corticosteroids (n=6) were excluded from this analysis. From the remaining 729 individuals, 40 (15.6%) and 29 (11.6%) white European men and women respectively were detected as having T2DM, while these values were 19 (15.3%) and 13 (13%) in South Asians men and women. The corresponding values for glucose intolerance were 129 (50.6%) and 122 (48.8%) in White European men and women, with 54 (43.5%) and 50 (50%) in South Asian men and women. There were no significant differences in people with T2DM or glucose intolerance between these groups. There were no participants with a serum creatinine value greater than 140µmol/l, taking thyroid medications or with a self-reported history of liver disease.
6.4 Statistical analysis

SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis, except that XLSTAT version 2011.5 (Addinsoft, Paris, France) was used for ROC curve analysis. Baseline demographics of the analysed population were reported stratified by ethnic group and sex. The distribution of continuous variables was inspected for any outlying values, skewness and kurtosis. Means and standard deviations were reported for normally distributed variables. Non-normally distributed parameters were logarithmically transformed and results expressed as geometric mean with 95% confidence intervals. Discrete variables were analysed using chi-squared tests. A high triglyceride level was defined as ≥ 1.7mmol/l and low HDL level as <1.0mmol/l and <1.3mmol/l in men and women respectively (IDF, 2006).

Within each sex and ethnic group, the triglyceride-to-HDL cholesterol ratio was divided into thirds (tertiles) and tested for association with markers of insulin resistance using analysis of covariance modelling with adjustment for confounding variables. Insulin resistance was assessed using the following four markers. Homeostasis model assessment of insulin resistance (HOMA1-IR) was calculated as fasting glucose (mmol/l) x fasting insulin (µIU/ml) / 22.5 which correlates well with insulin resistance (Matthews et al., 1985). A HOMA1-IR value above 75th percentile was defined as insulin resistance, following similar use in other studies (Sumner et al., 2010). A second definition of insulin resistance consisted of a fasting insulin level above 75th percentile (hyperinsulinaemia) in
people without diabetes (Li et al., 2008; Balkau et al., 1999). Thirdly, quantitative insulin sensitivity check index (QUICKI) represents a strong measure of insulin sensitivity and was calculated using the formula: $\frac{1}{\log \text{insulin, } \mu IU/ml, + \log \text{glucose, mmol/l}}$ (Katz et al., 2000). Finally, the fasting glucose: fasting insulin ratio, a good marker of insulin sensitivity, was calculated (Legro et al., 1998). The covariates included in the model were selected from potential confounders using a backward elimination process with variables removed if they were not significant at the 5% level. Using this method, age, systolic blood pressure, body mass index and LDL-cholesterol were included in the model, while deprivation level, diastolic blood pressure, waist circumference and creatinine were excluded. The analysis was repeated with an interaction between sex and triglyceride-to-HDL cholesterol ratio tertiles after pooling male and female datasets in each ethnic group. Models were checked for absence of collinearity of variables using variance inflation factor and normality of residuals was tested with Shapiro-Wilk tests.

Finally, we analysed performance of the triglyceride-to-HDL cholesterol ratio to detect insulin resistance, as measured by the cohort HOMA-IR ≥ 75th percentile (3.08), by calculating the area under the receiver operating characteristic (AUROC) curve and its 95% confidence intervals (DeLong et al., 1998). An AUROC > 0.7 is generally considered an acceptable test performance whereas < 0.7 is an indication of weaker and unacceptable performance (Hosmer, 2000). The optimal cut-point was calculated as the best balance in a trade-off between sensitivity and specificity, using maximal values derived from the Youden Index.
(sensitivity + specificity - 1) (Perkins et al., 2005). A two-sided p-value of <0.05 was considered statistically significant.

6.5 Results

Baseline demographics of the cohort investigated are shown in Table 6.1. In both ethnic groups, women presented with a lower mean height, weight, waist circumference, diastolic blood pressure, creatinine, triglyceride-to-HDL cholesterol ratio and higher mean HDL level than men. Additionally in the White European group, women (n=250) demonstrated a lower mean fasting plasma glucose, HOMA1-IR and higher mean age and QUICKI level compared to men (n=255). Whereas in the South Asian group, women (n=100) presented with a lower mean systolic, total and LDL-cholesterol, triglyceride, non-HDL cholesterol and higher body mass index compared to men (n=124).

Incremental tertiles of the triglyceride-to-HDL cholesterol ratio in White European women and men and South Asian men demonstrated a significant positive association with insulin level, hyperinsulinaemia, HOMA1-IR level and HOMA1-IR≥ 75th percentile and a negative association with QUICKI (p-values in Table 6.2). However, based on smaller numbers there were no significant associations in South Asian women. When men and women in each ethnic group were pooled, the analyses indicated a significant interaction between triglyceride-to-HDL cholesterol ratio and sex in South Asians on insulin (p=0.02), HOMA-IR
(p=0.04) and QUICKI (p=0.03). The same analyses in White Europeans revealed no significant interactions (all p>0.2).

The AUROC curve for triglyceride-to-HDL cholesterol ratio to detect insulin resistance was 0.73 (95% confidence intervals 0.65 to 0.80), 0.71 (0.64 to 0.78), 0.74 (0.62 to 0.83) and 0.68 (0.56 to 0.80) in White European men and women, South Asian men and women respectively, figure 6.1. For triglyceride alone, the corresponding values were 0.71 (0.64 to 0.78), 0.68 (0.630 to 0.74), 0.73 (0.64 to 0.82) and 0.62 (0.51 to 0.74) respectively. The optimal triglyceride-to-HDL cholesterol ratio cut-points for detecting insulin resistance were 1.7 and 0.9 in mmol/l SI unit (equivalent of 3.8 and 2.0 in mg/dl conventional unit) in White European men and women respectively; whilst in South Asian men and women these were 1.2 and 1.1 in mmol/l (2.8 and 2.5 in mg/dl) respectively. When the same analysis was performed for triglyceride, the optimal cut-points were 1.8 and 1.4mmol/l (159 and 124mg/dl) in White European men and women; while in South Asian men and women these were both 1.6mmol/l (142mg/dl) respectively.
<table>
<thead>
<tr>
<th>Measure</th>
<th>White Europeans (n=505)</th>
<th>South Asian (n=224)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men (n=255)</td>
<td>Women (n=250)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Men (n=124)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Women (n=100)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.4 (9.0)</td>
<td>52.2 (10.4)</td>
</tr>
<tr>
<td></td>
<td>62.1 (8.9) †</td>
<td>21.0 (14.1-26.7)</td>
</tr>
<tr>
<td>Deprivation level score</td>
<td>14.7 (13.5-16.0)</td>
<td>19.6 (16.9-25.3)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 (0.1)</td>
<td>1.69 (0.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>90.9 (15.2)</td>
<td>77.0 (12.2)</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>29.5 (4.2)</td>
<td>27.0 (3.6)</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>104.0 (11.2)</td>
<td>96.4 (9.3)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>143.9 (18.1)</td>
<td>140.6 (18.0)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>88.7 (9.5)</td>
<td>88.7 (11.3)</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>94.0 (11.7)</td>
<td>94.7 (13.8)</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.4 (1.3-1.5)</td>
<td>1.3 (1.3-1.6)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.2 (1.2-1.3)</td>
<td>1.3 (1.2-1.4)</td>
</tr>
<tr>
<td>Triglyceride-to-HDL Chol ratio</td>
<td>1.2 (1.1-1.3)</td>
<td>1.3 (1.1-1.4)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.8 (0.9)</td>
<td>3.6 (0.9)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.9 (1.8)</td>
<td>5.5 (1.5)</td>
</tr>
<tr>
<td>2 hour plasma glucose (mmol/l)</td>
<td>7.6 (4.0)</td>
<td>8.0 (4.5)</td>
</tr>
<tr>
<td>Fasting Insulin (µIU/ml)</td>
<td>7.6 (7.0-8.2)</td>
<td>8.7 (7.8-9.8)</td>
</tr>
<tr>
<td>HOMA1-IR</td>
<td>1.9 (1.7-2.1)</td>
<td>2.2 (1.9-2.5)</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.62 (0.61-0.64)</td>
<td>0.60 (0.58-0.63)</td>
</tr>
<tr>
<td>% High triglyceride ≥1.7mmol/l</td>
<td>33.3</td>
<td>40.3</td>
</tr>
<tr>
<td>% Low HDL</td>
<td>11.4</td>
<td>16.9</td>
</tr>
</tbody>
</table>

Continuous variables presented as mean (standard deviation) or geometric mean (95% confidence intervals) after initial log transformation of non-normally distributed variables. Low HDL defined <1.0 and 1.3mmol/l in males and females respectively. * p<0.001, ‡ p<0.01, † p<0.05
### Table 6.2. The Association of triglycerides-to-HDL ratio (THR) tertiles with markers of insulin resistance (IR)

<table>
<thead>
<tr>
<th>Insulin marker</th>
<th>Tertile of THR</th>
<th>White European</th>
<th>South Asian</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Insulin, µIU/ml</td>
<td>Lowest</td>
<td>6.8 (6.1-7.7)</td>
<td>5.7 (5.1-6.4)</td>
<td>6.7 (5.6-8.0)</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>7.2 (6.5-8.0)</td>
<td>6.7 (5.9-7.5)</td>
<td>9.3 (7.9-11.1)</td>
</tr>
<tr>
<td></td>
<td>Highest</td>
<td>9.1 (8.1-10.3)</td>
<td>8.1 (7.1-9.1)</td>
<td>10.6 (8.9-12.6)</td>
</tr>
<tr>
<td>Hyperinsulinaemia, %</td>
<td>Lowest</td>
<td>14.3</td>
<td>6.3</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>17.3</td>
<td>13.7</td>
<td>20.0</td>
</tr>
<tr>
<td></td>
<td>Highest</td>
<td>36.1 ‡</td>
<td>30.9</td>
<td>35.5‡</td>
</tr>
<tr>
<td>HOMA1-IR</td>
<td>Lowest</td>
<td>1.7 (1.5-1.9)</td>
<td>1.4 (1.2-1.5)</td>
<td>1.7 (1.4-2.0)</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>1.8 (1.6-2.0)</td>
<td>1.6 (1.4-1.8)</td>
<td>2.3 (1.9-2.8)</td>
</tr>
<tr>
<td></td>
<td>Highest</td>
<td>2.5 (2.2-2.8) †</td>
<td>1.9 (1.7-2.2) ‡</td>
<td>2.7 (2.2-3.3) ‡</td>
</tr>
<tr>
<td>HOMA1-IR ≥ 75th</td>
<td>Lowest</td>
<td>14.6</td>
<td>9.6</td>
<td>12.2</td>
</tr>
<tr>
<td>percentile, %</td>
<td>Middle</td>
<td>13.8</td>
<td>24.1</td>
<td>19.5</td>
</tr>
<tr>
<td></td>
<td>Highest</td>
<td>46.5 †</td>
<td>41.7 *</td>
<td>42.9 ‡</td>
</tr>
<tr>
<td>QUICKI</td>
<td>Lowest</td>
<td>0.65 (0.62-0.67)</td>
<td>0.69 (0.66-0.72)</td>
<td>0.65 (0.62-0.69)</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.64 (0.62-0.66)</td>
<td>0.65 (0.62-0.68)</td>
<td>0.59 (0.56-0.62)</td>
</tr>
<tr>
<td></td>
<td>Highest</td>
<td>0.58 (0.56-0.6) *</td>
<td>0.63 (0.6-0.65) †</td>
<td>0.57 (0.54-0.60) ‡</td>
</tr>
<tr>
<td>Glucose: Insulin ratio</td>
<td>Lowest</td>
<td>0.83 (0.74-0.94)</td>
<td>0.92 (0.82-1.03)</td>
<td>0.84 (0.71-1.00)</td>
</tr>
<tr>
<td></td>
<td>Middle</td>
<td>0.77 (0.69-0.85)</td>
<td>0.82 (0.74-0.92)</td>
<td>0.61 (0.52-0.72)</td>
</tr>
<tr>
<td></td>
<td>Highest</td>
<td>0.67 (0.75-0.59) †</td>
<td>0.62 (0.56-0.70)*</td>
<td>0.53 (0.45-0.63) ‡</td>
</tr>
</tbody>
</table>

The cut-points for tertiles of THR were as follows. White European men: <0.83, 0.83 to 1.50, ≥1.51; White European women: < 0.67, ≥ 0.67 to < 1.14, ≥ 1.14; South Asian men: < 0.91, ≥ 0.91 to < 1.70, ≥ 1.70; South Asian women: < 0.73, ≥ 0.73 to <1.18, ≥ 1.18. Continuous variables presented as geometric mean (95% confidence intervals) including adjustments for age, BMI, LDL-cholesterol and SBP. P-values for trend across the tertiles: * p<0.001, ‡ p<0.01, † p<0.05. Key: HOMA1-IR = Homeostasis model assessment of insulin resistance, IR = Insulin resistance, QUICKI = quantitative insulin sensitivity check index THR = triglyceride-to-HDL ratio.
Figure 6.1 Receiver operating characteristic (ROC) curves of the triglyceride-to-HDL cholesterol ratio (THR) to detect insulin resistance, defined as cohort HOMA-IR $\geq 75^{\text{th}}$ percentile, in White European and South Asian men and women.
6.6 Discussion

The main findings of this study demonstrate that the triglyceride-to-HDL cholesterol ratio is associated with insulin resistance measures in White Europeans and South Asian men. However this relationship appeared to be less established in South Asian women. The overall test performance of triglyceride-to-HDL cholesterol ratio to detect insulin resistance was variable, with the 95% confidence intervals of the area under the ROC curve falling below the acceptable range in all four groups tested. Finally the optimal cut-points for triglyceride-to-HDL cholesterol ratio and triglyceride to detect insulin resistance were 0.9 to 1.7 and 1.4 to 1.8mmol/l respectively.

Previous studies have reported the triglyceride-to-HDL cholesterol ratio is a valid marker of insulin resistance, particularly in White populations (Giannini et al., 2011; Li et al., 2008; McLaughlin et al., 2003; Fan et al., 2007). However in African-Americans this lipid ratio can demonstrate a weak or non-significant association with insulin resistance, particularly in women (Giannini et al., 2011; Sumner et al., 2005; Kim-Dorner et al., 2010; Bovet et al., 2006; Sumner et al., 2010). The AUROC to detect the upper quartile of HOMA-IR in African-American women was 0.66 compared to 0.77 in African-American men (Sumner et al., 2010). African-Americans produce high levels of the enzyme lipoprotein lipase clearing triglyceride-rich lipoproteins from blood circulation, even in insulin resistant states (Després et al., 2000). Therefore a relatively lower triglyceride level is maintained. In Hispanic populations, the association between the lipid
ratio and insulin resistance needs further clarification as studies generally not stratified by sex show opposite findings (Giannini et al., 2011; Li et al., 2008; Quijada et al., 2008). Only one previous study in South Asians has analysed the relationship between the triglyceride-to-HDL cholesterol ratio with a single insulin resistance marker and failed to show an association (Gasevic et al., 2012). However the results were not stratified by sex. This study demonstrated a significant association in South Asian men but not women, which was also suggested by a significant sex interaction. Therefore, this appears to demonstrate a similar result to African-Americans. Why this occurs is not fully understood. It may involve the relatively high proportion of South Asian women (48%) classified as having a low HDL cholesterol level compared to White European women (24%, Table 6.1). Furthermore, the relative proportion of HDL subtypes is known to vary between these ethnic groups (Bhalodkar et al., 2004). Despite this novel finding, I acknowledge the number of South Asian women in the present study was limited to 100 and therefore results may be somewhat prone to power issues. The percentage of South Asian women with insulin resistance defined by fasting insulin and HOMA-IR values in the upper quartile appeared to show increasing trend across incremental triglyceride-to-HDL cholesterol ratio tertiles but neither result achieved statistical significance. Therefore these results should be considered as hypothesis generating and investigated further in well-designed prospective studies. Furthermore, other potential confounding factors cannot be accounted for which may have influenced the results. These include physical activity levels and dietary intake,
including alcohol consumption. In addition, the South Asian population was a mix of first and second generation immigrants. Finally, medications which affect insulin or HDL-cholesterol levels, such as hormone replacement therapy / post-menopausal oestrogens, were not known as only cardiovascular medications were electronically recorded in ADDITION-Leicester databases. However South Asian women were not over-represented in exclusions due to lipid lowering therapies; also there were no exclusions made due to out-lying insulin values in South Asian women.

I acknowledge the HOMA-IR values in the present study may appear to be lower than in some, but not all cohorts investigating the same topic (Di Bonito et al., 2012; Quijada et al., 2008; Sumner et al., 2010; Gasevic et al., 2012). One explanation may be the lack of an international standardisation of insulin assays, with the possibility of large inter-assay variation (Wallace et al., 2004; Robbins et al., 1996). Also most epidemiology studies use single insulin samples to calculate HOMA-IR values, possibly introducing further variation (Wallace et al., 2004).

There are a number of strengths of this study. I investigated a multi-ethnic cohort of White Europeans and South Asians, where all participants underwent phenotyping as well insulin measurements. Secondly, I reduced the large intra-individual variation of triglyceride by using fasting samples. Thirdly, the results were stratified by sex and tested for an interaction effect with triglyceride-to-HDL cholesterol ratio. Regarding potential limitations, I was unable to measure insulin resistance directly in this cohort of 729 individuals, a recognised problem in similar studies (Li et al., 2008; Quijada et al., 2008; Fan et al., 2007; Kim-Dorner
et al., 2010; Bovet et al., 2006; Gasevic et al., 2012). However I assessed four different validated or recommended markers of insulin resistance/sensitivity to reduce bias and inaccuracies from any one single marker. Secondly, lipid profiles and insulin levels were measured using single venous samples.

The study demonstrated the optimal cut-points for the triglyceride-to-HDL cholesterol ratio to detect insulin resistance were 1.7 and 0.9 in mmol/l international (SI) unit (or 3.8 and 2.0 in mg/dl conventional unit) for White men and women, and 1.2 and 1.1 mmol/l (2.8 and 2.5 mg/dl) for South Asian men and women. Previous studies have demonstrated the same optimal cut-points to detect insulin resistance are between 2.0 to 2.5 mg/dl in African-Americans and 3.0 mg/dl in both non-Hispanic Whites and Mexican-Americans without diabetes. Using international units, a separate study has reported optimal cut-points for detecting insulin resistance of 0.9, 1.1, 1.1 and 1.8 mmol/l for Aboriginal, Chinese, European and South Asian populations. The reported differences between optimal cut-points in various studies may represent a mix of ethnic differences and/or different methods employed, such as measuring insulin resistance directly or using HOMA-IR models.

6.7 Potential implications for clinical practice

The potential translational value of the triglyceride-to-HDL cholesterol ratio into clinical practice has yet to be determined. Triglycerides and HDL-cholesterol are included within diagnostic criteria of the metabolic syndrome separately. A combined lipid ratio may better reflect the overall interaction between lipid/
lipoprotein fractions, and therefore associations with insulin resistance (Millán et al., 2009). The study showed performance of triglyceride-to-HDL ratio to detect insulin resistance was stronger than either triglyceride alone. However the 95% confidence intervals of AUROCs suggested a sub-optimal performance in all sub-groups investigated. Furthermore, this study and others have demonstrated the triglyceride-to-HDL cholesterol ratio may not associate with insulin resistance in women from ethnic minority groups (Giannini et al., 2011; Sumner et al., 2010). This appears to be the first study to demonstrate the optimal cut-points for triglyceride level detecting insulin resistance in South Asians are lower than those used in the metabolic syndrome, also termed the insulin resistance syndrome (IDF, 2006). International organisations have recommended using lower ethnic specific cut-points for waist circumference and body mass index in the metabolic syndrome for South Asians in response to high levels of diabetes (WHO, 2004; WHO 2008). Future studies should explore if recommendations on lower ethnic specific triglyceride cut-points are required in South Asians.

6.8 Conclusion to chapter 6
The triglyceride-to-HDL cholesterol ratio is a novel tool for identify people with insulin resistance and therefore at risk of T2DM, however further evidence of is required to determine its utility, especially it may not add anything beneficial beyond using triglyceride itself in this cohort. There are other novel risk factors which can be investigated and I will undertake this in chapter 7.
Chapter 7

Novel risk factors for the progression of impaired glucose regulation to Type 2 diabetes: use of statins and liver function tests
7.1 Chapter Overview

In Chapters 3 to 5, I investigated HbA1c as a diagnostic tool for T2DM and IGR; then in Chapter 6 I investigated the triglyceride-to-HDL cholesterol ratio as a novel risk marker for insulin resistance. In Chapter 7, I continue the theme of investigating novel risk factors for T2DM but focus on novel risk factors for the progression of IGR to T2DM.

7.2.1 Introduction

IGR is a metabolic condition that develops when blood glucose levels rise above the normal defined range but below the threshold used to diagnose T2DM (WHO, 1999). Therefore people with this intermediate metabolic state are at increased risk of T2DM. The reported prevalence of IGR is approximately 19% in specific western populations and is expected to rise; furthermore its progression to T2DM is 5 to 10% annually (Unwin et al., 2002; Tabák et al., 2012; International Diabetes Federation, 2011). With such high rates of IGR expected in the future, there is a need to investigate novel risk factors for the progression of IGR to T2DM, as these may help to understand more about why this disease is increasing or may form a target for prevention of T2DM.

7.2.2 Statins and T2DM
The use of statins is well established as an effective therapy for primary and secondary prevention of CVD (Baigent et al., 2005; Baigent et al., 2010). However recent research has also demonstrated exposure to statin therapy may be associated with an increased risk of incident T2DM (Sattar et al., 2010; Preiss et al., 2011; Ridker et al., 2012; Culver et al., 2012; Wang et al., 2012). A meta-analysis of 13 trials reported statin exposure over an average of four years was associated with a 9% increased risk for future T2DM (odds ratio: 1.09, 95% confidence intervals 1.02 to 1.17) (Sattar et al., 2010). Participants recruited to these trials were generally at high CVD risk or had experienced prior CVD events. Less information is known about people with IGR, who are at high risk of developing T2DM. Furthermore, there is a lack of data from studies conducted in real world settings that were designed specifically for glycaemic outcomes. Secondly, it remains unclear why this increased risk for future T2DM was observed (Sattar et al., 2010). The nature of such an analysis was not able to include a comprehensive adjustment of confounding risk factors for T2DM. Furthermore, recent research has suggested use of statins may affect or interact with lifestyle factors, in particular physical activity levels (Mikus et al., 2013; Kokkinos et al., 2013). Therefore, the interaction between statins and lifestyle factors is of interest; however, these have not been investigated for T2DM outcomes (Kokkinos et al., 2013). As the incidence of T2DM and IGR are set to rise globally, it is likely that many people with IGR will be also prescribed statin therapy, as they also possess risk factors for CVD (International Diabetes
Federation, 2011; Mostafa et al., 2010). Therefore there is need to elucidate what impact this commonly prescribed therapy has on this high risk group.

7.2.3 Liver enzymes and T2DM

In addition to statins, liver enzymes levels are well known to predict incident T2DM independent of traditional risk factors associated with developing glucose intolerance (Vozarova et al., 2002; André et al., 2005; Lee et al., 2003; Perry et al., 1998; Nannipieri et al., 2005; Ford et al., 2008; Wannamethee et al., 2005; Sattar et al., 2004; Nakanishi et al., 2004; Fraser et al., 2009). Therefore there is recent interest regarding the value of these routinely measured hepatic enzymes possess in the future prediction of T2DM. Previous research in this area has focused on the general population; there is less information about whether hepatic enzymes independently predict progression to T2DM in high risk people with IGR. Secondly, it is not known whether baseline liver enzymes can predict reversion of IGR back to normal glucose levels. Thirdly, the magnitude of change of liver enzyme levels from baseline to follow-up corresponding to a diagnosis of T2DM or normal glucose tolerance has not been previously reported.

It is also debated which hepatic enzymes are the better predictors of T2DM. Alanine aminotransferase (ALT) and gamma-glutaryltransferase (GGT) both independently predict T2DM towards the upper end of their normal physiological ranges in most reports. However there is contrasting information on which of the two enzymes is the better predictor (Vozarova et al., 2002; André et al., 2005;
Lee et al., 2003; Nannipieri et al., 2005; Ford et al., 2008; Wannamethee et al., 2005; Fraser et al., 2009). Furthermore, a significant association may vary by ethnic group as noted by Wannamethee et al. and shown by data from Korea, Japan, and Hispanic populations (Lee et al., 2003; Nannipieri et al., 2005; Wannamethee et al., 2005; Nakanishi et al., 2004). Lee et al. suggested GGT had a stronger relationship with incident T2DM, not ALT; whereas Nannipieri et al. and Nakanishi et al. both suggested GGT is an independent predictor of future T2DM, but not ALT. In contrast Vozarova et al., suggested ALT is the better predictor of incident T2DM in White Europeans.

The relationship of liver enzymes and incident T2DM is generally not well reported in non-white Europeans and has not been investigated to date in South Asians, an ethnic group with a high burden of T2DM and IGR (Chapter 2 and 3). Within this in mind, there is a need to identify those at highest risk of incident T2DM; liver function enzymes may form an additional tool for this purpose.

The aim of this chapter was to investigate (a) the effect of statin therapy on the progression from IGR to T2DM after appropriate adjustment for confounders. In addition, I wished to analyse whether the relationship between statin use and progression to T2DM was modified by lifestyle factors, specifically physical activity and body mass index.

(b) Secondly, I investigated whether baseline liver function tests independently predict progression from intermediate hyperglycaemia to diabetes or reversion to
normal glucose tolerance in a western contemporary multi-ethnic cohort. In addition, I analysed the magnitude of change in hepatic enzymes levels from baseline to final follow-up for each respective diagnosis.

7.3 Research Design and Methods

The analysis within Chapter 7 uses longitudinal data from a subset of the ADDITION study called the IGR cohort. The study methods, formation of this prospective subset and laboratory assays are all described in detail in Chapter 3. Figure 7.1 is an extension of Figure 3.0 and outlines how the IGR cohort was formed and specifically how many people were present in each analysis.

7.4 Statistical analysis

7.4.1 Statins

The statistical analysis for the statins topic was initially performed by me, but final analyses was led by Dr Danielle Morris. Stata version 12.1 (StataCorp, Texas, US) was used to perform statistical analysis. Baseline characteristics were reported in the total cohort of 910 people with IGR and then stratified by use or non-use of statin therapy at baseline. Means and standard deviations were reported for normally distributed variables, with non-statin and statin users
compared using t-tests. Non-normally distributed continuous parameters were expressed as median (inter-quartile range) and groups were tested using Kruskal-Wallis tests. For categorical variables, number (percentage) was reported and groups compared using $\chi^2$ tests.

The number of incident T2DM cases and total person-years at risk were calculated separately for each category of statin use at baseline. People contributed person-years from baseline until their last annual screening test. Cox proportional hazards models were used to calculate hazard ratios with 95% confidence intervals for the risk of progression to T2DM for statin use at baseline, compared to non-statin use, with T2DM as the outcome of interest and censoring of people who did not develop T2DM. Initially, an unadjusted hazard ratio was estimated. Following this, adjustment was made for each potential confounder in turn so that its effect on the association between statin use and T2DM could be seen. The potential confounders considered were fasting and 2-hour plasma glucose, HDL-cholesterol, triglycerides, body mass index, history of CVD, physical activity, social deprivation, age, ethnicity, sex, hypertension (defined as systolic blood pressure $\geq 140$mmHg, diastolic blood pressure $\geq 90$mmHg or taking ACE-inhibitors, beta-blockers, calcium channel blockers or diuretics) and family history of T2DM. The best fitting model was then found using forward selection with the entry criteria set to significance at 5%. The adjusted hazard ratio for statin use at each step of the forward selection process is shown. An interaction analysis was performed between lifestyle factors, defined as physical activity and body mass index (both as continuous variables), and use of statins in
relation to T2DM outcomes. In all models, missing data were replaced using multiple imputation methods, thus all models included 910 observations. Continuous variables were imputed using predictive mean matching and binary variables using logistic regression. A two-sided p-value of <0.05 was considered statistically significant throughout these analyses.

7.4.2 Liver enzymes

From the IGR sub-cohort of 910 participants, 26 people with an alcohol intake of exceeding 25 units (250 grams) per week were excluded, following similar exclusions in other studies (Nannipieri et al., 2005), Figure 7.1. From the remaining 884 people, 765 had a baseline ALT measurement available, however one individual had an ALT result greater than three times the local upper limit reference limit (159IU/L) and was excluded, following similar exclusions in previous reports, leaving 764 people for the final analysis of ALT (Vozarova et al., 2002; Wannamethee et al., 2005; Nakanishi et al., 2004). Of the 884 participants, 639 people also had a GGT test result available, with 16 people excluded for having a GGT level greater than three times the local upper reference range limit (150IU/L), leaving the final GGT population analysed at 623 people. All of those with a GGT measurement also had ALT measured.
SPSS version 20.0 (IBM SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. Baseline characteristics of the analysed population were reported as total cohort first and then stratified by ethnic group consisting of White Europeans (n=453) and South Asians (n=159). The remaining 152 people were not analysed because they were ethnic groups with low numbers or they did not disclose their ethnic group. Means and standard deviations were reported for normally distributed variables; non-normally distributed parameters were reported as median (interquartile range). Ethnic groups were compared using chi-squared tests for discrete variables and t-tests for continuous variables. ALT and GTT were divided into tertiles (threes). Cox proportional hazards models were used to calculate hazard ratios with 95% confidence intervals for the risk of progression to T2DM or reversion to normal glucose tolerance in each tertile, with censoring of people who did not develop the outcome of interest. The analysis was performed in the total cohort, and then stratified by White Europeans and South Asians. Unadjusted and adjusted hazard ratios were calculated. The covariates included in the adjusted model were selected from potential confounders added to ALT using a stepwise forward selection process if they were significant at the 5% level. The process was then repeated for GGT. The covariates considered were age, sex, ethnicity, social deprivation, family history of T2DM, alcohol intake, smoking body mass index, HDL-cholesterol, triglycerides, systolic and diastolic blood pressure and use of statins. A two-sided p-value of <0.05 was considered statistically significant throughout these analysis.
Figure 7.1 Participants used in chapter 7 analyses

ADDITION-Leicester T2DM screening study of 6749 people from 2004 to 2009

1080 (16.1%) people detected with IGR

Invited for annual re-screening visit

912 (84.4%) entered cohort study, i.e. attended at least one re-screen

910 (99.8%) participants in IGR cohort

Declined further invitation for cohort study = 168 (15.6%)

Missing data on OGTT outcome = 2 (0.2%)

Statins analysis

143 (15.7%) developed T2DM

Liver function test analysis

767 (84.3%) were T2DM free after average follow-up of 3.5 years

884 people

26 people with alcohol intake > 25 units / week excluded

765 had a baseline ALT value recorded

639 had a baseline GGT value recorded

1 person with ALT > 3 times upper reference range limit excluded

16 people with GGT > 3 times upper reference range limit excluded

764 people in ALT analysis

623 people in GGT analysis
7.5 Results

7.5.1 Statins results

The baseline characteristics of the 910 individuals in this cohort are shown in Table 7.1. The median age was 61 years, 47.8% of the participants were men, 69.3% were White European, and 27.7% were South Asian. There were 134 (14.7%) people on statin therapy at baseline, of whom 102 (76%) were still using therapy at final follow-up. Among these 134 people there were 49% taking Simvastatin, 42% on Atorvastatin and 9% on other statins (Rosuvastatin, Fluvastatin and Pravastatin). In contrast, there were 776 (85.3%) people not taking statin therapy at baseline, of whom 642 (83%) remained a non-statin user at final follow-up. Statin users were more likely to be older (p<0.001), male (p<0.01), have a past history of CVD (p<0.001) and hypertension (p<0.001), HDL (p=0.01) and LDL (p<0.001) cholesterol and triglycerides (p=0.04).

The median follow up was 3.5 years (interquartile range 2.2 to 4.5 years) with 3014 person-years of follow up. By final follow-up, 145 (15.7%) people had progressed to T2DM. Among non-statin users, 127 developed T2DM over 2588.1 person-years of follow-up (incidence rate: 49.1 per 1000 person-years; 95% confidence interval 41.2, 58.4). Among statin users, 18 developed T2DM over 426.1 person-years of follow-up (incidence rate: 42.2 per 1000 person-years; 26.6, 67.1). The hazard ratio of developing T2DM associated with baseline statin
use was non-significantly lower in unadjusted analyses (0.87, 0.53 to 1.43, Table 7.2). On adjusting for potential confounding variables individually, the hazard ratios suggested use of statins at baseline was associated with a lower risk of incident T2DM compared with non-statin use, but was not non-statistically significant (Table 7.2). The best fitting model (Table 7.3) included terms for FPG, triglycerides, 2-hour plasma glucose, body mass index, CVD history, HDL-cholesterol and physical activity. Table 7.3 shows each step of the forward selection process; the last model was adjusted for all of these confounders, but still demonstrated a non-significantly lower risk for T2DM among statin users, 0.60 (0.32 to 1.12). This model was also restricted to people who did not change their statin user category (n=744) which produced a hazard ratio of 0.33 (0.15 to 0.72, p=0.005).

7.5.2 Interaction analysis

There was a significant interaction between statin use and physical activity (p=0.04). To investigate this relationship further, we performed the analyses stratified by physical activity (low levels: <28 METS hours/ week, high levels: ≥ 28 METS hours/ week, based on the median level) with adjustment for the confounders selected during the forward selection process, but not physical activity. For the low METS category, the unadjusted hazard ratio associated with statin use was non-significantly lower, 0.49 (0.19 to 1.25; p=0.136), however
upon adjusting for confounders this became significant, 0.13 (0.04 to 0.40; p<0.001). For the high METS category, both the unadjusted and adjusted hazard ratios associated with statin use were not significant, 1.29 (0.63 to 2.63; p=0.489) and 0.98 (0.32 to 3.00; p=0.963) respectively. In contrast, there was no significant interaction between statin use and body mass index (p=0.804) and therefore I did not investigate this further.

7.5.3 Sensitivity analysis

To investigate the effect of the multiple imputation, the analyses in Table 7.3 were repeated without imputing missing data. As before, statin use at baseline was associated with a reduced risk of T2DM, but this was not significant after adjustment for FPG, triglycerides, 2-hour plasma glucose, body mass index, history of CVD and HDL-cholesterol (0.60, 0.32 to 1.13, p=0.112). However, when physical activity was added to this model the hazard ratio became significant, 0.37 (0.18 to 0.77), p=0.008. Furthermore, in the sensitivity analyses, the interaction of statin use with physical activity remained significant (p=0.04), and with body mass index remained non-significant (p=0.804). The adjusted hazard ratios for the low and high physical activity categories were similar to the main analyses, 0.13 (0.04 to 0.39; p<0.001) and 0.97 (0.32 to 2.99; p=0.963) respectively.
Table 7.1 Selected baseline characteristics of the total cohort and stratified by statin use at baseline

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<tr>
<th></th>
<th>N missing</th>
<th>All</th>
<th>Non-statin users</th>
<th>Statin users</th>
<th>P-value a</th>
</tr>
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<td>60 (52-68)</td>
<td>66 (60-70)</td>
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<tr>
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<td></td>
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</tr>
<tr>
<td>Male</td>
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<td>435 (47.8)</td>
<td>357 (46.0)</td>
<td>78 (58.2)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
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<td>475 (52.2)</td>
<td>419 (54.0)</td>
<td>56 (41.8)</td>
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</tr>
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<td>Ethnicity</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>533 (69.3)</td>
<td>431 (67.9)</td>
<td>102 (76.1)</td>
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<tr>
<td>South Asian</td>
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<td>213 (27.7)</td>
<td>184 (29.0)</td>
<td>29 (21.6)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
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<td>23 (3.0)</td>
<td>20 (3.2)</td>
<td>3 (2.2)</td>
<td>0.170</td>
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<tr>
<td>IMD Score b</td>
<td>37</td>
<td>16.8 (9.8-26.9)</td>
<td>16.8 (10.4-26.9)</td>
<td>15.0 (7.3-26.1)</td>
<td>0.122</td>
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<tr>
<td>BMI, kg/m²</td>
<td>148</td>
<td>29.7±5.2</td>
<td>29.6±5.4</td>
<td>30.3±4.3</td>
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<td>IFG only</td>
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<td>163 (17.9)</td>
<td>136 (17.5)</td>
<td>27 (20.2)</td>
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<td>619 (68.0)</td>
<td>531 (68.4)</td>
<td>88 (65.7)</td>
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<td>128 (14.1)</td>
<td>109 (14.1)</td>
<td>19 (14.2)</td>
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<td>Type of statin</td>
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<td></td>
<td></td>
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<td></td>
<td>776 (86.1)</td>
<td>776 (100.0)</td>
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<td>61 (48.8)</td>
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<td>Atorvastatin</td>
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<td>53 (42.4)</td>
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<tr>
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<td>LDL-cholesterol, mmol/l</td>
<td>16</td>
<td>3.5±1.0</td>
<td>3.6±0.9</td>
<td>2.6±0.7</td>
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<td>HDL-cholesterol, mmol/l</td>
<td>7</td>
<td>1.3±0.4</td>
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<td>1.2±0.4</td>
<td>0.011</td>
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<td>5</td>
<td>1.4 (1.0-1.9)</td>
<td>1.4 (1.0-1.9)</td>
<td>1.5 (1.1-2.2)</td>
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</tr>
<tr>
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<td>186 (29.5)</td>
<td>7 (5.2)</td>
<td>&lt;0.001</td>
</tr>
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<td></td>
<td>572 (74.8)</td>
<td>445 (70.5)</td>
<td>127 (94.8)</td>
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</tr>
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<td>Family history of T2DM</td>
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<td></td>
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<td></td>
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<td>484 (62.4)</td>
<td>85 (63.4)</td>
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<td>341 (37.5)</td>
<td>292 (37.6)</td>
<td>49 (36.6)</td>
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<td>History of CVD</td>
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<tr>
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<td>2 (1.5)</td>
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<td>Yes</td>
<td></td>
<td>394 (43.3)</td>
<td>262 (33.8)</td>
<td>132 (98.5)</td>
<td></td>
</tr>
<tr>
<td>Physical activity, mets-hours/week</td>
<td>283</td>
<td>1685 (396-4158)</td>
<td>1782 (462-4158)</td>
<td>1337 (297-2970)</td>
<td>0.064</td>
</tr>
<tr>
<td>Total</td>
<td>910</td>
<td>776 (100.0)</td>
<td>134 (100.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Data are n (%), mean ± standard deviation or median (Inter-quartile range). Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IMD, Index of multiple deprivation.

a P-values compare statin users and non-statin users and were estimated using $\chi^2$ tests for categorical variables, t-tests for normally distributed variables and Kruskal-Wallis for non-normal variables. b A measure of social deprivation. Higher scores suggest higher deprivation. c Defined as systolic blood pressure ≥140mmHg, diastolic blood pressure ≥90mmHg or taking ACE-inhibitors, beta-blockers, calcium channel blockers or diuretics.
Table 7.2 Hazard ratios showing the risk of conversion from IGR to T2DM associated with statin use at baseline in unadjusted form and then with single adjustments for individual risk factors.

<table>
<thead>
<tr>
<th>Model</th>
<th>HR(^a)</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>0.87</td>
<td>0.53, 1.43</td>
<td>0.593</td>
</tr>
<tr>
<td><strong>Past medical history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for past CVD event</td>
<td>0.59</td>
<td>0.32, 1.11</td>
<td>0.101</td>
</tr>
<tr>
<td>Adjusted for family history of diabetes</td>
<td>0.87</td>
<td>0.53, 1.43</td>
<td>0.588</td>
</tr>
<tr>
<td><strong>Glucose variables</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for fasting plasma glucose</td>
<td>0.80</td>
<td>0.49, 1.30</td>
<td>0.365</td>
</tr>
<tr>
<td>Adjusted for 2-hour plasma glucose</td>
<td>0.87</td>
<td>0.53, 1.43</td>
<td>0.588</td>
</tr>
<tr>
<td><strong>Lifestyle factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for body mass index</td>
<td>0.86</td>
<td>0.53, 1.42</td>
<td>0.564</td>
</tr>
<tr>
<td>Adjusted for physical activity</td>
<td>0.89</td>
<td>0.54, 1.46</td>
<td>0.640</td>
</tr>
<tr>
<td><strong>Biomedical factors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for hypertension</td>
<td>0.86</td>
<td>0.52, 1.42</td>
<td>0.562</td>
</tr>
<tr>
<td>Adjusted for HDL-cholesterol</td>
<td>0.80</td>
<td>0.49, 1.31</td>
<td>0.375</td>
</tr>
<tr>
<td>Adjusted for triglycerides</td>
<td>0.84</td>
<td>0.51, 1.38</td>
<td>0.501</td>
</tr>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for age</td>
<td>0.97</td>
<td>0.59, 1.61</td>
<td>0.916</td>
</tr>
<tr>
<td>Adjusted for ethnicity</td>
<td>0.91</td>
<td>0.56, 1.49</td>
<td>0.713</td>
</tr>
<tr>
<td>Adjusted for sex</td>
<td>0.86</td>
<td>0.53, 1.42</td>
<td>0.566</td>
</tr>
<tr>
<td>Adjusted for IMD</td>
<td>0.89</td>
<td>0.54, 1.46</td>
<td>0.644</td>
</tr>
</tbody>
</table>

Missing data were imputed; therefore there were 910 observations for each model.
Table 7.3. Hazard ratios (HR) adjusted for baseline confounders in a stepwise forward selection model

<table>
<thead>
<tr>
<th>Confounders included in the model</th>
<th>HR of statins vs. non-statins</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose</td>
<td>0.80</td>
<td>0.49, 1.30</td>
<td>0.365</td>
</tr>
<tr>
<td>Above plus triglycerides</td>
<td>0.78</td>
<td>0.48, 1.28</td>
<td>0.332</td>
</tr>
<tr>
<td>Above plus 2-hour plasma glucose</td>
<td>0.80</td>
<td>0.49, 1.32</td>
<td>0.387</td>
</tr>
<tr>
<td>Above plus body mass index</td>
<td>0.83</td>
<td>0.50, 1.36</td>
<td>0.454</td>
</tr>
<tr>
<td>Above plus CVD history</td>
<td>0.63</td>
<td>0.33, 1.18</td>
<td>0.147</td>
</tr>
<tr>
<td>Above plus HDL-cholesterol</td>
<td>0.58</td>
<td>0.31, 1.08</td>
<td>0.087</td>
</tr>
<tr>
<td>Above plus physical activity</td>
<td>0.60</td>
<td>0.32, 1.12</td>
<td>0.110</td>
</tr>
</tbody>
</table>

Only significant confounders were left in the final model. Missing data were imputed; therefore there were 910 observations for each model.
7.5.4 Liver enzymes

Baseline characteristics of the cohort analysed are described in Table 7.4. In the total cohort, the mean age and body mass index were 60.1 years and 29.7 kg/m$^2$ respectively, 46.6% were male, 59.3% of white European origin and 20.8% South Asian. The mean cohort ALT and GGT were 28.4 IU/L (SD 17.5) and 34.3 IU/L (22.1) respectively. On stratification by ethnic group, South Asians were younger (p<0.001), had higher levels of social deprivation (p<0.001) and had lower levels of HDL-cholesterol (p<0.001), systolic blood pressure (p<0.001), alcohol consumption (p<0.001) and ALT (p<0.05) than White Europeans. Over a median of 3.6 years (total 3022 person-years follow-up of), 124 (15.5%) people progressed to T2DM (incidence of 41 per 1000 person-years), corresponding to 61 (12.8%, 39 per 1000 person-years) and 41 (24.1%, 76 per 1000 person-years) of White Europeans and South Asians over 1554.9 and 537.0 person-years follow-up respectively. In contrast, 353 (44.2%) of the total cohort reverted back to normal glucose tolerance, including 224 (47.1%) and 62 (36.5%) of White Europeans and South Asians respectively.

When analysing the risk of T2DM according to incremental tertiles of ALT levels in the total cohort, there was a significantly higher risk in the upper tertile compared to the lowest, with hazard ratios of 1.69 (95% confidence intervals 1.08 to 2.64, p=0.021) and 1.74 (1.05 to 2.89, p=0.031) for unadjusted and after adjusting for triglycerides, ethnicity and body mass index respectively (Table 7.5). In White Europeans the same unadjusted and adjusted hazard ratios were 4.33
(1.92 to 9.79, p<0.001) and 3.35 (1.47 to 7.64, p=0.004) without adjusting for ethnicity. However there was no significantly increased risk for T2DM in South Asians across incremental ALT tertiles. The rate of reversion back to normal glucose tolerance in white Europeans was significantly lower in the highest ALT tertile compared to lowest, with hazard ratios of 0.62 (0.44 to 0.88, p=0.007) and 0.66 (0.47 to 0.94, p=0.02) for unadjusted and after adjusting for HDL-cholesterol respectively. These same hazard ratios were not significantly lower for the total cohort or for South Asians.

The highest tertile of GGT was associated with an increased risk of T2DM compared to the lowest tertile in the total cohort, hazard ratios of 2.08 (1.22 to 3.54, p=0.007) and 1.97 (1.15 to 3.35, p=0.013) for unadjusted data and after adjusting for HDL-cholesterol respectively (Table 7.6). In White Europeans, a similar pattern was observed where the highest tertile had hazard ratios of 3.22 (1.19 to 8.73, p=0.022) and 2.81 (1.04 to 7.64, p=0.043) for unadjusted data and after adjusting for HDL, respectively. The same results were not significant in South Asians. Incremental tertiles of GGT levels were not associated with significantly reduced rate of reverting back to normal glucose tolerance in any of the populations analysed.

Finally, on analysing the change in liver enzymes levels from baseline to final follow-up in white Europeans, there was a significant decrease in mean ALT and GGT levels of 2.1 and 3.3IU/L (p=0.046 and 0.015 respectively) for those reverting back to normal glucose tolerance, but only non-significant increases for those who developed incident T2DM (Table 7.7). South Asians demonstrated a
similar significant decrease of mean ALT and GGT of 5.3 and 5.9IU/L (p=0.002 and 0.004) for reversion back to normal glucose; however for incident T2DM there was also a significant increase in ALT only of 7.0IU/L (p=0.002).
Table 7.4 Baseline characteristics of the total cohort and stratified by ethnic group.

<table>
<thead>
<tr>
<th></th>
<th>Total (n=764)</th>
<th>WE (n=453)</th>
<th>SA (n=159)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.2 (9.9)</td>
<td>62.5 (8.4)</td>
<td>53.2 (11.0)***</td>
</tr>
<tr>
<td>% male</td>
<td>46.6</td>
<td>44.8</td>
<td>49.1</td>
</tr>
<tr>
<td>Social deprivation</td>
<td>16.6 (17.4)</td>
<td>14.8 (14.0)</td>
<td>23.4 (15.0)***</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>29.7 (5.3)</td>
<td>30.0 (5.1)</td>
<td>29.0 (5.4)</td>
</tr>
<tr>
<td>Alcohol (units/week)</td>
<td>3.4 (5.7)</td>
<td>5.0 (6.2)</td>
<td>1.8 (4.5) ***</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.3 (0.4)</td>
<td>1.3 (0.4)</td>
<td>1.2 (0.3) ***</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.4 (0.9)</td>
<td>1.4 (1.0)</td>
<td>1.4 (0.9)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>143.2 (20.1)</td>
<td>145.3 (20.3)</td>
<td>137.2 (18.0)***</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>87.5 (10.4)</td>
<td>87.4 (10.4)</td>
<td>87.8 (10.7)</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>28.4 (17.5)</td>
<td>29.0 (17.4)</td>
<td>25.5 (16.5)*</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>34.3 (22.1)</td>
<td>35.2 (22.9)</td>
<td>31.6 (20.3)</td>
</tr>
<tr>
<td>% Current smoker</td>
<td>7.9</td>
<td>8.7</td>
<td>5.1</td>
</tr>
<tr>
<td>% statin user</td>
<td>18.8</td>
<td>21.0</td>
<td>14.5</td>
</tr>
</tbody>
</table>

p<0.05 *, p<0.01 **, p<0.001***. Data presented are mean (standard deviation), median [interquartile range] (indicated by a), or %. Key: BP, blood pressure; ALT, alanine aminotransferase; GGT, gamma-glutaryltransferase.
Table 7.5 The risk of T2DM or reversion to normal glucose tolerance in ALT tertiles

<table>
<thead>
<tr>
<th>Final diagnosis on last OGTT</th>
<th>Model</th>
<th>Lowest tertile</th>
<th>Middle tertile</th>
<th>Highest tertile</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Cohort</strong></td>
<td>Unadjusted</td>
<td>31 (12.8)</td>
<td>36 (13.7%)</td>
<td>51 (19.9)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>1.00 (0.58-1.74)</td>
<td>1.74 (1.05-2.89) *</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>White European</td>
<td>Unadjusted</td>
<td>9 (6.0)</td>
<td>14 (9.4)</td>
<td>38 (21.8)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>1.89 (0.78-4.60)</td>
<td>4.34 (1.92-9.79) ***</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>South Asian</td>
<td>Unadjusted</td>
<td>18 (27.7)</td>
<td>0.77 (0.37-1.60)</td>
<td>0.87 (0.39-1.93)</td>
<td>0.773</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>0.734 (0.35-1.56)</td>
<td>0.80 (0.36-1.80)</td>
<td>0.703</td>
<td></td>
</tr>
<tr>
<td><strong>Normal glucose tolerance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total Cohort</strong></td>
<td>Unadjusted</td>
<td>124 (50.6)</td>
<td>123 (46.8)</td>
<td>98 (38.3)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>0.95 (0.74-1.22)</td>
<td>0.82 (0.63-1.07)</td>
<td>0.321</td>
<td></td>
</tr>
<tr>
<td>White European</td>
<td>Adjusted</td>
<td>79 (59.4)</td>
<td>0.87 (0.66-1.14)</td>
<td>0.535</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>0.98 (0.77-1.23)</td>
<td>0.87 (0.66-1.14)</td>
<td>0.535</td>
<td></td>
</tr>
<tr>
<td>South Asian</td>
<td>Unadjusted</td>
<td>27 (36.0)</td>
<td>17 (36.2)</td>
<td>18 (43.9)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>1.01 (0.56-1.84)</td>
<td>1.35 (0.73-2.53)</td>
<td>0.585</td>
<td></td>
</tr>
</tbody>
</table>

P<0.05 *, p<0.01 **, p<0.001 ***. The lowest, middle and highest tertiles for ALT were < 20, 20 to < 29 and ≥ 29 IU/L respectively. Models were adjusted for triglycerides, body mass index and ethnicity (the latter for total cohort only) for diabetes models and HDL-cholesterol for normal glucose tolerance.
Table 7.6 The risk of T2DM or reversion to normal glucose tolerance in GGT tertiles

<table>
<thead>
<tr>
<th>Final diagnosis on last OGTT</th>
<th>Model</th>
<th>Lowest tertile</th>
<th>Middle tertile</th>
<th>Highest tertile</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diabetes</strong></td>
<td>Total Cohort</td>
<td>N (%)</td>
<td>21 (10.4)</td>
<td>34 (16.0)</td>
<td>41 (18.2)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Reference</td>
<td>1.71 (0.99-2.95)</td>
<td>2.08 (1.22-3.54)**</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>1.59 (0.92-2.75)</td>
<td>1.97 (1.22-3.54)**</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>White European</td>
<td>N (%)</td>
<td>5 (11.9)</td>
<td>18 (42.9)</td>
<td>19 (45.2)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Reference</td>
<td>3.01 (1.12-8.10) *</td>
<td>3.22 (1.19-8.73) *</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>2.53 (0.93-6.83)</td>
<td>2.81 (1.04-7.64) *</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>South Asian</td>
<td>N (%)</td>
<td>11 (19.0)</td>
<td>12 (32.4)</td>
<td>11 (23.9)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Reference</td>
<td>1.87 (0.83-4.25)</td>
<td>1.37 (0.58-3.20)</td>
<td>0.323</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>1.89 (0.83-4.30)</td>
<td>1.36 (0.58-3.20)</td>
<td>0.311</td>
</tr>
<tr>
<td><strong>Normal glucose tolerance</strong></td>
<td>Total Cohort</td>
<td>N (%)</td>
<td>102 (50.7)</td>
<td>97 (46.9)</td>
<td>83 (38.6)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Reference</td>
<td>0.95 (0.71-1.25)</td>
<td>0.96 (0.72-1.28)</td>
<td>0.916</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>0.97 (0.73-1.29)</td>
<td>0.98 (0.73-1.31)</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>White European</td>
<td>N (%)</td>
<td>55 (57.3)</td>
<td>65 (49.6)</td>
<td>50 (42.0)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Reference</td>
<td>0.95 (0.66-1.37)</td>
<td>0.89 (0.60-1.30)</td>
<td>0.827</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>0.97 (0.68-1.40)</td>
<td>0.91 (0.62-1.33)</td>
<td>0.872</td>
</tr>
<tr>
<td></td>
<td>South Asian</td>
<td>N (%)</td>
<td>24 (41.4)</td>
<td>14 (37.8)</td>
<td>14 (31.1)</td>
</tr>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Reference</td>
<td>0.87 (0.44-1.72)</td>
<td>0.74 (0.38-1.44)</td>
<td>0.678</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td></td>
<td>0.87 (0.44-1.72)</td>
<td>0.73 (0.38-1.43)</td>
<td>0.656</td>
</tr>
</tbody>
</table>

P<0.05 *, p<0.01 **, p<0.001 ***. The lowest, middle and highest tertiles for GGT were <23, 23 to <35 and ≥ 35IU/L respectively. a All models were adjusted for HDL-cholesterol.
Table 7.7 Change in liver enzymes from baseline to final follow up according to final diagnosis

<table>
<thead>
<tr>
<th>Final Diagnosis</th>
<th>ALT (IU/L)</th>
<th>GGT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diabetes</td>
<td>Normal glucose tolerance</td>
</tr>
<tr>
<td>White Europeans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>40.4 (26.0)</td>
<td>26.8 (15.3) *</td>
</tr>
<tr>
<td>Final follow-up</td>
<td>42.5 (26.1)</td>
<td>24.7 (14.0)</td>
</tr>
<tr>
<td>Change in value (95% CI)</td>
<td>2.1 (-3.8, 8.0)</td>
<td>-2.1 (-4.17, -0.4)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.480</td>
<td>0.046</td>
</tr>
</tbody>
</table>

South Asians

<table>
<thead>
<tr>
<th>Final Diagnosis</th>
<th>ALT (IU/L)</th>
<th>GGT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>25.4 (20.3)</td>
<td>26.8 (16.5)</td>
</tr>
<tr>
<td>Final follow-up</td>
<td>32.3 (25.7)</td>
<td>21.5 (8.2)</td>
</tr>
<tr>
<td>Change in value (95% CI)</td>
<td>7.0 (2.7, 11.3)</td>
<td>-5.3 (-8.6, -2.0)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.002</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are expressed in mean (standard deviation) and compared using paired t-tests. Baseline liver enzymes compared for final follow-up diagnosis using unpaired t-tests, p<0.05 * and change from baseline to final visit using paired tests. Key: ALT, alanine aminotransferase; GGT, gamma-glutaryltransferase.
7.6 Discussion

7.6.1 Statins

The main findings from Chapter 7 suggest that exposure to statin therapy is associated with a non-significantly lower risk of progression from IGR to incident T2DM in both unadjusted and adjusted analyses. This association strengthened when analyses were restricted to those who did not change their statin use status at the end of the study. Furthermore, the study demonstrated that the effect of statins on incident T2DM was modified by physical activity. Further exploration of this relationship demonstrated that within individuals who were less physical active, use of statins was associated with a significantly reduced risk of T2DM. In contrast, in those who performed more physical activity, there was no association between use of statins and incident T2DM, suggesting there is no additive benefit of taking statins on reducing T2DM risk in this sub-group.

In the present study, the median length of follow-up was 3.5 years which is similar to a recent meta-analysis over 4 years (Sattar et al., 2010). Over 90% of the statins prescribed in this cohort were Simvastatin and Atorvastatin, which is representative of UK national prescribing rates during the years of longitudinal follow-up (NICE, 2008). These two statins are suggested to be of similar or relatively higher potency compared to most other statins, bar Rosuvastatin, and therefore are more likely to be representative of statin trials within a recent meta-analysis (Sattar et al., 2010; Helfand et al., 2006). Furthermore, the ratio of people classified as using statin therapy compared to non-users was 1:6 in the present study and similar to other reports from real world settings (Wang et al., 2012).
Exposure to statin therapy has been shown to be associated with a 9% increased risk of T2DM in people generally at high cardiovascular risk or who experienced prior CVD events in randomised controlled trials (Sattar et al., 2010). However this meta-analysis was not able to consider interactions of statins with physical activity for T2DM outcomes, which may be because physical activity was not measured in individual trials (Sattar et al., 2010; Preiss et al., 2011). To my knowledge this is the first study to consider the role of physical activity in the relationship between statins and T2DM. The results in people with IGR demonstrated the opposite trend of reducing risk of T2DM in those who perform lower levels of physical activity, but not higher physical activity levels. These findings may mirror the WOSCOPS study which found use of Pravastatin therapy led to a significant 30% reduction in T2DM incidence (adjusted hazard ratio 0.70, 0.50 to 0.99), although this study did not contain a standardised diagnosis of T2DM; in addition physical activity levels were not assessed (Freeman et al., 2001).

There is no fully accepted mechanism explaining why statins may increase risk for T2DM. In contrast, statins may increase levels of HDL-cholesterol and reduce levels of triglycerides and inflammatory markers (including interleukin-6, TNF-alpha and C-reactive protein), which may contribute to lowering risk for T2DM (Rosenson et al., 1999; Egashira et al., 1994; Albert et al., 2001). However, recent research has demonstrated that within people who aimed to increase physical activity levels the action of statins may attenuate cardiorespiratory fitness and reduce skeletal muscle mitochondrial content (Mikus et al., 2013). This suggests statins may interact detrimentally with, and blunt the positive effects of, higher levels of physical activity. This may explain the finding that statins significantly reduced T2DM incidence in those with lower physically levels but no additional significant benefit was observed.
in those who performed higher levels of physical activity. However, in contrast to these findings, another recent study reported that higher physical activity levels in combination with statins may be associated with lower total mortality than either factor alone in older individuals with dyslipidaemia, even after adjusting for cardio-metabolic risk factors (Kokkinos et al., 2013). Overall, the relationship between statins and physical activity levels appears to be complex and many other aspects/factors explaining the relationship may be unidentified in this area, therefore there is a need for more research in this area to clarify the relationship and mechanism between statins and physical activity.

7.6.2 Liver enzymes

The principal findings suggested that in people at high risk of T2DM baseline ALT and GGT levels predict progression to T2DM in White Europeans, at levels that begin within the high-normal physiological range of each enzyme. People in the highest category of baseline ALT and GGT levels demonstrated a two to three fold higher risk of progression compared to the lowest tertile. Furthermore, White Europeans in the highest ALT tertile demonstrated a lower risk of reverting back to normal glucose levels compared to the lowest tertile. To my knowledge this is the first study to report that a baseline hepatic enzyme can predict incident T2DM or a reversion back to normal glucose levels in people with IGR, suggesting ALT may have a role in assessing risk for T2DM.

The present cohort focused on IGR at baseline, therefore the baseline mean ALT and GGT were relatively high at 28 and 34 IU/L in contrast to population based
studies with baseline levels of 20 and 25 IU/L respectively (Ford et al., 2008). Despite the higher ALT values in the present high risk cohort, there was still a two to three-fold increased risk for T2DM in the highest ALT category compared to the lowest category. Previous research in population based studies suggests the risk of incident T2DM is two to five times higher in the highest compared to lowest categories of ALT and/or GGT levels (Perry et al., 1998; Ford et al., 2008).

In addition, the change in levels of hepatic enzymes from baseline to final follow-up for incident T2DM in White Europeans were mild and non-significant increases of 2.1 and 5.6 IU/L for ALT and GGT levels respectively. This appears to be the first report to comment on the change in liver enzymes from baseline for T2DM outcomes. As the changes in these enzymes levels were only relatively small in progressing from intermediate hyperglycaemia to T2DM, this may suggest larger changes potentially occur in progressing from normal glucose to IGR.

Regarding ethnic differences, neither baseline ALT nor GGT levels predicted T2DM in South Asians in the hazard models investigated. The proportions of South Asians who developed incident T2DM in each ALT and GGT tertile were approximately equal without any obvious incremental increase. Secondly, baseline ALT levels were non-significantly lower in South Asians who developed T2DM compared to those who developed normal glucose tolerance, another potential indication that ALT may not be a predictor of T2DM in the same way as occurs in White Europeans. Similar findings have already been noted in other non-white European populations from the Far East (Lee et al., 2003; Nannipieri et al., 2005; Wannamethee et al., 2005; Nakanishi et al., 2004).

Explaining these results may require understanding of the main proposed mechanisms linking liver enzymes and incident T2DM. ALT and GGT levels correlate
with increasing hepatic fat, even within their normal physiological range, therefore suggesting these hepatic enzymes may be markers of excess liver fat deposition associated with conditions including non-alcoholic fatty liver disease, obesity and the metabolic syndrome (Tiikkainen et al., 2003). Liver fat deposition leads to increased gluconeogenesis and decreased storage of liver glycogen (Samuel et al., 2004). Secondly, liver enzyme levels may also correlate with certain inflammatory processes, where liver fat deposition induces inflammatory cytokine production, particularly interleukin-6 and TNF alpha, which are associated with increased risk for T2DM (Vozarova et al., 2002; Day et al., 2003; Pradhan et al., 2001). Thirdly, GGT mediates intracellular uptake of glutathione required for antioxidant defences, where its expression is increased by oxidative stress which may increase the risk for T2DM (West et al., 2000; Penn et al., 1983; Lee et al., 2009). Therefore GGT may be a sensitive, but not specific marker of liver damage. To my knowledge it is not known whether these mechanisms operate in a similar fashion in South Asians, who demonstrate higher levels of T2DM at lower levels of obesity. Liver enzymes, as surrogate measures of liver fat deposition and inflammation, may not be as important in development of T2DM in South Asians as White Europeans, where other disease processes may be operating at a faster rate. Alternatively, ALT and GGT may be poor markers of liver fat deposition and inflammation in South Asians. Further research in this area is required.

7.6.3 Limitations and strengths
Despite the novel findings in the statin analysis, there are a few limitations to acknowledge. Firstly, a potential limitation in the statins analysis is the relatively small sample size, which could have resulted in low power to detect significant associations. This probably explains why the effect sizes associated with statin use were fairly large but non-significant. However, this potential limitation should be considered when interpreting these findings and indicate that these analyses require repetition in a larger study. Secondly, participants were not randomly allocated to a statin user or non-user group; however differences in baseline characteristics between the two groups where accounted for in statistical analyses as far as possible. Thirdly, participants may not have been statin naïve prior to recruitment, a potential problem in most similar studies (Sattar et al., 2010; Preiss et al., 2011; Wang et al., 2012). In addition, we did not have information regarding compliance to therapies or the dose of statins; however the results are likely to reflect real life practice. UK national prescribing guidelines reflecting the relevant time period recommended starting Simvastatin at 40mg once daily (or other statin of similar efficacy) for both primary and secondary CVD prevention (NICE clinical guideline 67, 2008). If lipid targets were not achieved, then these guidelines recommended titration of Simvastatin to 80mg daily (or other statin of similar efficacy) for secondary prevention (NICE clinical guideline 67, 2008). Therefore I may assume relatively higher doses of statins were prescribed in most cases. Finally, physical activity was based on participant self-report questionnaires.

There are some strengths of this study. It was conducted in a real life setting where participants were not given any interventions. Previous findings are predominantly from randomised controlled trials, where participants may have been encouraged or reminded to take their statin therapy in a regular manner that may not reflect real life
practice (Sattar et al., 2010; Ridker et al., 2012). Secondly, this study was designed for glycaemic outcomes and therefore included robust detection of T2DM performed on an annual basis, as per international recommendations, using an OGTT (ADA Executive summary, 2010). Most other studies in this area were trials designed to report on cardiovascular outcomes and the unstandardised methods of T2DM diagnosis were in some cases based on physician testing (occasional use of FPG test), presence of prescribed anti-diabetic medications or participant self-report measures. Thirdly, use of the OGTT for baseline screening allowed detection of IGT and / or IFG. Fourth, as the study was designed for T2DM outcomes, I measured important potential confounding factors and so were able to include them in the analysis. These included physical activity levels, family history of T2DM and 2-hour plasma glucose which were not considered in other reports (Sattar et al., 2010; Preiss et al., 2011; Wang et al., 2012).

Regarding the liver enzymes analysis there are also some limitations to acknowledge. Liver enzymes may be influenced by other liver pathologies, such as stable chronic hepatitis, which I was unable to account for, similar to most other reports (André et al., 2005; Lee et al., 2003; Perry et al., 1998; Nannipieri et al., 2005; Ford et al., 2008; Wannamethee et al., 2005; Sattar et al., 2004; Nakanishi et al., 2004; Fraser et al., 2009). I excluded liver enzyme test results that were three times higher than the local reference range upper limit and were able to account for alcohol intake and use of statins, even if they were not in the final adjusted models to due non-significance. In addition, people with any active diseases (e.g. acute hepatitis, liver malignancies, cirrhosis) were not screened until they had recovered; those with terminal illnesses were not screened. Secondly, regarding the ethnic comparisons, the number of South Asians analysed was small and thus may be
underpowered, suggesting they may need repeating in a larger study. However the overall number of T2DM cases in South Asians was 24%, representing the nature of this high risk cohort. Thirdly, regarding the final diagnosis of normal glucose tolerance, I reported those who had achieved this glycaemic status according to their last visit. In theory, they may have achieved this status before their final visit; however as the diagnosis may have changed once again afterwards, I focused on the diagnosis at the final visit only.

In contrast, there are some advantages of the liver enzymes study. This cohort was designed for T2DM outcomes and participants did not undergo any specific intervention during the period of follow-up. Secondly, as the measured levels of hepatic enzymes at all visits I was able to investigate the change in enzyme levels from baseline; the liver enzymes were all measured in the same laboratory using the same assay.

In summary, baseline levels of ALT predict both progression to incident T2DM and restoration of normal glucose levels in White Europeans with intermediate hyperglycaemia, suggesting this enzyme may have a role in assessing risk for T2DM.

7.7 Conclusion to Chapter 7

In this chapter, I found use of statin therapy was associated with a non-significantly lower risk of progression from IGR to T2DM. There was evidence that statin users had a significantly lower risk of progressing to T2DM among people with low physical activity levels, but not among those with high physical activity levels. This suggestion
that statin use and physical activity do not have an additive effect requires further investigation. Secondly, regarding liver enzymes, baseline levels of ALT predicted both progression to incident T2DM and reversion to normal glucose levels in White Europeans with IGR, suggesting this enzyme may have a role in assessing increasing or decreasing risk for T2DM.

Regarding risk factors for T2DM, I have looked at the triglyceride-to-HDL ratio, liver enzymes and statins in Chapters 6 and 7. For the final chapter of this thesis I will investigate Vitamin D as novel risk factor for T2DM, however this will be in the form of setting up a prospective randomised controlled trial.
Chapter 8

Vitamin D deficiency,
the risk of T2DM and
the VITALITY feasibility study
8.1 Chapter overview

In chapters one to seven I have investigated using various risk markers for T2DM including HbA1c, the triglyceride-to-HDL cholesterol ratio and liver enzymes. In the last major chapter of this thesis, I will introduce Vitamin D deficiency (VDD) as a novel risk marker for T2DM. Secondly, I will focus on the feasibility of a pilot study known as the VITALITY (Can Vitamin D replacement reduce Insulin resistance in South Asians with vitamin D deficiency?), a proposed 6 month double blind randomised controlled trial (RCT). The main objective is to evaluate and analyse the potential of this proposed pilot study in order to determine if further research or a larger trial should go ahead.

8.2 Introduction

8.2.1 Vitamin D Deficiency

There has been a resurgence of interest in the recognition and treatment of VDD beyond established roles in metabolic bone disease. Epidemiological studies implicate vitamin D and calcium homeostasis in a plethora of non-skeletal immune-based chronic diseases (Lee et al., 2008). Proposed mechanisms accounting for these pleiotropic actions focus upon well-characterised in-vitro immuno-modulatory effects, intracellular calcium signalling and the recent finding that the Vitamin D receptor (VDR) is ubiquitous. The emerging science connecting biochemical deficiency, molecular or cytokine responses and disease pathogenesis is well-reviewed (Lee et al., 2008; Holick, 2008). VDD appears to predispose to vascular,
rheumatic and neuropathic inflammation, hypertension, metabolic syndrome and atherosclerosis.

8.2.2 Vitamin D sources, production, metabolism and actions

Up to 70 to 90% of vitamin D in human body is acquired via direct exposure to sunshine, particularly the ultraviolet B rays at wavelengths of 270 to 300nm (Holick, 2006; Hume et al., 1927). These wavelengths are typically common in equator and tropical regions of the world, but tend to decrease proportionally with shifting latitudes away from the equator, suggesting lower amounts are available at temperate and arctic regions. In contrast, only a small amount of Vitamin D (up to 30%) is obtained via the diet, reflecting that only a few foods contain natural Vitamin D (Holick, 2004). Secondly, these foods with Vitamin D only contain a relatively small amount, including oily fish and cod liver oil, therefore diet is unlikely to maintain optimal levels of vitamin D. Nutritional supplements and foods artificially fortified with vitamin D may provide extra sources of vitamin D.

Exposure to sunlight leads to photochemical conversion of a molecule produced in large amounts by the skin, known as 7-dehydrocholesterol; the product of this reaction is pre-vitamin D3. The latter molecule undergoes spontaneous isomerisation to become Vitamin D3 (cholecalciferol) in approximately 12 days (Holick, 2004). Cholecalciferol undergoes two subsequent hydroxlations in the liver and kidney respectively (Deluca et al., 2001). The first hydroxylation is facilitated by a microsomal enzyme produced by hepatocytes, 25-hydroxylase, which in turn produces the pro-hormone 25-OH Vit D3, also known as calcidiol (Mathieu et al.,
This is commonest form of Vitamin D3 measured in clinical practice as it has a longer half-life (i.e. possesses a slower rate of clearance) and is a better estimator of total body stores than the substance it is converted into (Zittermann, 2003). The second hydroxylation is catalysed by another enzyme predominantly in the proximal tubules of the kidney, 1-alpha-hydroxylase, to produce 1, 25-dihydroxyvitamin D3 which is also referred to as calcitriol, the biologically active form of vitamin D (Mathieu et al., 2005; Deluca et al., 2001; Zittermann, 2003). Calcitriol is released into the bloodstream and where it is a potent ligand of a plasma carrier protein, the vitamin D-binding protein. This binding protein carries calcitriol to its numerous target organs and is essential for endocytosis and metabolism which permits calcitriol to exert its various biological effects (Mathieu et al., 2005). The target organs include bone, parathyroid, liver, kidney, brain, heart, stomach, adrenal glands, thyroid, skin and pancreatic beta cells (Bikle, 1992). The main roles of calcitriol include maintaining mineral homeostasis of calcium and phosphate through enhancing their intestinal absorption, increasing calcium reabsorption in the kidney and therefore playing a pivotal role in regulating bone remodelling (Deluca et al., 2001; Zittermann, 2003).

Both food and nutritional supplements may provide an alternative form of vitamin D, known as D2 or ergocalciferol rather than D3. It is found typically in certain types of mushrooms. This form of vitamin D eventually joins the same metabolic pathway to produce active Vitamin D, avoiding the first hydroxylation in liver, but undergoing the second hydroxylation in the kidney to produce calcitriol.

It is suggested that people with pigmented skin are less efficient at making Vitamin D3 as a result of the melanin in skin hindering Vitamin D synthesis; however other factors including genetic predisposition may also play a role (Signorello et al., 2010).
8.2.3 Definitions of Vitamin D status

There are various definitions regarding categorising levels of vitamin D; for the purposes of this thesis a Vitamin D level in the optimum range is defined as a serum 25-hydroxy vitamin D (25(OH) Vit D) >75 to 80nmol/l, an adequate level is 50 to 75nmol/l, vitamin D insufficiency is 25 to 50nmol/l and VDD as <25nmol/l (Pearce et al., 2010).

8.2.4 Cross-sectional associations between VDD and T2DM/ CVD risk factors

Data from large observational cohorts consistently demonstrate inverse relationships between serum 25(OH) Vit D and prevalent metabolic syndrome, T2DM, glycaemic levels or CVD risk factors (Ford et al., 2005; Scragg et al., 2007; Martins et al., 2007; Kositsawat et al., 2010; Pinelli et al., 2010). Scragg et al. demonstrated the adjusted odds ratio for T2DM in the highest 25-OH vit D quartile (≥ 81nmol/l) compared to those in the lowest quartile (< 43.9nmol/l) were 0.25 (95% CI 0.11 to 0.60) for Europeans and 0.17 (0.08 to 0.37) for Mexican Americans (Scagg et al., 2007). Similarly, Martins et al. showed the adjusted prevalence of T2DM using odds ratios was 1.98 (1.57 to 2.51) in the lowest quartile compared to the highest. Using a small group of 126 people who were glucose tolerant, Chiu et al. demonstrated 25-OH vit D was positively correlated with insulin sensitivity on hyperglycaemic clamps and negative correlated with fasting and two hour plasma glucose (Chiu et al., 1994).
Cross-sectional studies are limited as they cannot confirm the direction of causality. However Forouhi et al. analysed 10 year changes in glucose levels in 524 people. Using multiple linear regression analysis and adjusting for confounders, baseline 25(OH) vit D was associated inversely with hyperglycaemia on fasting glucose: and 2-hour glucose, fasting insulin and insulin resistance (using HOMA-IR). Similarly, in a nested case control study of women, Pittas et al. demonstrated the adjusted odds ratios for incident T2DM was 0.52 (0.33 to 0.83) in the highest compared to lowest quartile (Pittas et al., 2010). Additionally, previous research has suggested in people with T2DM the presence of VDD is associated with microvascular complications and predicts increased risk of all-cause and CV mortality independent of traditional CVD risk factors (Suzuki et al., 2006; Joergensen et al., 2010). Finally, a systematic review and meta-analysis of observational studies suggested a consistent association between low vitamin D status and T2DM with an odds ratio of 0.36 (0.16 to 0.80) for highest vs. lowest 25 OH-vit D levels (Pittas et al., 2007).

8.3 Mechanisms linking Vitamin D and T2DM/ glucose homeostasis

8.3.1 Pancreatic actions

Both Vitamin D receptors and Vitamin D-dependent calcium-binding proteins are known to exist in beta pancreatic cells, suggesting a role for Vitamin D in insulin secretion (Ishida et al., 1988; Johnson et al., 1994). Further evidence suggests Vitamin D is required for insulin release in response to increasing glucose loading; conversely, VDD leads to lower insulin secretion from the pancreas (Inomata et al.,
1986; Orwoll et al., 1994; Gedik et al., 1986). The possible reasons for this include Vitamin D influencing intracellular calcium concentrations in beta pancreatic cells, activating endopeptidases responsible for converting pro-insulin into insulin, allowing exocytosis of insulin granules into the bloodstream (Chiu et al., 2004). Furthermore, vitamin D may also stimulate insulin synthesis by activating protein synthesis pathways in beta cells (Borissova et al., 2003).

8.3.2 Immune actions

Vitamin D is known to have a varied range of immune actions which are possible as Vitamin D receptors are known to exist in activated T-lymphocytes, macrophages and thymus tissue (Chiu et al., 2004; Hewison et al., 2001). This suggests Vitamin D has a role in immuno-modulation, including promoting monocyte differentiation, reducing antigen-presenting activity of macrophages to lymphocytes and preventing dendritic cell maturation (Zittermann et al., 2003; Hewison et al., 2001, Mauricio et al., 1996). Furthermore, Vitamin D has been shown to down-regulate the production of certain cytokines including IL-6, interferon gamma and TNF-alpha, which may suggest Vitamin D influences low grade chronic inflammatory levels, however more evidence is required (Mauricio et al., 1996; Lemire et al., 1995).

8.3.3 Genetic risk
Polymorphisms consisting of genetic sequence variations are known to exist in genes responsible for producing (a) the vitamin D receptor, (b) the Vitamin D-binding protein and (c) the Vitamin D 1 alpha-hydroxylase enzyme (the latter leads to production of the active form of Vitamin D, calcitriol) which contribute to genetic predisposition to abnormal insulin secretion and therefore glucose intolerance (Uitterlinden et al., 2004; Malecki et al., 2003; Malecki et al., 2002).

### 8.4 South Asians and VDD

Migrant South Asians living in the UK have among the highest rates of VDD (Ford et al., 2006; Hamson et al., 2003; Webb et al., 2012; Hull et al., 2010). Pilot data performed in our research group suggested 72% of Leicester South Asians have a 25(OH) Vit D level of < 20nmol/l (Webb et al., 2012). Recognised causes of VDD susceptibility in South Asians include 1) pigmented skin colour (melanin acting as a potential barrier to preventing ultraviolet rays B penetrating through), 2) lack of sunlight exposure (which may be related to cultural practices of covering up high proportions of the body), 3) vegetarianism, 4) adipose tissue vitamin sequestration, and 5) less time spent in the sunshine.

Despite the high prevalence of Vitamin D Deficiency in South Asians, there is lack of evidence supporting a specific dosing regime in this Vitamin D deplete population. Consensus algorithms for replacement based predominantly on bone-related outcomes in white Europeans are unlikely to adequately address VDD in South Asians.
South Asians also have a high prevalence of T2DM and it is plausible that this relates directly to sub-optimal Vitamin D status (Boucher et al., 2006). Therefore South Asians form an ideal group to test a Vitamin D intervention with the aim of reducing risk of T2DM.

8.5. Interventional studies of vitamin D replacement and its effect on glycaemia or T2DM

While VDD is associated with future T2DM, less is known about what impact vitamin D therapeutic replacement has on future T2DM incidence or on levels of glycaemic markers in people with or without T2DM. There are a limited number of Vitamin D replacement intervention studies to date which have reported such results; many of these studies were not designed primarily for glycaemic outcomes. Table 8.2 outlines a summary of Vitamin D intervention studies and the impact these had on either glucose measures or surrogates markers of insulin resistance and sensitivity. Some of these studies have been limited by small sample sizes, inadequate replacement doses of vitamin D, short intervention times and selecting populations who are not VDD. The lowest number of people investigated in a single study was 11 (Selimoglu et al., 2010) and only three studies had over 100 people (Pittas et al., 2007; de Boer et al., 2010; Davidson et al., 2013). The mean or median 25 OH-Vit D level was less than 25nmol/l in only one study (Von Hurst et al., 2010). Where reported, the total dose of Vitamin D3 administered was between 40,000 units to 1,004,000 units. The length of studies varied from single doses on one day to 3 years.
The most promising results for changes in glycaemia have come from two studies. Daily administration of 700 units of vitamin D3 in combination with 500mg calcium citrate for three years, led to a slower rise in final FPG compared to the placebo group (0.02 vs. 0.34mmol/l) in people with IFG, but not normal glucose tolerance (Pittas et al., 2007). Secondly, 106 people with IGR received an average of 88,865 units per week of vitamin D3 or placebo for 1 year which led to an HbA1c decrease of 0.2% in the intervention group (Davidson et al., 2013). While the two studies have used vastly different doses of vitamin D3, it should be noted they are both longer term studies of at least one year and reported positive results in people with IGR who are at risk of T2DM. Shorter term vitamin D intervention studies or those using people with T2DM or normal glucose tolerance do not appear to report changes in glucose markers (Pittas et al., 2007; Pittas et al., 2010; Jorde et al., 2009). For the case of T2DM, it is suggested that vitamin D replacement may need to be administered earlier in the disease process before the onset of T2DM, as by the time this disease is diagnosed the opportunity to gain benefits from vitamin D replacement may have been lost (Jorde et al., 2009). A systematic review of eight trials revealed no effect of vitamin D replacement on glycaemia or incident T2DM; however only two trials were rated good quality and five consisted of people with normal glucose tolerance at baseline (Pittas et al., 2010). While changes in glucose levels may not have been generally reported, there have alternate changes in markers of insulin resistance, secretion and sensitivity in Table 8.1 (Von Hurst et al., 2009; Harris et al., 2012; Nagpal et al., 2009; Selimoglu et al., 2010; Borissova et al., 2003; Inamoto et al., 1986).
8.5.1 Interventional studies of vitamin D replacement in South Asians

There have two vitamin D intervention studies in South Asians, Table 8.2. The first was a six month randomised controlled trial in migrant females based in New Zealand (Von Hurst et al., 2009). This study used daily 4,000 units of vitamin D3 or placebo and did not find any improvements in glucose levels, but reported reduction in insulin resistance (HOMA-IR, median change from baseline -0.2 vs. 0.2 for intervention vs. control) and fasting serum insulin (-1.3 vs. 1.1) and additionally, increases in insulin sensitivity (HOMA2 %S, -1.3 vs. 1.1). Within the second study Indian males were administered three doses of 120,000 units of vitamin D3 or placebo on fortnightly (Nagpal et al., 2009). There was an improvement in postprandial insulin sensitivity (oral glucose insulin sensitivity), but no effect on other markers of insulin sensitivity, insulin resistance and beta cell function.
<table>
<thead>
<tr>
<th>Study</th>
<th>Population sampled</th>
<th>Size (n)</th>
<th>Study design</th>
<th>Baseline Vit D (nmol/l) *</th>
<th>Vit D regime</th>
<th>Total Vit D intervention dose (units)</th>
<th>Main result in relation to glycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jorde et al., 2009</td>
<td>T2DM on Metformin Insulin</td>
<td>36</td>
<td>RCT</td>
<td>60</td>
<td>40,000IU/ week for 6 months or Placebo.</td>
<td>1,004,000</td>
<td>No change in levels of FPG, Insulin, C-peptide, fructosamine, HbA1c</td>
</tr>
<tr>
<td>Grimes et al., 2011</td>
<td>People aged 30-75yrs</td>
<td>94</td>
<td>DB RCT</td>
<td>40.3</td>
<td>Twice weekly 20,000IU for 6 months</td>
<td>1,004,000</td>
<td>No changes in HbA1c, insulin sensitivity, insulin secretion</td>
</tr>
<tr>
<td>Witham et al., 2010</td>
<td>T2DM</td>
<td>61</td>
<td>RCT</td>
<td>~45</td>
<td>Single dose of placebo 100,000 or 200,000IU</td>
<td>100,000 or 200,000</td>
<td>At 16 weeks: no change in HbA1c or HOMA-IR.</td>
</tr>
<tr>
<td>Von Hurst et al., 2010</td>
<td>South Asian, Insulin resistant women without DM</td>
<td>81</td>
<td>DB RCT</td>
<td>21</td>
<td>Daily 4000IU vit D or placebo for 6 months</td>
<td>728,000</td>
<td>↑ Insulin sensitivity, ↓ insulin resistance and ↓ fasting insulin in the intervention arm</td>
</tr>
<tr>
<td>Harris et al., 2012</td>
<td>Overweight African Americans with IGR or T2DM</td>
<td>89</td>
<td>RCT</td>
<td>40</td>
<td>4000 IU/day for 12 weeks or placebo</td>
<td>336,000</td>
<td>↓ Insulin secretion by 12% in the intervention arm. No changes in other glucose measures.</td>
</tr>
<tr>
<td>Nagpal et al., 2009</td>
<td>South Asian males</td>
<td>71</td>
<td>DB RCT</td>
<td>36.5</td>
<td>120,000 units once a fortnight x 3</td>
<td>360,000</td>
<td>Increase in post prandial insulin sensitivity</td>
</tr>
<tr>
<td>Selimoglu et al., 2010</td>
<td>Women with PCOS</td>
<td>11</td>
<td>Trial</td>
<td>42.5</td>
<td>Single dose of 300,000 units</td>
<td>300,000</td>
<td>3 weeks after dose: HOMA-IR ↓ from 4.41 to 3.67. No changes in glucose + insulin levels</td>
</tr>
<tr>
<td>Pittas et al., 2012</td>
<td>Caucasian</td>
<td>314</td>
<td>DB RCT</td>
<td>81.4</td>
<td>Combined daily vit</td>
<td>766,500</td>
<td>IFG group: intervention FPG</td>
</tr>
<tr>
<td>Year</td>
<td>Group</td>
<td>Intervention</td>
<td>Sample Size</td>
<td>Design</td>
<td>Dose</td>
<td>Outcome</td>
<td>Notes</td>
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<tr>
<td>2007</td>
<td>Adults without Diabetes</td>
<td>700 IU/ + calcium 500 mg/d or placebo for 3yrs</td>
<td>Variable - determined by body weight</td>
<td>DB RCT</td>
<td>55</td>
<td>Mean weekly dose 88.865IU or placebo</td>
<td>HbA1C ↓ 0.2% in intervention arm. No change in T2DM incidence, glucose or insulin secretion/ sensitivity levels.</td>
</tr>
<tr>
<td>2013</td>
<td>African Americans and Hispanics with IGR</td>
<td>109</td>
<td>DB RCT</td>
<td>55</td>
<td>Mean weekly dose 88.865IU or placebo</td>
<td>HbA1C ↓ 0.2% in intervention arm. No change in T2DM incidence, glucose or insulin secretion/ sensitivity levels.</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Females: T2DM vs non T2DM</td>
<td>27</td>
<td>Trial</td>
<td>35.3</td>
<td>1332 units daily for 1 month</td>
<td>34.3% increase in 1st phase of insulin secretion</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>T2DM</td>
<td>34</td>
<td>DB RCT</td>
<td>38.3</td>
<td>Single dose 100,000 vit D2 or placebo</td>
<td>No change in HbA1c or insulin sensitivity</td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>Post-menopausal women</td>
<td>33,951</td>
<td>DB trial</td>
<td>&lt;50nmol/l in 61% of population</td>
<td>Daily 1,000mg calcium and 400units vit D3 or placebo</td>
<td>Varied</td>
<td>7 years: no reduction in risk for T2DM</td>
</tr>
<tr>
<td>1986</td>
<td>T2DM</td>
<td>14</td>
<td>Not a trial</td>
<td>unknown</td>
<td>1 alpha (OH)-vitamin D3 2mcg for three weeks or placebo</td>
<td>61mcg</td>
<td>Increase in insulin secretion, but not glucose</td>
</tr>
<tr>
<td>1987</td>
<td>Men with IGR</td>
<td>65</td>
<td>Not a trial</td>
<td>unknown</td>
<td>0.75 micrograms alpha-calcidol or placebo for 3 month</td>
<td>~ 67.5mcg</td>
<td>No effect on glucose, insulin or insulin measures</td>
</tr>
<tr>
<td>1994</td>
<td>T2DM</td>
<td>35</td>
<td>DB RCT (cross-over)</td>
<td>35.0</td>
<td>1.25-dihydroxyvitamin D 1mcg for 4 days</td>
<td>4mcg</td>
<td>No effect on glucose, insulin or insulin measures</td>
</tr>
<tr>
<td>2013</td>
<td>People with IGR</td>
<td>45</td>
<td>RCT</td>
<td>Group (a) 77.5, (b) 80, (c) 44.8</td>
<td>Once to twice weekly: a)50,000 units vit D3/ 500 mg calcium; b) 300,000 units Vit D3; c)500 mg calcium</td>
<td>Variable</td>
<td>Insulin sensitivity improved in group (a), no other changes in glucose or insulin resistance.</td>
</tr>
<tr>
<td>Study</td>
<td>Intervention</td>
<td>N</td>
<td>Study Design</td>
<td>Mean/Median</td>
<td>Dose</td>
<td>Outcome Description</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------------</td>
<td>----</td>
<td>--------------</td>
<td>-------------</td>
<td>------</td>
<td>-------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Tai et al., 2008</td>
<td>Vit D Insufficiency, no T2DM</td>
<td>33</td>
<td>Not a trial</td>
<td>39.9</td>
<td>2 x 100,000 Vit D3, separated by 2 weeks</td>
<td>200,000 After 2nd dose: No change in levels of glucose, insulin, insulin sensitivity or insulin resistance markers</td>
<td></td>
</tr>
<tr>
<td>Mozaffari-Khosravi et al., 2012</td>
<td>Post-partum women with GDM</td>
<td>45</td>
<td>RCT</td>
<td>80% of cohort had VDD</td>
<td>Single dose 300,000 units Vit D3</td>
<td>300,000 3 months later: No change in glucose, insulin resistance or insulin sensitivity markers</td>
<td></td>
</tr>
</tbody>
</table>

* mean or median: nmol/l, † if data available. Key: DB = double blind; RCT = Randomised controlled trial, FPG = fasting plasma glucose, GDM = gestation diabetes mellitus, HOMA-IR = Homeostatic model of Assessment Insulin resistance, IFG = impaired fasting glycaemia, IGR = impaired glucose regulation, NFG = Normal fasting glucose, PCOS = polycystic ovarian syndrome, Vit D = Vitamin D, ↑ = increase /rise, ↓ = decrease/ fall.
8.6 The potential clinical benefits gained from research around Vitamin D replacement and T2DM

It is suggested that Vitamin D replacement in people with VDD may aid the prevention of T2DM. The prospect of using this commonly prescribed therapy as a potential intervention for T2DM prevention carries considerable public health implication; therefore there is a need to determine the potential of this simple, safe and generally well tolerated therapy. However, it must be shown that it is feasible to undertake such a study. For the remainder of this chapter, I focus on the viability of undertaking such a pilot study and uncover the strengths and weaknesses of this proposed project and provide conclusions on whether a larger study should go ahead.

8.6.1 Hypothesis and objectives

The hypothesis formed suggests that the risk of T2DM in South Asians is explained, at least in part, by the presence of VDD. Therefore if the VDD is reversed/‘normalised into optimum target range’ using Vitamin D therapy in high risk individuals, then this risk of T2DM should reduce from baseline. However, current UK recommended doses of Vitamin D do not adequately replenish VDD into the optimum target range (defined as 25(OH) Vit D level > 75nmol/l) (Pearce et al., 2010). Therefore only higher pharmacological doses are able to replace VDD adequately and reduce risk of T2DM. To test this hypothesis, I set out to design a small pilot study in the form of a double blind randomised controlled trial.
Objectives of this chapter:

- To design, describe and justify methods for a pilot study in the form of a six month randomised controlled trial comparing high dose Vitamin D3 replacement to UK standard dose Vitamin D3 replacement to reduce insulin resistance in South Asians with VDD. This is preceded by a screening phase to detect eligible participants.
- To gain regulatory approvals for this proposed pilot study.
- To present the results which were obtained.
- To describe the feasibility of setting up and conducting the VITALITY study in a reasonable time period, including the strengths and weaknesses and potential for success if larger studies were performed.

8.7 Methods: rationale and justification for the VITALITY pilot study

8.7.1 Target population

The risk of incident T2DM is inversely proportional to baseline Vitamin D levels, suggesting replacement interventions should focus on those populations with the lowest Vitamin D levels in order to achieve the most positive outcomes. Previous Vitamin D replacement intervention studies that selected populations with Vitamin D insufficiency (defined as 25(OH) Vit D level of 25 to < 50nmol/l) or those with Vitamin D levels within the acceptable physiological range (defined as 25(OH) Vit D level > 50nmol/l) have generally not detected any significant changes in glycaemia, Table 8.1. Therefore I propose to recruit participants with the lowest Vitamin D levels (defined as 25(OH) Vit D level <25nmol/l, VDD). South Asians living in Leicestershire will meet this criterion, as there appears to be an abundance of VDD in this ethnic
group (Webb et al., 2010). By focusing on a single ethnic group, this allows us to investigate a relatively homogeneous population.

8.7.2 Basis of primary outcome variable

Previous research has demonstrated only a limited number of studies where Vitamin D replacement has been used to reduce glucose levels or decrease the incidence of T2DM. This may be because it is necessary to give Vitamin D supplementation over a number of years before changes in glucose levels are seen. Pittas et al. who administered Vitamin D replacement over three years, observed a slower rise of glucose levels in people who took Vitamin D and calcium supplementation compared to placebo of 0.02 mmol/l vs. 0.34 mmol/l in those with impaired fasting glycaemia; this was one of the only studies that noticed positive effects on glucose levels (Pittas et al., 2007). Studies which are designed to last shorter time periods (e.g. six months) may need to focus on other markers of glycaemia, particularly those markers whose changes relating to risk of T2DM predate changes in glucose levels. An example of this includes markers of insulin resistance, such as homeostatic model assessment of insulin resistance (HOMA-IR, Matthews et al., 1985; Levy et al., 1998). In people who eventually develop T2DM, HOMA models are thought to alter up to 4 to 6 years before the corresponding changes in glucose levels (Tabák et al., 2009). Furthermore, a study of South Asian females observed that Vitamin D replacement did not lead to a significant reduction in glucose levels but led to a significant reduction in HOMA2-IR (Von Hurst et al., 2010). Therefore, I propose using HOMA2-IR, as a surrogate marker of insulin resistance, as our primary outcome. Additionally, South Asians are thought to develop T2DM predominantly
due to high levels of insulin resistance, suggesting HOMA-IR is highly relevant variable of interest to this ethnic group (McKeigue et al., 1993; Hall et al., 2008).

HOMA-IR is a validated method to measure insulin resistance from one fasting glucose and insulin. The original model HOMA1-IR index is calculated by the formula: \[
\frac{\text{fasting plasma insulin (μU/ml) x fasting plasma glucose (mmol/L)}}{22.5}
\]
has been widely used in research (Mattews et al., 1985; Levy et al., 1998). This was updated with some physiological adjustments to a computer version to form HOMA2-IR providing a more accurate index (HOMA Calculator, Oxford Centre for Diabetes).

### 8.7.3 Length of the study

Most of the previous research in this area has been conducted in short term studies that lasted from 1 week to 3 months and have generally shown negative findings (Table 8.1). Those studies that have lasted six months or longer appear to be more likely to demonstrate at least some changes in markers of glycaemia (Von Hurst et al., 2010; Pittas et al., 2007). This may be because the actions of Vitamin D replacement may require some time before influencing glycaemia. Therefore I propose the VITALITY study randomised controlled trial phase will be conducted over six months. The screening phase to detect the 100 participants for the randomised controlled trial necessary for the trial is expected to take 12 to 18 months, as some reports have suggested that recruiting for vitamin D intervention trials will take a long time (Tripolt et al., 2013).
8.7.4 Baseline levels of insulin resistance

South Asians recruited to the VITALITY study will have both VDD and a pre-defined level of insulin resistance. Regarding the latter, there is no accepted global or regional HOMA-IR definition of insulin resistance for any ethnic group. My initial idea was to use a definition derived from an Indian urban population (HOMA-IR ≥ 1.93) as this population was thought to provide the closest data estimate to participants in the VITALITY study population; also this specific definition has been adopted by other studies (Von Hurst et al., 2010; Deepa et al., 2002). However the lack of international standardisation of insulin assays/kits suggests any one HOMA-IR value may not be the same in other locations where different types of insulin assays and methods are used and have different co-efficient of variances. Therefore using pilot data conducted during the initial run of the VITALITY study, I determined a local HOMA-IR cut-point for insulin resistance by adopting the 50th percentile of all HOMA-IR values collected up to March 2013 (n=40). This gave a slightly lower cut-point of HOMA-IR of 1.4 compared to the urban Indian population, but this method has the advantage of defining insulin resistance according to our own insulin kits and reflects our local South Asian population.

8.7.5 Doses of Vitamin D therapies and target Vitamin D levels
Previous studies have used up to 600,000 units of Vitamin D in a single dose without any reported complications (Cipriani et al., 2010). A study of South Asians living in New Zealand found a daily dose of 4000 Vitamin D3 units per day for 6 months (728,000 units in total) did not lead to any reported problems regarding toxicity (Von Hurst et al., 2010). Furthermore, in this study the highest reductions in HOMA-IR were achieved when the final 25 (OH) Vit D level was >75 to 80nmol/l, however many participants did not reach this value by the end of six months using a daily regime of 4000 units only. Finally, in a separate study, a single administered dose of vitamin D3 of 100,000 units raised a relatively high mean baseline 25(OH) vitamin D level of 67.5nmol/l to a post dose peak at one week of 105nmol/l (Liahi et al., 2008), Figure 8.1. However the 25(OH) vitamin D levels began to gradually reduce once again from approximately 10 to 15 days after the dose was given suggesting repeat doses are required in time to keep a 25(OH) Vit D level at a particular target range.

**Figure 8.1 Levels of calcidiol (25-OH vit D) after a single dose of 100,000 units Vit D3 cholecalciferol.** Taken from Liahi et al., 2008.
Based on this information, in the VITALITY study I propose the aim will be to replace the 25-OH Vit D levels of intervention arm participants to this threshold of > 75 to 80nmol/l rapidly and maintain this level over the six months. Therefore I propose the intervention arm will be administered periodic high dose Vitamin D3 (cholecalciferol) of 200,000 or 100,000 units once every six to eight weeks over the six months (a total of four high dose preparations). This will be in the form of oral liquid oil. The higher 200,000 units dose will be given initially when the 25-OH vit D levels will be at their lowest. This will lead to a rapid correction of VDD in the intervention arm, such that participants should achieve or be close to achieving the target 25-OH vit D level of > 75 to 80nmol/l early in the study. If participants are not achieving the target of > 75 to 80nmol/l at interim analysis at 3 months I propose they can be administered an extra amount of Vitamin D3 therapy during their third dose (see Visit 5 below). When the intervention arm receives the oral liquid oils, the comparator arm will receive a matched placebo oil to maintain blinding.
8.7.6 Safety elements in the VITALITY study

There will be various safety criteria. Firstly, as we are focusing on people with VDD as our target population, which theoretically can include some people with very low 25-OH Vit D levels, it is unethical to have a comparator arm receiving only placebo based medications within the study, as these people may be at risk of metabolic bone disease complications (e.g. Osteomalacia related fractures). Therefore I will introduce a control arm, rather than a placebo arm, and they will receive Vitamin D3 replacement at the UK standard doses (1000 units once daily tablets). This will reduce the risk of such complications occurring. Furthermore, the intervention arm will also receive this daily tablet to maintain blinding within the study. The proposed VITALITY study will be an intervention versus control trial. The hypothesis suggests only higher doses of Vitamin D3 therapy will influence glycaemic markers over six months, therefore lower doses such as 1000 units per day should not substantially alter the primary outcome result.

Secondly, participants with a history of bone problems and fractures will be excluded, as it may be suggested that these individuals will need high dose Vitamin D3 replacement (see below). Thirdly, it is important to avoid Vitamin D toxicity in the intervention arm. Published reports suggest 25(OH) Vit D levels must rise above 750nmol/l to produce Vitamin D toxicity; however hypercalcaemia occurs above 375 to 500nmol/l (Jones, 2008). A more prudent upper limit of 250nmol/l is recommended to ensure a wider safety margin (Jones, 2008), but in the VITALITY study the limit will be 220nmol/l based on the advice of the UK Medicines and Healthcare products
Regulatory Agency (MHRA). We estimate that Vitamin D toxicity should not be encountered in this study, especially as people with pre-existing kidney and liver disease are excluded before the study, as are those on medications that are known to interfere with Vitamin D metabolism (see exclusions below). Exposure to sunlight is not expected to contribute to Vitamin D toxicity. Total-body sustained sun exposure may somewhat increase Vitamin D levels, however whether British South Asians may experience this is questionable with variable exposure to sunlight, skin pigmentation blocking penetration of certain ultra-violet rays and cultural practices of covering up many areas of the body when outdoors. Fourthly, unblinding sealed envelopes with participant treatment allocation are kept for emergency circumstances with the external drug company packaging the medications, a local on-call pharmacist and a departmental individual who is not involved directly in the study. Finally, there will be checks of levels of Vitamin D, parathyroid hormone, calcium (blood and urine), renal and liver function at baseline and/ or thought out the study.

Adverse events, serious adverse events and suspected unexpected serious adverse reactions will be recorded and reported to the appropriate regulatory authorities according to guidelines set out in Good Clinical Practice and the University of Leicester guidelines (sponsor). Annual safety reports will be sent to regulatory bodies. A data monitoring committee will be organised to oversee elements of the study on a periodic basis.

8.7.7 Regulatory approvals

The VITALITY study received ethical approval from Leicestershire, Northampton and Rutland research ethics committee first in March 2011 and subsequently for major
amendments in September 2012. MHRA approval (EudraCT Ref: 201002421331) was granted in October 2012; research and development approval was subsequently granted in November 2012. Finally, university of Leicester approval was granted as the sponsor in December 2012. The original PhD time started in November 2010 meaning there were large delays in gaining approvals (see conclusions below). The VITALITY study has also been registered on public databases (Clinical trials.gov, NCT01385345, and Current controlled trials ISRCTN 8522562).

8.7.8 Maintaining blinding during the randomised controlled trial

As mentioned earlier, both the intervention and control arms will be given daily 1000 unit Vitamin D3 tablets and therefore this IMP will not be blinded. However when intervention arm participants are administered Vitamin D3 oils, the control arm will take the equivalent volume of placebo oil. Both sets of oils, their packaging/ labelling, prescription pads and overall treatment allocation will be blinded to the main study team. The only exception to this will be at visit 5 where a different set of unblinded doctors and nurses will be conducting the visit as an extra dose of Vitamin D3 may be administered (see below). Secondly, safety blood tests taken during the randomised controlled trial (e.g. 25-OH vit D and renal function) will potentially reveal treatment allocation; therefore these results will be checked by a local unblinded endocrinology doctor and nurse. Any copies of clinical letters with these specific results that are placed in the patient’s hospital notes will be kept in a sealed envelope.
8.7.9 Investigational medicinal products in the VITALITY study

All investigational medicinal products (IMPs) used in the VITALITY study have been sourced, packaged and labelled by Bilcare Global Clinical Supplies (Europe) Limited (now known as Sharp Clinical Services). All labelling of IMPs was performed according to the Annex 13 national guidelines (Good manufacturing practices; Manufacture of investigational medicinal products). The active Vitamin D3 cholecalciferol solution is Vigantol oil ® 20,000 IU/ml oral drop solution (Merck Sono, Gmbh), the placebo oil is Miglyol and Vitamin D3 cholecalciferol tablets are Vigantoletten® 1,000IU (Merck Sono, Gmbh). All products are suitable for vegetarians. These will be transferred to University Hospitals of Leicester Pharmacy department and kept in a secure storage facility between 15 to 25 degrees Celcius until dispensing.

8.8 Outcomes of the VITALITY study

8.8.1 Primary outcome

This pilot study will test the hypothesis that 6 months of periodic high dose Vitamin D3 replacement (200,000 and 100,000 units cholecalciferol, oral liquid drops at 6 to 8 week intervals) followed in between by daily Vitamin D3 1000 units, decreases insulin resistance by HOMA2-IR ≥ 0.36, in comparison to control, standard dose
Vitamin D3 1000IU/day for 6 months, in south Asians with both VDD (25 OH vit D < 25nmol/l) and insulin resistance (HOMA1–IR ≥ 1.4).

In terms of translating the primary outcome into clinical practice, HOMA-IR as a surrogate measure of insulin resistance, is a risk marker that for T2DM. Therefore any reduction in HOMA-IR naturally reflects a risk reduction for T2DM; however by how much this is reduction is expected to be should this study reach the primary outcome is unknown. Therefore this is a proof of concept study.

8.8.2 Sample size: power required for the primary outcome variable

A study with 50 participants randomly allocated to each treatment arm would have 80% power, at the 5% level of statistical significance, to detect an underlying difference in HOMA2-IR (change-from-baseline to 6 months) of 0.36 between the two treatment groups, allowing for 20% participants being lost to follow-up. For this calculation, we derived an estimate of the standard deviation in HOMA2-IR change-from-baseline of 0.573 using the quartile data presented in Table 2 of von Hurst et al., 2010.

8.8.3 Secondary outcomes

1) To investigate the effects of 6 months of periodic high dose pharmacological Vitamin D3 (cholecalciferol, oral liquid drops) replacement followed by daily 1000 units in comparison to control, standard dose Vitamin D3 1000IU/day (2) for 6 months in insulin resistant, Vitamin D deficient south Asians for improvements/
changes in: (a) insulin measures: HOMA1-IR, (b) Fasting glucose, 2 hour plasma glucose and HbA1c 2)

In finding participants who are Vitamin D deficient for the final RCT, we wish to test whether a targeted approach of using GP database searches for eligible participants (using a specifically created search criteria) is more effective than a general approach where anyone can self-refer to our screening sessions.

8.9 Methods: recruitment and study design for the VITALITY study

8.9.1 Location of the study

This study will be conducted at University Hospitals of Leicester NHS Trust (Leicester, UK) which covers a population of over 950,000; one third of whom are resident in the city of Leicester. In the 2001 census, in the city of Leicester, approximately 30% of people classed themselves as belonging to Indian, Pakistani, Bangladeshi or Sri Lankan ethnic groups (Office of national statistics, census, 2001).

8.9.2 Recruitment strategies

There are two main strategies for recruitment of participants, Figure 8.2. The first method of recruitment is a self-referral method that allows anyone to express interest and attend a screening session. These participants will be responding to
various advertisements, posters and flyers (in English and a variety of South Asian including Gujarati, Hindi, Urdu, Punjabi and Bengali) which will be placed within local general practices, pharmacies, community centres, places of worship and University Hospitals of Leicester. Also, the study will be advertised on media services (e.g. local radio stations, magazines and newspapers), on internet health websites and at local health promoting events. Furthermore, people who have been involved in previous research studies in our department may be asked to participate.

The second strategy is to utilise a targeted method consisting of searching primary care databases using a MIQUEST search which has been specifically created for this study by Dr. David Shephard (Leicester City PCT). MIQUEST Enquirer software (Crown Copyright) is distributed under license by NHS Connecting for Health, UK. This search is effectively acting as a filter to select those people who eligible for the study (e.g. those not prescribed Vitamin D therapies and not previously diagnosed with T2DM, see below for full list of inclusion and exclusion criteria) or people at the highest risk of entry criteria (e.g. central obesity for insulin resistance criteria). The search criteria will only apply to those variables that are recorded in the database and assumes they are correctly coded. The search criteria is listed below:

- Age 25 to 75 years
- South Asian
- Central obesity by body mass index or waist circumference using south Asian cut-points for these indices (Snehalatha et al., 2003)
- IGR or HbA1c 5.7 to 6.4% if recorded
- No previous T2DM diagnosis
- Not prescribed vitamin D and/or calcium therapy
- Not currently pregnant or breast feeding
- Not currently involved in another interventional research study
- No history of osteoporosis, osteomalacia
- Chronic kidney disease stages 3, 4 and 5, dialysis, chronic renal failure or hyperparathyroidism.

For either method of recruitment, the participant will sign and return a reply slip after reading the participant information sheet (either directly handed to a patient or sent in the post via the general practitioner).
Method 1: **Population**
- Advertisements through local media (internet, radio, magazines)
- Posters, leaflets, flyers in Community centres, places of worship, Hospital, pharmacies, general practices
- Word of mouth through friends

Participant response via Telephone or email

Invitation letters sent to participant
Patient information sheet
Reply Slip

Method 2: **Targeted method** through General practice database MIQUEST searches:
- Age 25-75 years
- South Asian prediabetes, centrally obese
- No Diabetes diagnosis
- Not on Vitamin D or calcium therapy
- Not pregnant/breast feeding
- No significant bone or renal disease
- Not taking part in other studies

Information pack sent via GP

Participant returns reply

Telephone call – answer further questions
Brief checks (age/ South Asian/ diabetes/ Vitamin D/calcium tablets/ pregnancy criteria/ certain medications).

Participant interested/ eligible

**Screening appointment:**
- Consent / Past medical history / medication + contraceptive checks
- Bloods: Glucose/ Insulin/ Vitamin D levels, Renal/ liverfunctions, Bone + lipids

Qualify for baseline visit (HOMA-IR ≥ 1.4 + Vit D < 25nmol/l + safety requirements met)

Not qualify: discharged to GP with a results letter
8.9.3 Study design

After identification of a VDD and insulin resistant population, eligible participants will be recruited into a double blind, randomised controlled trial for 6 months, Figure 8.3. This will be a feasibility trial and has been developed in line with CONSORT guidelines for parallel randomised controlled trials.

8.9.4 Randomisation

This will be performed by the unblinded statistician and departmental researcher who has access to the unblinding envelopes. Individuals will be randomised to the intervention or control arms using randomisation lists, stratified by age (25 to 49 years vs. 50 to 75 years), gender (male vs. female) and the season of study entry (winter and spring vs. summer and autumn).

8.9.5. Eligibility to participate in the VITALITY randomised controlled trial

**Inclusion criteria:** People will be included if they meet all criteria:

a. 25 to 75 years old south Asian (Bangladeshi, Indian, Pakistani or other South Asian country) man or woman.

b. A low vitamin D level (defined by 25(OH) VitD<25 nmol/L)

c. Insulin resistance, defined as HOMA1-IR ≥ 1.4.
**Exclusion criteria:** People will be excluded if they have **any** of the following:

**a.** Previous or newly detected diabetes (Type 1 or 2) using WHO criteria 2011 (WHO, 2011).

**b.** Prior use of calcium and/or Vitamin D tablets (D2 ergocalciferol or D3 cholecalciferol, alfacalcidol, calcitriol, paricalcitol, dihydrotachysterol) / therapy or previous adverse reaction to Vitamin D (D2 or D3). Participants must be off these therapies for at least one to two months. All other medications are permitted.

**c.** People with an allergy to nuts, as Vitamin D liquid preparations may contain trace amounts of peanut oil.

**d.** Pregnancy, breast feeding, females or males whose female partners are actively intending to become pregnant during the planned six month trial. A urine pregnancy test will be performed prior to initiation of IMPs. Also, females / males whose female partners are of child bearing potential who are not using a single adequate contraceptive method will be excluded. These include established use of oral, injected or implanted hormonal methods of contraception; intrauterine devices and systems; condoms/ occlusive cap (diaphragm or cervical/vault caps) together with spermicide; male sterilisation or true abstinence.

**e.** Pre-existing or newly detected hypercalcaemia/ hypocalcaemia, hyperparathyroidism, kidney stones or other kidney problems, estimated glomerular filtration rate \(< 60\text{ml/min/1.73m}^2\), liver disorders or granulomatous conditions that may interfere with Vitamin D and calcium metabolism.

**f.** A history of known bone diseases (e.g. osteoporosis, osteomalacia, osteopetrosis) or muscle diseases. People with a history of a low trauma fractures or multiple falls
will also be excluded. A raised PTH will be considered in the clinical context of symptoms, ALP and Vitamin D level (i.e. may or may not be excluded).

g. Terminal illness, malignancy or physical inability to give consent (not language barriers).

h. Taking medications which may interfere with Vitamin D metabolism (anti-convulsants: phenytoin, carbamazepine; primidone, thiopental and barbituates), potentially leading to other problems (thiazide and related diuretics, digoxin, digitalis and anti-tuberculosis medications) or influencing hyperglycaemia/insulin resistance (e.g. oral or intravenous corticosteroids).

i. Participants unable to commit time for the six month study (e.g. holiday abroad, work commitments).

j. Actively taking part in another interventional study (e.g. medication, lifestyle RCTs); observational and cross sectional studies are still permitted.

8.9.6 Participant discontinuation criteria and withholding of IMPs

Participants will not be allowed to continue the study if they become pregnant (females), are not following contraceptive guidelines, experience intolerable adverse events (as possible side effects of vitamin D3 or other new symptoms) or investigator judgement for any further issues.

The Vitamin D IMPs will be withheld during the course of the randomised controlled trial if the 25(OH) Vit D safety tests taken during the study are > 220nmol/l; serum adjusted calcium above the upper limit of normal defined as ≥ 2.7mmol/l (confirmed on a repeat test with one week); impaired kidney function defined as serum
Creatinine >140 µmol/L or a drop in estimated glomerular filtration rate by > 30ml/min/1.73m² (both confirmed by a repeat test within one week); urinary calcium level > 7.5mmol/24 hours or if participants are not adhering to contraceptive advice. Finally, if a participant has a high parathyroid hormone during the baseline visit that does not reduce by approximately 20% at 3 months, this may suggest primary hyperparathyroidism and may require further management (e.g. an ultrasound scan of the parathyroid glands). A diagnosis of primary hyperparathyroidism is an exclusion criterion.

8.10 Participant visits

Prior to the all study visits, participants were sent a letter through the post with their appointment date and time. All appointments were carried out at a local Leicester hospital and research facility. Interpreters and transport were organised if necessary. Any non attendances at appointments were given up to two re-arranged appointments. Participants can only proceed to the next visit if all entry criteria and all safety requirements are met.

8.10.1 Screening visit: V¬2

Participants attended an early morning appointment after an overnight fast of minimum 8 to 10 hours (excluding drinking plain water). Participants were asked to provide written informed consent for entering the study. All participants were given a
copy of the consent form; additional copies were retained in the participant case report file, NHS notes and a copy sent to the general practitioner.

The blood tests performed at screening included FPG, an insulin level, 25-OH vit D, urea and electrolytes, bone test, liver function test and lipid profile (see Table 8.2). Furthermore, past medical history, medications, allergies and contraceptive checks were performed, as well ascertaining whether the participant came via the targetted or population method of recruitment. People with HOMA-IR ≥ 1.4 and 25-OH vit D< 25nmol/l, who meet all additional entry criteria, were invited to the baseline visit.

8.10.2 Baseline Visit (V - 1)

Participants attended an early morning appointment after an overnight fast of minimum 8 to 10 hours. Participants underwent blood tests including an oral glucose tolerance test (OGTT, 75 gram oral glucose load consisting of 410mls Lucozade or equivalent product) as well as measurement of blood insulin levels (at 0 minutes and 120 minutes), parathyroid hormone and an HbA1c. A urine pregnancy test was performed in females of child bearing potential. Anthropometric measurements were taken in accordance with standardised operating procedures by trained staff members. Height was measured to the nearest 0.1cm with a rigid stadiometer. Body weight was measured in light indoor clothing to the nearest 0.1kg using a Tanita scale (Tanita, Europe) which also measures percentage body fat, muscle mass, fat free mass and visceral rating. Waist circumference was measured with a soft tape on standing, mid-way between the lowest rib and iliac crest) all measured to the nearest 0.1cm. Percentage body fat, muscle mass, fat free mass and visceral rating
was measured using a Tanita bio-impedence machine. Brachial blood pressure was measured on three occasions from the right arm of the patient in a sitting position after 3 minutes rest, using standardised Omron M7 digital sphygmomanometers (Omron Healthcare, Milton Keynes, UK). The average of the second and third blood pressure measurements was noted down. Pulse was noted down using the same equipment. Additionally, I will see each participant to perform a brief general examination particularly focusing on evidence of bone disease or those at risk of falls and fractures. Also participants were asked to fill out a questionnaire relating to lifestyle habits (smoking and alcohol intake), education levels, sunshine exposure, health outcomes using EuroQol, EQ-5D (Greiner et al., 2005), dietary habits using Dietary Instrument for Nutrition Education (Roe et al., 1994), as well as foods that contain Vitamin D.

8.10.3 Visit (V1): Dosing visit – start of the RCT

The intervention arm participants will be administered 200,000 units Vitamin D3 in 10ml oral liquid drops mixed in with a drink of the patient’s choice (e.g. water, tea, coffee) and the control participants will be given a similar volume of placebo oral liquid in a similar fashion, both under direct observed therapy. An initial supply of a bottle of 46 x daily 1000 units tablets were given provided for both arms to take home, as well as a spare bottle of 46 x Vitamin D3 1000 units tablets as cover for the study should a participant miss / delay future appointments where further daily Vitamin D3 1000 unit tablets were to be given out. Additionally a study support pack
was given to participants including a diary of any missed tablet dose and any new symptoms.

8.10.4 – Visit 2 (V2): 1 week post-dose follow up

Participants underwent safety blood tests (25-OH Vit D, urea and electrolytes and bone test) in a non-fasting state. Results were chased up by an unblinded local endocrinology specialist doctor, who had the option of discussing the result with the data monitoring committee if there were any concerns (this same process occurred for all other potentially unblinding blood tests throughout the study). Furthermore, the participant had the opportunity to discuss the nature of any new symptoms written in the diary and there were checks of contraceptive use where appropriate (this was also be discussed at all future appointments). If all safety requirements were met the participant allowed to proceed to Visit 3.

8.10.5 Visit 3 (V3): 1.5 month visit

During this non-fasting visit, the second large dose of Vitamin D3 was given to intervention arm participants of 100,000 units in 5ml of oral liquid drops mixed in with a drink of the patient’s choice (e.g. water, tea, coffee) under direct observed therapy. The control group was given 5ml of placebo liquid drops in a similar fashion. A 25-OH Vit D level was checked as well as a 24 hour urine calcium collection.
8.10.6 - Visit 4 (V4): 2.5 month follow-up

Participants returned for non fasting blood tests of 25-OH Vit D levels, urea and electrolytes and bone test. The 25-OH Vit D levels determined the dose of Vitamin D3 at Visit 5 for intervention arm participants.

8.10.7 - Visit 5 (V5): 3 month follow up: Interim analysis

This visit was conducted by unblinded nurses and doctors. At interim analysis, blood tests performed included FPG and an insulin level to calculate HOMA-IR, as well as 25-OH Vit D level and parathyroid hormone. Intervention arm participants will be given 100,000 units of vitamin D3 (5ml oral solution) or placebo oral solution of similar volume for control, under direct observed therapy. Secondly, based on the 25-OH vit D level taken at Visit 4, participants in the intervention arm also received an additional dose of Vitamin D3 simultaneously. If this 25-OH Vitamin D level remained below <30nmol/l then the participant received an extra 100,000 units (5ml of oral solution, giving total volume of 10ml). Those with a 25-OH Vit D level between 30 to < 50nmol/l received an extra 50,000 units (2.5ml) and 2.5ml of placebo oil to give a final volume of 10ml again. Finally those with a 25-OHVit D >50nmol/l did not receive an extra dose, but only 5ml of placebo oil (total volume 10ml). All final solutions were mixed into a drink of the participant’s choice to conceal the volume of IMP administered. Daily Vitamin D3 1,000 unit tablets for the next 3
months were handed to the participant. A 25-OH Vit D level will be checked as well as a 24 hour urine calcium collection.

8.10.8 - Visit 6 (V6): 5 month follow-up

Participants attended for the fourth and final high dose of Vitamin D3, this time at 100,000 units (5ml oral solution) for intervention arm and placebo 5ml oral solutions for control arm under direct observed therapy.

8.10.9 - Visit 7 (V7): 6 month: final visit

Participants attended the final visit after an overnight fast and underwent an OGTT and insulin levels at 0 and 120 minutes. Other bloods taken included parathyroid hormone, 25-OH Vit D levels, urea and electrolytes, liver function test, bone test and lipid profile. Similar to the baseline visit (V-1), anthropometric measurements, blood pressure and pulse were taken, as well as the participant filling out the questionnaire again. Participants had now finished the study, however they were asked to keep a diary for one month (see below). A final results letter was sent to the participant and their general practitioner. If the Vitamin D level was still low (25-OH vit D3 level <25 nmol/l) despite 6 months of high dose Vitamin D3 therapy, then the general practitioner was advised to refer for an opinion from a metabolic bone expert. Advice for other participants varied according to the treatment allocation arm and the 25-OH vitamin D3 level.
8.10.10 - Post study 1 month follow up

A telephone conversation took place one month after the study end with the opportunity to discuss the diary or new symptoms. If there were any issues, the participant was brought to a local hospital for an appointment with the study doctor.
**Figure 8.3 Consort schematic demonstrating participant flow through the VITALITY STUDY 6 month randomised controlled trial**

**Dosing visit V1:** **TIME 0:** RANDOMISATION (direct observed therapy)

**Routine Arm:**
Placebo Oil + 1,000IU/Day throughout 6 months

**1.5 months**
PLACEBO

**1.5 months**
100,000 units

**V2:** Toxicity check 1 week post dose

**V3:** VIT D Efficacy level/ oral drops

**V4:** 2.5 MONTHS
Efficacy/ Toxicity Vit D check

**V5:** 3.0 months
PLACEBO

**V5:** 3 MONTHS:
100,000 units

**V6:** TIME 5 MONTHS
PLACEBO

**V6:** 5 MONTHS
100,000 units

**V7:** TIME 6.0 months
FINISH

**V7:** 6.0 months
FINISH

**Intervention Arm:**
Vit D3 Oil
200,000IU STAT, (1000 units/day throughout 6 months)

**Option of extra Vit D3 (on top of 100,000) for intervention arm:**
1) 100,000 if Vit D3 < 30nmol/l
2) 50,000 if Vit D3 30 to < 50nmol/l
3) PLACEBO if Vit D3 > 50nmol/l

**Vit D level**
OGTT
HOMA-IR
Table 8.2 Measurements and blood tests to be taken throughout the VITALITY study

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Screening (V 2)</th>
<th>Baseline (V 1)</th>
<th>Dosing (V 2)</th>
<th>Visit 2 (V 3)</th>
<th>Visit 3 (V 4)</th>
<th>Visit 4 (V 5)</th>
<th>Visit 5 (V 6)</th>
<th>Visit 6 (V 7)</th>
<th>Visit 7 (V 7)</th>
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<td>Time (weeks)</td>
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<td>-1</td>
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8.11 Laboratory assays

The laboratory assays for measurements for HbA1c, glucose levels and liver function enzymes were described in chapter 3. Insulin levels were measured using a different assay to chapter 3. These were taken in lithium heparin tubes and spun immediately at 3000 RPM for 10 mins, then pipetted into aliquots and frozen at -20 degrees C. I contributed to measuring of Insulin samples initially under the supervision of Dr. Balu Webb (University of Leicester). Levels of insulin within plasma samples were quantitatively determined using the Mercodia (Uppsala, Sweden) Ultra-sensitive insulin ELISA using protocols provided by the manufacturer. The technique used was a direct sandwich assay, in which two monoclonal antibodies are directed against separate antigen sites of insulin molecule.

Plasma samples stored at -80°C were allowed to defrost and were centrifuged at 10,000g for 5 min to remove unwanted cellular debris that may affect ELISA results. Plasma samples and calibrators (the latter to construct a standard curve) were accurately pipetted on to the ELISA plate which was coated with a primary insulin antibody and was left to incubate for one hour.

A secondary insulin antibody conjugated to peroxidise enzyme was then added to each well and following a 30 minute incubation, unbound secondary antibody was removed from the plate by washing six times with wash buffer. Such a vigorous regimen was used to ensure that only secondary antibody attached to insulin molecules remained attached, reducing the chance of non-specific background staining.
In the final step 3,3',5,5'-tetramethylbenzidine was added to each well. This colourless substrate reacts with the enzyme (peroxidase) conjugated to the secondary antibody and produces a blue product which is proportional to the concentration of the secondary antibody which in turn is proportional to insulin levels.

After the 3,3',5,5'-tetramethylbenzidine was left for 15 minutes the reaction was stopped with 0.5 molar sulphuric acid. The solution changed colour from blue to yellow in equivalent proportions to the amount of insulin molecules present. The ELISA plate was read immediately after the addition of sulphuric acid on a spectrophotometer for colourimetric endpoints using a Bio-RAD I mark TM microplate reader at 450nm with a differential filter of 630nm. Manufacturer assessment shows that the intra-assay coefficient of variation for an insulin mean value of 11mU/L was 3.4% whilst the inter-assay value was 3.6%. All ELISA plates were run with control sera for the internal quality control, where the control sera (Mercordia Diabetes Antigen Control) were analysed parallel to the patient sera in order to evaluate the accuracy and the precision of the analyses performed.

25 (OH) Vit D was measured using Liquid chromatography mass spectrophotometer, Agilent Technology 6410 Triple Quad. The inter batch coefficient of variance at low vitamin D3 levels was 8.3% and high levels is 4.0%; the intra batch co-efficient of variance was 7.5% (low), 5.9% (medium) and 8.6% (high). Urea & electrolytes and bone profile was performed using the Advia 2400 Chemistry System, Siemens Healthcare Diagnostics. For calcium, a mean of
2.55mmol/l has a 1.89% CV. For ALP, a median 184 has CV of 2.7%. PTH will be measured on a Siemens Sentaur machine using Chemiluminescent Immunoassay technique which has a CV of 6.9% at mean 57.7pg/ml.

All the samples throughout the study were analysed in the same laboratory (except insulin levels) using stable methodology standardised to external quality assurance reference values. Regarding the sub-studies blood samples, the EDTA sample which was collected for storage of plasma was spun within two hours of venepuncture at 1000g for 15 minutes and plasma saved into aliquots. The serum samples stood for 30 minutes before centrifugation (spun at 1000g for 15 minutes) and pipetted into in aliquots. Blood samples for insulin and biomarkers was stored in a locked freezer for future analysis. Samples were initially stored in a -20 to -30 Celsius freezer and then transferred to a -70 to -80 Celsius freezer within 2 weeks, if not analysed by then.

**8.12 Statistical analysis**

SPSS version 20.0 (IBM SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. Baseline demographics of the cohort presenting for screening sessions were reported as a whole and also by recruitment strategy (targeted vs. population strategy). Means and standard deviations were reported for normally distributed variables; non-normally distributed parameters were reported as median and inter-quartile range. Discrete variables were analysed using chi-squared tests. Trial data characteristics will be summarised in both intervention and treatment arms at baseline and final follow up. The change in measured
HOMA2-IR, from baseline to 6 months, will be analysed using linear regression modelling, with the stratification variables and baseline values as covariates. All analyses will be by intention-to-treat; therefore missing data will be imputed using a suitable method. A two-sided p-value of <0.05 was considered statistically significant throughout the study.

8.13 Results

There were 63 individuals who presented for screening between December 2012 and September 2013. The mean age of the screening cohort was 48.5 years and 31.7% of these people were male, Table 8.3. The mean vitamin D level was 32.9nmol/l (SD 20.7) and 23 (36.5%) people were VDD, with a further 27 (42.9) classified as having vitamin D insufficiency (25-OH vitamin D 25 to <50nmol/l). Only 13 (20.6%) individuals had a vitamin D level in the adequate or optimal range. The mean FPG was 5.8mmol/l (SD 0.8); overall there were 4 cases of newly diagnosed T2DM.

On comparing individuals recruited via the targeted strategy (n=21) to the population strategy, there was no difference in age, Table 8.4. However the targeted strategy consisted of 61.9% of males, whereas the population strategy only had 19% males (p=0.001). The targeted method had 47.6% of individuals screened in winter and spring, however the population method had 90.5% screened during this time. There was no difference in the proportion of people prescribed vitamin D therapy in the last two (19.0% and 33.3% for targeted and
population methods respectively, p=0.19). Furthermore, there were no significant differences in recruitment categories for mean Vitamin D level (31.9nmol/l (22.0) vs. 34.4nmol/l (20.8), p=0.68) and the proportion of people detected with VDD or combined VDD and VDI.

From the 63 participants screened by October 2013, only eight people were found to be eligible to proceed to the baseline visit (12.7%). Three individuals did not want to enter the RCT phase and therefore were not invited to the baseline visit. Five people attended the baseline visit; two of these individuals developed T2DM on OGTT therefore were excluded. Three participants entered the RCT, of which one was lost to follow-up despite being sent letters to attend appointments. The remaining two participants were mid-way through the RCT by September 2013.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number, percentage or mean/ median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population number</td>
<td>63</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.6 (11.8)</td>
</tr>
<tr>
<td>% Male</td>
<td>31.7</td>
</tr>
<tr>
<td>% screened in Winter or spring</td>
<td>78.3</td>
</tr>
<tr>
<td>25-OH Vit D (nmol/l)</td>
<td>32.9 (20.7)</td>
</tr>
<tr>
<td>VDD, N (%)</td>
<td>23 (36.5)</td>
</tr>
<tr>
<td>VDI, N (%)</td>
<td>27 (42.9)</td>
</tr>
<tr>
<td>Adequate Vitamin D level, N (%)</td>
<td>7 (11.1)</td>
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<td>Optimal Vitamin D level, N (%)</td>
<td>6 (9.5)</td>
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<tr>
<td>FPG (mmol/l)</td>
<td>5.1 (0.8)</td>
</tr>
<tr>
<td>Insulin (µIU/ml) *</td>
<td>7.36 (4.61, 9.97)</td>
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<tr>
<td>HOMA-IR *</td>
<td>1.58 (0.99, 2.30)</td>
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<tr>
<td>Total cholesterol (mmol/l)</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
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<td>LDL-cholesterol (mmol/l)</td>
<td>2.9 (0.7)</td>
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<td>Triglycerides (mmol/l) *</td>
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<td>Creatinine (µmol/l)</td>
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<td>ALT (IU/L)</td>
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<td>GGT (IU/L)</td>
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<td>Calcium (adjusted) (mmol/l)</td>
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<td>Phosphate (inorganic) (mmol/l)</td>
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<td>Undiagnosed T2DM, N (%)</td>
<td>4 (6.3)</td>
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* median (IQR). Key: T2DM = Type 2 diabetes mellitus; VDD, Vitamin D deficiency; VDI, Vitamin D insufficiency
Table 8.4 Comparison of characteristics according to target and population strategies

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<th>Targeted</th>
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<th>P-value</th>
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<td>42</td>
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<tr>
<td>Age (years)</td>
<td>46.8 (13.9)</td>
<td>49.5 (10.9)</td>
<td>0.42</td>
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<tr>
<td>% male</td>
<td>61.9</td>
<td>19.0</td>
<td>0.001</td>
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<tr>
<td>% screened in winter and spring</td>
<td>47.6</td>
<td>90.5</td>
<td>&lt;0.001</td>
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<td>% prescribed Vitamin D therapy in last two years</td>
<td>19.0</td>
<td>33.3</td>
<td>0.19</td>
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<td>25-OH Vit D (nmol/l) *</td>
<td>31.9 (22.0)</td>
<td>34.4 (20.8)</td>
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<td>VDD, %</td>
<td>33.3</td>
<td>38.9</td>
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<tr>
<td>VDD and VDI, %</td>
<td>80.9</td>
<td>76.2</td>
<td>0.53</td>
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</table>

* expressed as mean (SD). Key: VDD, Vitamin D deficiency; VDI, Vitamin D insufficiency
8.14 Discussion

8.14.1 Screening Data

The baseline results of the VITALITY screening in a high risk group of South Asians aged 25 to 75 years without T2DM demonstrate that over 80% of South Asians who present for a screening test had either VDD or VDI. The mean vitamin D level was relatively low at 32.9nmol/l, but not as low as previous data in the same region taken in 2006 to 2009 had shown the mean was 20.3 ± 10.3nmol/l, with 72% of Leicester South Asians having a 25(OH) Vit D level of < 20nmol/l (Webb et al., 2012). However, overall the recent results suggest that there is a still high burden of relatively low vitamin D levels in the South Asian general population and is similar to other reports (Ford et al., 2006; Hamson et al., 2003; Hull et al., 2010).

Secondly, using a targeted strategy in comparison to a population strategy did not lead to any improved rates for detecting VDD (or combined VDD and VDI). This result remained significant even after adjusting for variables that may confound the odds ratios. Thirdly, the 25-OH Vit D levels were similar between the two strategies which formed further evidence of the lack of effectiveness from using a targeted strategy.

8.14.2 Feasibility and reflections of the VITALITY pilot study
By the end of September 2013 only 63 people had been screened and only three of these people were recruited to the main RCT phase, despite the PhD research time starting in November 2010. Therefore it was not a successful/feasible study for the relative time period, which can be attributed to the lengthy period for setup of the study. Subsequently, this did not leave long enough for conducting the study. Many of the delays are described below in chronological order and are also in Figure 8.4.

a) Changes in the protocol (June 2011).

The original format of the pilot study used IMPs which were not the high supra-physiological doses of 200,000 and 100,000 units of vitamin D3, but instead lower doses of 8,000 units per day. However during the course of 2010 and 2011, there was published literature suggesting high dose Vitamin D replacement was required to quickly reverse very low 25-OH vitamin D levels and then these levels should be maintained. Therefore after much deliberation between the study team members including the senior leads, it was decided there should be a protocol amendment to change the IMPs to the higher doses in the current methods section and reflect the new literature. Unfortunately this led to contract negotiation delays with the external pharmaceutical company supplying the drugs (see below). The protocol amendment involved working with Bilcare GCS to see what licensed IMPs were available on the market that would be deemed
acceptable for use in a trial by MHRA, subsequent re-signing of contracts with Bilcare GCS (see below), re-writing the protocol followed by a sponsor review by the University of Leicester, re-submitting the study documents for ethical approval and re-writing and submitting the MHRA CTA form. These changes took 13 to 14 months to make. On a personal reflection, I was less in favour of making this protocol change, because I had seen first-hand how long it had taken to get to study to this point in time and my main concern was remaining time to conduct the study, given my PhD finish time date of October 2013. However simultaneously I did fully acknowledge that the newer current protocol was much better and more likely to achieve the primary outcome.

Other amendments were made based on these changes in the IMPs, in particular an increase in the number of visits (visit 5 was added where an extra dose of vitamin D3 may be administered in the intervention group which would be determined by 25 OH-vitamin D level at visit 4) and an increased number of safety checks conducted in the study as a result of using higher doses of Vitamin D3 which could lead to toxicity (visit 2 and 4). The inclusion of visit 5 led to a requirement of both blinded and unblinded doctors and nurses; this created more logistical problems which took time to solve, however it was a good research experience.

On reflection of this now, I would not have opted for these amendment changes in the protocol. I have learnt that there will always be updates in literature available and whilst it is tempting to update the protocols to reflect this new
information, it must be carefully balanced out against the potential time delays and the remaining available to implement these changes.

b) Contract problems with Bilcare GCS (April 2012). In preparing the new protocol from June 2011 onwards, Bilcare GCS were asked to find new Vitamin D3 and placebo IMPs. They mentioned it would take some time to identify appropriate for clinical trials regarding licensing. Once identified these new IMPs were imported by Bilcare GCS and a new contract for these costs was agreed. However at a later date the qualified person from Bilcare GCS responsible for signing off products as double blind, noticed the two sets of bottles which contained these two liquid preparations (Vitamin D3 and placebo liquid oils) had very minor differences in bottles at the necks /shoulders and was not happy to sign these products off as appropriate in double blind study. Bilcare GCS insisted a new contract must be drawn up in April 2012, which included extra costs of decanting the placebo liquid from its current bottles and placing them in exactly matching bottles to the Vitamin D3 IMP. As a contract had already been agreed, I wondered if I should be accountable for this new cost, as it seemed to be an additional cost of their making. Therefore I consulted with the sponsor and reviewed the terms of agreement. I set-up a meeting with the study leads, university sponsors, university business development section (who reviewed and signed the contracts on my behalf) and research and development leads. Overall, the joint advice was to agree to the new contract suggested by Bilcare, otherwise
I would have to find a new supply company. The business development manager informed the meeting group that Bilcare GCS had not broken. Unfortunately, this delay lasted three to four months in total which in hind sight was a long time period. Overall this highlights the potential problems of signing contracts with external companies in academic research studies and the importance of involving the university to read through the terms and agreements.

c) Delays from the university sponsor / clinical trial pharmacists (2011 to 2012).
Prior to 2010/2011, most studies similar to VITALITY would have been hosted by the University Hospitals of Leicester as the sponsor. However this was switched to the University of Leicester from 2010/ 2011 onwards, which reflected the time in which the VITALITY study was set-up. During the meeting described directly above, the university sponsors were surprised at the number of ongoing delays and suggested they should be involved more directly. They also suggested that if the switching of sponsors taken place earlier, they would have been in a position to offer effective support at an earlier date.
Secondly, during the site initiation meeting with the sponsor (November 2013), the university clinical trial pharmacists suggested more information was required about the procedures for requesting the IMP dispensing, randomisation of the blinded IMP packs and the administration of the additional dose of IMP at visit 5. Once these documents were produced, a second site initiation meeting was organised to run these procedures and then sponsor approval was granted one to two months later. On personal reflection, this is one area I should have got
correct first time around (i.e. checking the trial pharmacists were happy with procedures in place), however it was not previously flagged up by the trial pharmacists in prior meetings, so my impression was that were satisfied with the procedures. Overall, this was not a major delay.

d) Changing of the inclusion criteria (April 2013). Once the screening phase of the VITALITY study had begun in December 2012, the recruitment from screening phase to the trial phase was lower than expected. The main problem appeared to be the HOMA-IR inclusion criteria was initially too high using HOMA-IR ≥ 1.93. This was reduced to reflect the 50th percentile of data collected so far (see section 8.4.4). This change involved re-writing a portion of the protocol, acquiring permission from our data monitoring committee to make this change, then applying for approvals from ethics, MHRA and R+D. The delay was three months but overlapped with the MHRA inspection (below). On a personal reflection this was a change that had to be made, as the uptake of people reaching the HOMA-IR ≥ 1.93 criteria was low, which may have been due to differences in insulin measuring kits and also the population tested. Therefore I chose the 50th percentile of HOMA-IR data (1.4) collected up until April 2013 and this subsequently improved the number of people who met the HOMA-IR criteria.

e) MHRA Good Clinical Practice inspection (June 2013). The University of Leicester underwent a MHRA Good Clinical Practice inspection in June 2013 and the VITALITY study was selected by MHRA as a study for inspection. I spent three months prior to this to prepare for the MHRA inspection, including
undergoing monitoring visits conducted by the University of Leicester Clinical Trials Unit in order to prepare for MHRA inspection. During the MHRA inspection the VITALITY study had minimal feedback and findings. On personal reflection, this was very useful for my training in research, however in terms of progressing with the study it was another delay. I have learnt that keeping documents, note keeping and filing immaculate for the level required for a MHRA inspection in the first place is extremely important, although it may be difficult to achieve in reality.

Many of the delays mentioned above were beyond my control; however these can be used as learning experiences. Problems with similar short term Vitamin D replacement intervention studies have discussed elsewhere, where only two of nineteen studies registered on clinical trials.gov have published their final data, despite half of the trials commencing at least three years ago (Tripolt et al., 2013). Therefore the overall delay in the VITALITY study has not an isolated event and similar problems are likely to have been experienced in other studies.

8.14.2 Feasibility and implications for future research in this area

The VITALITY study was unsuccessful in two main areas. Firstly, there was a low uptake to screening sessions, which may reflect the fact that the UK South Asians are less likely to engage in research studies (Stone, 2005). Anecdotally, during the VITALITY study some individuals reported they wanted to undergo a Vitamin D and diabetes test but did not want to enter the RCT or would not enter
the RCT unless they received the higher dose IMPs. Also, some individuals were already taking vitamin D therapies and therefore had to be excluded prior to screening.

Secondly, the number of individuals who passed the screening visit was lower than expected. The HOMA-IR criteria change is described directly above. However it should be mentioned that the lack of people meeting overall qualifying criteria for the screening visit was also because the mean 25-OH vitamin D levels were higher than expected, with less people with a level < 25nmol/l. Furthermore, many individuals had to be excluded from the screening visit because they were actively on vitamin D therapies. The most likely explanation is that the rise of clinical interest in vitamin D occurred in the years prior to the VITALITY study starting (especially from 2008 onwards), therefore by late 2013 many individuals with VDD had been detected and undergone at least some form of treatment, even if short term, by the own general practitioners. That may explain why the Vitamin D levels in the Leicestershire pilot data (Webb, 2012) were much lower than those I obtained (mean 25-OH of 32.9nmol/l in VITALITY vs 20.3nmol/l from Webb 2012).

Finally, I have mentioned above that the sponsor changed from the University Hospitals of Leicester to University of Leicester prior to 2010, which led to some re-organisation changes on their front and perhaps less ability to support this study. However simultaneously neither the host department nor I were able to identify early enough how much support I would require as a junior researcher, in order to setup this very challenging study. An experienced project manager was
appointed (Mrs. Stephanie Goldby in January 2012) by the host department which helped me with everyday questions, particularly related to MHRA forms and the setting up of a CTIMP. The host department as a whole were very supportive; however there may have been a slight inexperience in the area of vitamin D interventional trials prior to this study. Simultaneously any of these of criticisms could equally be directed at me for not picking up how to set up this study early enough or foreseeing potential delays before they occurred. Overall, this is should be seen as a learning experience that can be used for future research.

In terms of implications for future research in this field, my experiences together with the article mentioned above (where only two of nineteen studies registered on clinical trials.gov published their final data despite starting at least three years earlier, Tripolt et al., 2013) suggest there should be caution before beginning any interventional trials with Vitamin D replacement. From the prospective of the VITALITY study, our results suggest the mean 25-OH Vitamin D levels in Leicester South Asians have increased. It is unknown whether this finding translates to other populations who are previously thought as having the lower vitamin D levels, i.e. it is possible that VDD is less prevalent now. Furthermore, many individuals may be taking low dose vitamin D therapies (either prescribed or over the counter) suggesting the inclusion criteria may need careful thinking. Furthermore, future intervention trials may have to focus on both VDD and VDI (25-OH Vitamin D of 25 to <50nmol/l). Secondly, uptake to screening and the
RCT was lower than expected; the South Asian population has previously been reported as engaging less in research (Stone et al., 2005). Future studies in this area may need to consider this and consider alternate strategies to improve uptake including working with community leaders.

In order to complete a successful academic CTIMP study, there should be a good support system in place in regarding the host department and from the sponsor to aid the study setup together with relevant experience – this was not necessarily the case for VITALITY, but it is important to remember. In terms of protocol amendments, it is better to get a protocol correct first time around but perhaps not always possible to do so. During the course of any research period there will usually be updates in the literature; whether protocols should be amended to reflect these changes is a relative balance between the complexity of the changes, the impact on reaching the primary outcome and remaining time left to complete the work.

Finally the report by Tripolt et al., 2013 analysed the progress of studies which started a minimum of three years ago; it will be important to see how these studies are progressing at five and seven years (i.e. were they finished?). This will provide information on the timescale needed for such future studies. Alternatively, if many of these studies were terminated early, it suggests more caution for repeating the studies – however each study may have its own specific problems/ delays and therefore it would be useful to contact the individual project teams to find out what these were. If the studies were to report the same issues/
delays, it may represent a generalised problem in undertaking vitamin D intervention studies.

In conclusion, the VITALITY pilot study was not successful in terms of feasibility which was mainly due to the long delays in study setup. Future studies in this area should proceed with caution, especially given that most other studies in this appear to be experiencing similar problems.
Figure 8.4 VITALITY study timelines for progress and delays

<table>
<thead>
<tr>
<th>Project dates / progress</th>
<th>Delays</th>
</tr>
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<tbody>
<tr>
<td>PhD time started</td>
<td></td>
</tr>
<tr>
<td>Ethical approval gained</td>
<td>Protocol amendment to change IMPs June 2011</td>
</tr>
<tr>
<td>MHRA approval gained</td>
<td>Contract issues with Bilcre April 2012</td>
</tr>
<tr>
<td>R+D approval gained</td>
<td>Sponsor approval delay (clinical trial pharmacists)</td>
</tr>
<tr>
<td>Sponsor Approval gained</td>
<td></td>
</tr>
<tr>
<td>Screening started</td>
<td>Screening halted: inclusion criteria change April 2013</td>
</tr>
<tr>
<td>Screening re-started</td>
<td>MHRA GCP inspection preparation (March13)</td>
</tr>
</tbody>
</table>
Chapter 9

Overall summary to

this thesis
9.1 Conclusion to this thesis

In this thesis I have reviewed various topics related to T2DM and IGR. Firstly, use of HbA1c provides a good option as an additional diagnostic tool for T2DM and IGR, as there are some logistical advantages associated with its use such as test performance in the non-fasted state. However use of HbA1c ≥ 6.5% will detect more people as having T2DM in comparison to an OGTT in the Leicestershire region, with a similar observation for people with IGR using either HbA1c 5.7 to 6.4% or 6.0 to 6.4%. This impact on prevalence is more pronounced in South Asians as HbA1c is independently higher in this ethnic group compared to White Europeans by 0.2%, however similar independent differences also exist in FPG and two hour plasma glucose. There may be alternate options of incorporating HbA1c into diagnostic pathways for T2DM such as using a two cut-point strategy; however more evidence is required as these appear to be preliminary ideas.

I have additionally investigated novel risk factors for T2DM. The triglyceride-to-HDL cholesterol ratio associates with insulin resistance in White Europeans and South Asian males but not females. Regarding the progression of IGR to T2DM, use of statin therapy was associated with a significantly lower risk of progressing to T2DM among people with lower physical activity levels. Additionally, baseline levels of ALT predicted both progression to incident T2DM and reversion to normal glucose levels in White Europeans with IGR, suggesting this enzyme may have a role in assessing risk for T2DM. Finally, Vitamin D replacement may aid
the prevention of T2DM however there were many delays in setting up the study which prevented me from conducting much of the study.
## Appendix One: Contributions

Key to contributors

<table>
<thead>
<tr>
<th>Initials</th>
<th>Name</th>
<th>Role</th>
</tr>
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<tbody>
<tr>
<td>CW</td>
<td>Carrie Wilson</td>
<td>Project Nurse (blinded)</td>
</tr>
<tr>
<td>CM</td>
<td>Carolyn Maloney</td>
<td>University of Leicester</td>
</tr>
<tr>
<td>CA</td>
<td>Carol Ackroyd</td>
<td>Project Support</td>
</tr>
<tr>
<td>DM</td>
<td>Danielle Morris</td>
<td>Statistician (Blinded)</td>
</tr>
<tr>
<td>EB</td>
<td>Emer Brady</td>
<td>Project Support</td>
</tr>
<tr>
<td>HB</td>
<td>Helen Bray</td>
<td>Project Nurse (unblinded)</td>
</tr>
<tr>
<td>HM</td>
<td>Hamid Mani</td>
<td>Doctor (unblinded)</td>
</tr>
<tr>
<td>HT</td>
<td>Hannah Troughton</td>
<td>Project Nurse</td>
</tr>
<tr>
<td>JH</td>
<td>Jayne Hill</td>
<td>Ethics and R+D coordinator</td>
</tr>
<tr>
<td>JH</td>
<td>Jo Howe</td>
<td>Project Nurse</td>
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<tr>
<td>KK</td>
<td>Kamlesh Khunti</td>
<td>PhD supervisor</td>
</tr>
<tr>
<td>LB</td>
<td>Lesley Bryan</td>
<td>Project Health Care Assistant</td>
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<tr>
<td>LG</td>
<td>Laura Gray</td>
<td>Statistician (unblinded)</td>
</tr>
<tr>
<td>MB</td>
<td>Mike Bonar</td>
<td>Designer</td>
</tr>
<tr>
<td>MC</td>
<td>Mark Cooper</td>
<td>Collaborator</td>
</tr>
<tr>
<td>MJD</td>
<td>Melanie Davies</td>
<td>PhD supervisor</td>
</tr>
<tr>
<td>PC</td>
<td>Patrice Carter</td>
<td>Research Associate</td>
</tr>
<tr>
<td>SG</td>
<td>Stephanie Goldby</td>
<td>Project Manager</td>
</tr>
</tbody>
</table>
Overall programme of work

The author of this thesis, Dr Samiul Mostafa, performed the following activities in relation to each study contained within this thesis:

VITALITY study (Chapter Eight)

- obtained ethics, research and development, MHRA and sponsor approval
- developed the protocol and study documents
- managed the study logistics
- managed participant recruitment
- led all study visits with participants (except visit 5 – unblinded visit)
- communicated study results to the participants and their GPs
- analysed and interpreted data collected to September 2013
Review on HbA1c for diagnosis (Chapter Two)

- developed the search terms
- performed the searches, read the abstracts and full text articles
- interpretation of the data

The above activities were performed under the supervision of MJD and KK

Other activities mentioned in this thesis:

Chapter Eight: the VITALITY study

DW and MJD had the original idea for the study. SAM, DW, MJD, KK and MC were involved in the development of the protocol. Study investigators (MJD, KK) reviewed the study documents which I developed. MB and SJ helped format and design logos, posters, flyers and the participant information sheet.

JH and SG helped review and coordinate the ethics application, MHRA and R+D submission.

The protocol, study documents, ethics, R+D and MHRA applications were reviewed by CM and WG as part of the sponsor review process.

PB provided administrative support for the study, also he assisted/ performed MIQUEST searches on GP databases and was responsible for accelerometer/ ActivPAL data downloads.
EB provided project support during the setup of the study.
CA assisted with matter involving primary care research network.
Randomisation of participants was performed by PC and LG.
Statistical support during this study was provided by DM and LG.
SAM and LS conducted all visits, except at visit 5 (unblinded visit) which was conducted by the unblinded team of HB and DW. Some visits were additionally aided by CW and HT.
Blood tests were taken by LS, LB, CB and HT.
Results of blood and urine tests taken during the study were collected and interpreted by LS and SAM, except when participants entered the RCT; these test results were chased by the unblinded team of HB and DW.
Insulin samples were stored and frozen by LS, then measured by BW and assisted by SAM.
Finally, I am grateful to SG who was project manager for the VITALITY study since January 2012 and provided support and guidance during this time.

**Chapter Two: Review on HbA1c**

SS helped SAM develop the search strategy.
Appendix Two: Letters and documents related to the conduct of and
recruitment to the VITALITY study (Chapter Eight)

This appendix contains the following, in the order as listed:

- Clinical trial registration information
- Participant information sheet
- Consent form
- Questionnaire

Clinical trial registration

1) Clinical Trials.gov identifier: NCT01385345
   Available at: http://clinicaltrials.gov/show/NCT01385345

2) Current controlled trials ISRCTN 18522562
   Available at: http://www.controlled-trials.com/ISRCTN18522562
PARTICIPANT INFORMATION SHEET

The VITALITY Study: a study of Vitamin D replacement in South Asian people with insulin resistance and Vitamin D deficiency

Principal Investigators: Professor Melanie Davies, Professor Kamlesh Khunti, University of Leicester, UK

University of Leicester
University Hospitals of Leicester
NHS

VITALITY STUDY Patient Information Sheet Version 2 29/11/2012
Introduction/Question 1

Do you want to know more about your Vitamin D level and why it may be important for future health, particularly developing Type 2 diabetes? If yes please take a few minutes to read this information booklet.

What is Type 2 diabetes?

In a person without diabetes, part of our body called the pancreas produces an important hormone called insulin which helps reduce high glucose level in the blood, particularly after we eat or drink. The glucose is then stored in other areas of the body (muscles and the liver). In people with Type 2 diabetes, the pancreas does not work as effectively and will not produce as much insulin. Also, any insulin produced may not work effectively and blood glucose levels increase (this is known as insulin resistance). If blood glucose levels remain high (diabetes) people are at a risk of heart attacks, strokes and damage to the kidneys, eyes and feet.

The importance of preventing diabetes. Type 2 Diabetes is rapidly increasing, especially in South Asians. You may have already noticed this in your relatives or people within your local community. Long term problems associated with diabetes (such as a heart attack or stroke) can be detrimental to a person’s life, ability to function and on their families. Therefore if we can prevent diabetes developing in the first place, this is a real bonus. However prevention of diabetes is not always easy as you must maintain a healthy diet and be physical active, which is not easy to maintain in the long term. Therefore we are looking for new methods to reduce a person’s risk of developing diabetes. Our study focuses on South Asians at risk of diabetes (we cannot include anyone who has diabetes).
What is Vitamin D and how is it potentially related to diabetes?

Vitamin D is an important substance which our body uses to maintain strong healthy bones. Vitamin D may also protect us from developing diabetes, high blood pressure and heart disease. We know people with low Vitamin D levels are more likely to develop diabetes in the future. Therefore if people with low Vitamin D levels are given high doses of tablets, we may be able to reduce future risk of developing diabetes. However we will not know if this is true or not until it is tested in a formal research study.

South Asians and low Vitamin D levels.

Vitamin D is mainly gained from exposure to sunlight, although a smaller amount can come from diet (e.g. oily fish, cod liver oil).

British South Asians are known to have very low Vitamin D levels – in fact in Leicester we estimate 7 out of 10 South Asians have very low Vitamin D levels. This may be related to sunlight exposure because:

1. There is less sunlight available in UK
2. South Asians tend to cover more of skin with clothes
3. The colour of South Asian skin does not allow as much sunlight to penetrate through
4. UK South Asians do not generally spend enough time outdoors

“So is the answer to spend more time in the sun and eat certain foods?”

We cannot ask people to spend excessive time in the sun (which risks skin cancer) or to eat large portions of certain foods at every meal! Therefore using medication is an alternative option. The current recommended dose of Vitamin D medication (800 units per day; although 1,000 units is available at certain health shops) is currently being debated, as this is not high enough to increase a very low Vitamin D level. Therefore we may need to use higher doses of Vitamin D medication in South Asians.
Question 2:
Can we reduce the risk of developing diabetes by giving higher dose Vitamin D to South Asians with low Vitamin D levels?

This is the question our hopes to answer. But we can’t do this alone. We need the support of south Asian people in Leicester interested in finding the answer to this question. In fact we need 100 people for a six month study! Because joining a research study is an important decision, we have put together some information to explain why the research is being done and how it may benefit you. You can talk it over with your family or friends, and if anything is not clear, or you would like to know more, we have contact numbers at the end of the leaflet so you can talk directly to us about being invited to take part in this research study.

Why have I been chosen?

We are inviting south Asian individuals who participated in diabetes screening studies in Leicestershire who may have at least one risk factor for developing diabetes. Alternatively, your GP has identified you have a risk factor for developing diabetes in the future, even if it is something simple like age or family history of diabetes.

What dose of Vitamin D will be used?

We know using high doses of Vitamin D is safe and not harmful to the human body - in fact, researchers have used up to 600,000 units safety without any reported problems and doctors can use up to 300,000 units injections. We will not be using those doses but instead some people in the study may be taking 200,000 and 100,000 and units once every six to eight weeks in a vegetarian oral liquid solution followed by regular daily 1,000 units per day tablets. These doses are more effective at increasing very low Vitamin D levels and will be under monitored conditions. Only people with certain conditions (including liver or kidney problems, pregnancy/ breast feeding or history of muscle/bone problems) may not be able to take this. We will identify who these people are.
Will everyone take the same dose of Vitamin D?

No - Some people will take the 200,000/100,000 units dose while others will take 1,000 units tablet every day. We can not choose which dose you will take - a computer will randomly decide. Everyone will receive some form of Vitamin D medication and also general education on:

1. Vitamin D and
2. Reducing the risk of diabetes

To enter this study you must not be taking Vitamin D tablets or Calcium tablets for one to two months. You need to be aged 25 to 75 years to take part.

How long do I need to take the Vitamin D for?

The study lasts six months and involves taking four loads of oral liquid drops every six or eight weeks, then a daily Vitamin D tablet in-between. We hope the low Vitamin D level will be improved by the end.

The tablet can be taken at any time of the day (e.g. breakfast or before bed) - but we recommend it is the same time everyday. At the start of each six to eight weeks we may ask you to see come us when you have the oral liquid drops.

Do I have to take part?

No - Taking part is entirely up to you, although of course we hope that enough people will come forward to help us run the study. Even if you decide to take part now, and change your mind later, you can stop whenever you wish. And whatever decision you make will not affect the quality of care you receive.
What will happen to me if I take part?

1. **Initial contact** - First of all, we ask you to contact us either by email/telephone (see back page) or by sending us the reply slip attached to your invitation letter.

2. **Screening** - We will contact you and arrange a screening visit at a local hospital in Leicester or at a community practice.
   - If you need an interpreter to help you we can arrange this for you.
   - This visit will be first thing in the morning and will take around 1 hour.
   - We will ask you to fast (not eat any food after midnight, you may have plain drinking water) and bring a list of your current medications.
   - Initially we will ask you to sign a consent form.
   - Then we will take up to 20mls of blood (4 teaspoons) to determine if you can enter the study.
   - Once the tests have finished, we will of course provide you with a light snack and a cup of tea/coffee.
   - You will meet members of our team and can ask any questions you might have.
   - We will also ask you to complete a questionnaire about your health, eating habits and amount of time you spend in the sun.

If your results show:

1. Very low Vitamin D
2. You have some insulin resistance

We will arrange a longer detailed appointment as we will perform a full diabetes test (called an oral glucose tolerance test which may take up to three hours) at a second visit and take other blood samples (up to 32ml blood).

The study doctor will perform some examinations and a nurse will measure your height, weight, waist circumference and blood pressure.

If all tests and examinations are satisfactory; we will arrange to handover the first set of tablets (a short visit: up to 30 minutes) and a diary for anything you wish to fill in.

You have then entered the six month research study.
3. **Follow up** - We will arrange another appointment for you to take the oral liquid drops, then we would like to see you again one week afterwards to see how you are getting on and to check your Vitamin D level (requiring up to 10ml of blood, 2 teaspoons). This will last up to 30 minutes.

4. **At six weeks** - we will give you some more oral liquid drops and check your vitamin D level again (up to 5ml of blood).

5. **At three months** - we will check your Vitamin D level and insulin resistance level again, then give you more oral liquids drops.

6. **At six months** - The last appointment after six months will be the similar to the second visit.

At all visits we will keep you informed of results by writing (and your GP if you are happy) After the study if you still need more Vitamin D tablets we will write to your GP and recommend for these to go on your regular prescription.

**Optional studies**

There are also some optional studies - it is for you to decide whether or not you would like to be included.

1. Firstly, we may ask if you would like your physical activity level measured for two weeks (one week before the start and one at the end of the six months) - this is useful information on how active you are! It involves wearing a small device similar to a pedometer, but called an accelerometer.

2. Secondly, during the oral glucose tolerance test, we may wish to take another set of blood samples during the test for glucose and insulin levels.

3. Finally, we would like to store some of your blood samples long term and use them for future diabetes related research. Any such study that we want to do in future will have ethics committee approval to ensure we may do with samples is approved and regulated. We will store the sample in our secure freezers for up to 10 years, after which we will transfer them into a bio-bank. When you donate your samples you will be ‘gifting’ them to us, but you can at any time request them to be destroyed if they have not been used.
Will there be any side effects of taking Vitamin D?

Any medication may have side effects. Regarding Vitamin D, these usually occur if a person has taken too much - we will reduce the likelihood of this by excluding people prone to having high Vitamin D levels (such as those with kidney and liver problems). We will expect the vast majority of people to be fine.

The possible side effects reported include constipation or diarrhoea, nausea and/or vomiting, passing more urine than normal, thirst, weight loss and headaches. If you have unpleasant side effects, then we will simply stop the oral liquid drops/tablets. Also, the study involves having blood tests which occasionally lead to small problems of bruising, swelling and/or temporary discomfort.

For females of child bearing potential - it is important you do not become pregnant during the study, as the effects of high dose Vitamin D therapy on the early stages of foetal development are not fully known. Therefore we can only include those using adequate contraceptive methods or participating in true abstinence during the course of the study - this will be discussed during your first study visit. If you decide to participate in the study and meet all the inclusion criteria, a urine pregnancy test will be carried out to confirm that you are not pregnant. Females who are already pregnant, planning to become pregnant or breast feeding can not take part in this study.

For males who have a female partner of child bearing potential - it is important your partner does not become pregnant during the study. If you and your partner are planning to have a baby you can not take part the study. You must also ensure that you use adequate contraceptive methods or participate in true abstinence during the course of the study - this will be discussed during your first study visit. If your female partner is pregnant, then you must use condoms during your participation in the study and for four months afterwards. Also you should not make any sperm donations whilst you are participating in the study and for four months afterwards.
What are the possible benefits of taking part?

All people will find out their Vitamin D level and have a basic diabetes test. Those who enter attend the second visit will have a full diabetes test. If we find out you have new diabetes or other health problems, we will arrange for you to be followed up by a doctor.

You also have the opportunity to help us answer our initial study question and this could one day benefit the South Asian community. The results of the study may be published in a professional journal, but you will not be identified by name in any publications.

Will I get travelling expenses?

Reasonable travel costs will be reimbursed, which includes parking charges and public transport fares, so please keep all your receipts. If driving you will also need to record your mileage.

What if something goes wrong?

If you are harmed by taking part in this research project there are no special compensation arrangements. If you are harmed due to someone’s negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

Will my taking part in this study be kept confidential?

Absolutely! All information about you will be kept strictly confidential. All your research data may be handled by the study staff, the sponsors and regulation bodies.
Who is organising and funding the research?

This research is being undertaken by the University of Leicester Diabetes Research Team which is led by Professors Melanie Davies and Kamlesh Khunti. The study is coordinated by the University Hospitals of Leicester NHS Trust (UHL) and funded by Novo Nordisk Research foundation.

Who has reviewed the study?

All research that involves NHS patients or staff, information from NHS medical records or uses NHS premises/facilities must be approved by an NHS Research Ethics Committee before it goes ahead. Approval does not guarantee that you will not come to any harm if you take part. However, approval means that the committee is satisfied that your rights will be respected, that any risks have been reduced to a minimum and balanced against possible benefits where you have been given sufficient information to make an informed decision.

Further Information

If you require any further information about the study you can contact Dr. Samiul Mostafa or the VITALITY study team on:

T: 0116 258 8893/4389 (or email: samiul.mostafa@uhl-tr.nhs.uk)

If you would this leaflet in a different Language please let us know (e.g. Hindi). If you require more information about general research you can contact Research and Development Office on 258 8351 or Patient Information Liaisons Service (PILS) 08081 788337

Many thanks for taking the time to read this patient information booklet
The VITALITY Study Consent Form

Version 3, 07/03/2013

Chief Investigators: Professor Melanie J Davies, Professor Kamlesh Khunti

Study ID

1. I confirm that I have read and understand the patient information sheet V2 29/11/2012 for the above study. I have had the opportunity to consider the information, ask questions & have had these answered satisfactorily.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, without my medical or legal rights being affected.

3. I understand that sections of my medical notes and/or study data may be looked at by responsible individuals from the study team, from regulatory authorities, the sponsor or the NHS trust where it is relevant to me taking part in research. I give permission for these individuals to have access to my records.

4. I agree to my GP being informed of my participation, all blood tests & clinical consultations. I agree to the study team asking my GP for current medications/ past medical history if needed.

5. I wish to be informed of my test results.

6. I agree to take part in the above study.

The following questions are Optional for the VITALITY study:

i. I consent for my blood sample to be stored and used for future research. I understand this is a free choice and does not affect my participation in the study. I understand this is donated as a gift and I can ask for its destruction should I wish.

ii. I consent for my stored samples to be transferred to a biobank after 10 years of being stored if they have not been used.

iii. I understand that information held by the NHS and records maintained by the General Register Office may be used to keep in touch with me and follow up my health status.

iv. I agree to being contacted with details of future research and my details to be stored on a computer database for this purpose.

v. I consent for participation in the physical activity sub-study using an Accelerometer.

vi. I consent for participation in the oral glucose tolerance test sub-study, during which there are more blood tests may be taken.

Name of patient __________________ Date __________ Signature __________

Name of person taking the consent __________________ Date __________ Signature __________

VITALITY Study Consent Form Version 3 07/03/2013
1 copy participant; 1 copy researcher pack, 1 copy for medical notes (if they exist), 1 copy for GP record
Study ID:   

Initials:   

Date:  

Questionnaire Booklet

Please fill out all the questions contained in this booklet.
The answers you give are important to us & will be treated with the utmost confidentiality.

It is necessary to find out if it is safe for you to enter the VITALITY study 6 month trial.

Any questions you can not understand, please ask for help from a member of the study team.
SECTION A - Background information

A1. What is your highest education level achieved?

- None
- GCSE/O levels/GNVQ level
- A level/NVQ 3
- College/City & Guilds
- University degree
- Post graduate degree

A2. How much alcohol do you consume on average per week?

- [ ] 3 units/week
- [ ] 2 units/week
- [ ] 1 unit of 4% ale
- [ ] 9 units
- [ ] 1 pint of ginger beer
- [ ] 1 pint of 4% cider
- [ ] 1 litre of 5% cider
- [ ] 1 bottle of 5% lager
- [ ] 1 bottle of 5% wine

A3. What is your current work status?

- In work - full time i.e. more than 30 hours per week
- In work - part time i.e. less than 30 hours per week
- Keeping house
- Wholly retired from work
- Waiting to start a new job already obtained
- Unemployed and looking for work
- Out of work as temporarily sick
- Permanently sick or disabled
- Student
- Other

If other, please specify

A4. What is your legal marital status?

- Married/civil partnership/ co-habiting
- Unmarried
- Divorced/Separated
- Widow/Widower
A5. Have you ever smoked?

1. Yes
2. No (skip to question B1)

A6. Do you smoke now?

1. Not at all
2. Yes, occasionally
3. Yes, daily

A7. If you smoked earlier but do not smoke now, when did you quit smoking?

1. Less than 1 month ago
2. 1 – 5 months ago
3. 6 – 11 months ago
4. 1 – 4 years ago
5. 5 – 9 years ago
6. 10 or more years ago

A8. If you smoke daily, how much you smoke per day (Use numbers)?

1. Cigarettes _________ per day
2. Pipes _________ per day
3. Cigars _________ per day
4. Other _________ per day (Such as Sheesha, waterpipe, hookah, ..)
**Section B: Quality of Life (EQ-5D)**

These questions are about your general quality of life.

Please indicate which statement best describes your health state, today, by ticking one box in each group:

1. **MOBILITY**

I have no problems in walking about
I have slight problems in walking about
I have moderate problems in walking about
I have severe problems in walking about
I am unable to walk about

2. **SELF-CARE**

I have no problems washing or dressing myself
I have slight problems washing or dressing myself
I have moderate problems washing or dressing myself
I have severe problems washing or dressing myself
I am unable to wash or dress myself

3. **USUAL ACTIVITIES**
   (e.g. work, study, housework, family or leisure activities)

I have no problems doing my usual activities
I have slight problems doing my usual activities
I have moderate problems doing my usual activities
I have severe problems doing my usual activities
I am unable to do my usual activities

4. **PAIN / DISCOMFORT**

I have no pain or discomfort
I have slight pain or discomfort
I have moderate pain or discomfort
I have severe pain or discomfort
I have extreme pain or discomfort

5. **ANXIETY / DEPRESSION**

I am not anxious or depressed
I am slightly anxious or depressed
I am moderately anxious or depressed
I am severely anxious or depressed
I am extremely anxious or depressed
SECTION C: Physical Activity (IPAQ).

The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house & yard work, to get from place to place, & in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort & make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ days per week

   [ ] No vigorous physical activities ➔ Skip to question 3

2. How much time did you usually spend doing vigorous physical activities on one of those days?

   _____ hours per day
   _____ minutes per day

   [ ] Don't know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort & make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ days per week

   [ ] No moderate physical activities ➔ Skip to question 5
4. How much time did you usually spend doing **moderate** physical activities on one of those days?

    _____ hours per day
    _____ minutes per day

    [ ] Don’t know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work & at home, walking to travel from place to place, & any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

    _____ days per week

    [ ] No walking  ➔  **Skip to question 7**

6. How much time did you usually spend **walking** on one of those days?

    _____ hours per day
    _____ minutes per day

    [ ] Don’t know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work & during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

    _____ hours per day
    _____ minutes per day

    [ ] Don’t know/Not sure
SECTION D: Eating Habits Questionnaire (Dine)

Purpose
The purpose of this questionnaire is to get an idea of your usual eating habits. For the listed foods, we would like to know how many servings you eat in a typical day or week.
A serving is an average portion that would be served at a meal. If you usually eat more than one serving of the food at a time, you should count all the servings you eat.

Instructions
For each food listed, tick the box that describes the number of servings that you usually eat. If you never eat a particular food, tick the box under “None”.

Please do not leave any lines blank.

<table>
<thead>
<tr>
<th>Breads &amp; Rolls</th>
<th>None</th>
<th>Less than 1 a day</th>
<th>1 to 2 a day</th>
<th>3 to 4 a day</th>
<th>5 or more a day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. White bread or rolls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Brown or granary bread or rolls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Wholemeal bread or rolls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Chapatis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Breakfast cereals</th>
<th>None</th>
<th>Less than 1 a week</th>
<th>1 to 2 a week</th>
<th>3 to 5 a week</th>
<th>6 or more a week</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Sugared type: Frosties, Coco Pops, Rice Krispies Sugar Puffs&lt;br&gt;Rice or Corn type: Corn Flakes, Rice Krispies, Special K</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Porridge or Ready Brek&lt;br&gt;Wheat type: Shredded Wheat, Weetabix, Fruit 'n Fibre, Puffed Wheat, Nutri-grain, Start&lt;br&gt;Muesli type: Alpen, Jordan's</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Bran type: All-Bran, Bran flakes, Sultana Bran</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### About how many servings per week do you eat of the following foods? (Please tick one box on each line)

<table>
<thead>
<tr>
<th>Vegetable foods</th>
<th>None</th>
<th>Less than 1 a week</th>
<th>1 to 2 a week</th>
<th>3 to 5 a week</th>
<th>6 to 7 a week</th>
<th>8 to 11 a week</th>
<th>12 or more a week</th>
</tr>
</thead>
<tbody>
<tr>
<td>7. Pasta or rice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Potatoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. Peas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Beans (baked, tinned, or dried) or lentils</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Other vegetables (any type)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12. Fruit (fresh, frozen, canned)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### About how many servings per week do you eat of the following foods? (Please tick one box on each line)

<table>
<thead>
<tr>
<th>13. Cheese (any except cottage)</th>
<th>None</th>
<th>Less than 1 a week</th>
<th>1 to 2 a week</th>
<th>3 to 5 a week</th>
<th>6 or more a week</th>
</tr>
</thead>
<tbody>
<tr>
<td>14. Beefburgers or sausages</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15. Beef, pork, or lamb (for vegetarians: nuts)</td>
<td></td>
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</tr>
<tr>
<td>16. Bacon, meat pie, processed meat</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>17. Chicken or turkey</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Fish (NOT fried fish)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>19. ANY fried food: fried fish, chips, cooked breakfast, samosas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. Cakes, pies, puddings, pastries</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>21. Biscuits, chocolate, or crisps</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### About how much of the following types of milk do you yourself use in a day, for example in cereal, tea, or coffee? (Please tick one box on each line)

<table>
<thead>
<tr>
<th>Milks</th>
<th>None</th>
<th>Less than a quarter pint</th>
<th>About a quarter pint</th>
<th>About half a pint</th>
<th>1 pint or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>22. Full cream (blue top) or Channel Islands (gold top)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. Semi-skimmed (green top)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>24. Skimmed (red top)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>
### About how many rounded teaspoons per day do you usually use of the following types of spreads, for example on bread, sandwiches, toast, potatoes, or vegetables?

<table>
<thead>
<tr>
<th>Spreads</th>
<th>None</th>
<th>1 a day</th>
<th>2 a day</th>
<th>3 a day</th>
<th>4 a day</th>
<th>5 a day</th>
<th>6 a day</th>
<th>7 or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular margarine or butter or</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced fat spread such as sunflower or olive spread; Flora,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitalite, Clover, Olivio, Stork, Utterly Butterly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low fat spread such as Flora Light, St. Ivel Gold, Olivite, Half-fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>butter, Flora Pro-activ, Light spread</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

### What type of fat do you usually use for the following purposes?
(Please tick one box on each line)

<table>
<thead>
<tr>
<th></th>
<th>Butter, lard, or dripping</th>
<th>Solid cooking fat (White Flora, Cock Kee)</th>
<th>Soft margarine (sunflower, soya)</th>
<th>Reduced fat spread (olive, Flora Butterly, Olivio)</th>
<th>Vegetable oil or Low fat spread (Flora Light, Olivite, St. Ivel Gold)</th>
<th>No fat used</th>
</tr>
</thead>
<tbody>
<tr>
<td>27. On bread and vegetables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28. For frying</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29. For baking or cooking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SECTION E: Diet

Purpose: to get an idea of how much Vitamin D you get from your food

Instructions: For each question listed, tick the box that describes your pattern.

Please try to answer every question.

1. Are you vegetarian?
   Vegetarian
   Vegan
   Fish only, no meat
   No

2. Do you eat oily fish?   Yes
   No

   This includes: Salmon, Mackerel, Sardines, Tuna (fresh only), Shrimp, Catfish, Eel, Herring, Trout, Pilchards, Kipper, Anchovies, Whitebait, Swordfish, Carp, Jack fish.

   If YES, how often do you eat oily fish? (Please select only one option)
   Every day
   A few times a week
   Once a week
   Once a month
   Less than once a month

3. Do you take Pure Cod liver oil supplements (not refined)/ Other Fish Liver Oils?   Yes
   No

   If YES, how often?

   If YES, how often do you take these supplements? (Please select only one option)
   Every day
   A few times a week
   Once a week
   Once a month
   Less than once a month
4. Do you drink milk? (including breakfast cereals) Yes ☐
   No ☐
If YES, how often do you drink milk? (Please select only one option)
   Every day ☐
   A few times a week ☐
   Once a week ☐
   Once a month ☐
   Less than once a month ☐

5. Do you eat the areca nut/ betel leaf (‘Paan’)? Yes ☐
   No ☐
If YES, how often? (Please select only one option)
   Every day ☐
   A few times a week ☐
   Once a week ☐
   Once a month ☐
   Less than once a month ☐

SECTION F: Sunshine Exposure Questionnaire

Purpose: to get an idea of how much sun exposure you receive.
Please try to answer every question.

. How much time do you spend outdoors on average during the summer?

1a. How many hours per day during the week? ☐☐

1b. How many hours per day at the weekend? ☐☐

. How much time do you spend outdoors on average during the winter?

2a. How many hours per day during the week? ☐☐

2b. How many hours per day at the weekend? ☐☐

. Which time(s) of day do you usually go outside? (Tick as many as apply)

3a. Weekdays

   Morning (5am-12pm) ☐
   Midday (12pm-2pm) ☐
3b. Weekends

Morning (5am-12pm) ☐
Midday (12pm-2pm) ☐
Afternoon (2pm-6pm) ☐
Evening (6pm onwards) ☐

4. When you go outdoors in the summer do you usually cover the following? (Please tick all that apply)

- Forearms ☐
- Neck ☐
- Head ☐
- Face ☐
- Legs ☐
- Feet ☐

5. When you go outdoors in the winter do you usually cover the following? (Please tick all that apply)

- Forearms ☐
- Neck ☐
- Head ☐
- Face ☐
- Legs ☐
- Feet ☐

6. How much time does your job involve going outdoors during the day? ☐ hours and ☐ minutes per day

7. Do you use a sunbed? Yes ☐
    No ☐

SECTION G: Marshall & Sitting Surveys
Please estimate how many hours you spend SITTING EACH DAY in the following situations: (please write your answer)

<table>
<thead>
<tr>
<th>Activity</th>
<th>On a WEEK day</th>
<th>On a WEEKEND day</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. While travelling to and from places</td>
<td>Hours: □□</td>
<td>Minutes: □□</td>
</tr>
<tr>
<td>2. While at work</td>
<td>□□</td>
<td>□□</td>
</tr>
<tr>
<td>3. While watching Television</td>
<td>□□</td>
<td>□□</td>
</tr>
<tr>
<td>4. While using a computer at home</td>
<td>□□</td>
<td>□□</td>
</tr>
<tr>
<td>5. In your leisure time, NOT including television (eg visiting friends, movies, dining out, etc.)</td>
<td>□□</td>
<td>□□</td>
</tr>
</tbody>
</table>
Appendix Three: Supplementary material for the HbA1c review (Chapter Two)

Search terms used:

1. hba1c.ti
2. (glycated AND haemoglobin).ti
3. (glycated AND hemoglobin).ti
4. (hemoglobin AND A1c).ti
5. (haemoglobin AND A1c).ti
6. diabetes.ti
7. (type AND 2 AND diabetes).ti
8. NIDDM.ti
9. T2DM.ti
10. DM.ti
11. progression.ti
12. prediction.ti
13. (long AND term AND follow AND up).ti
14. (future AND risk).ti
15. (subsequent AND risk).ti
16. incident.ti
17. developing.ti
18. development.ti, ab
19. (long AND term AND follow AND up).ti

20. prognosis.ti

21.correlation.ti

22. 11 OR 12 OR 13 OR 14 OR 15 OR 16 OR 17 OR 18 OR 19 OR 20 OR 21

23. 6 OR 7 OR 8 OR 9 OR 10

24. 1 OR 2 OR 3 OR 4 OR 5

25. 22 AND 23 AND 24
Appendix Four: Publications related to work in this thesis

Papers

Original articles (first author papers listed first)


**Review articles**


Published abstracts (first author abstracts listed first)

Mostafa SA, MJ Davies, DH Morris, DR Webb, BT Srinivasan, K Khunti. Do baseline vitamin D levels independently predict four year progression from high risk states to Type 2 diabetes? Diabetic Medicine (2013): 30 (Suppl. 1), 76 (P157).

Mostafa SA, K Khunti, DH Morris, DR Webb, BT Srinivasan, MJ Davies. Is the use of statin therapy associated with higher risk of progression from high risk states to Type 2 diabetes over four years? Diabetic Medicine (2013): 30 (Suppl. 1), 6 (A15).

Mostafa SA, Khunti K, Webb D, Morris DH, Srinivasan BT, Davies MJ. Can Liver enzymes predict 3 year progression from prediabetes to Type 2 Diabetes


**Mostafa SA**, 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 2010; 27 (suppl. 1), 1: A1.

**Mostafa SA**, DR Webb, LJ Gray, MJ Davies, BT Srinivasan, MJ Davies, K Khunti. 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 2010; 27 (suppl. 1), 1: A65.


**Conference abstracts (non-published)**

Mostafa S, Khunti K, Webb D, Srinivasan BT, Gray LJ, Davies MJ. In a multi-ethnic UK population is using HbA1c ≥6.0 - <6.5% the optimal HbA1c cut-point for detecting people with impaired glucose regulation (IGR)? Presented at the World Congress on prevention of diabetes and its complications Conference 2010.

Mostafa S, Khunti K, Webb D, Srinivasan BT, Gray LJ, Davies MJ. Use of revised diagnostic criteria based on HbA1c≥ 6.5% increases prevalence of Type 2 Diabetes Mellitus (T2DM) in a UK multi-ethnic cohort. Presented at the Anglo-Danish-Dutch Diabetes Group Conference 2010.

Appendix five: Awards and media appearances

- Novo Nordisk Research Foundation UK Training Fellowship 2010 to undertake this PhD
- National Young Diabetologist Award at Diabetes UK APC Winner 2010; short-listed for award 2013.
- Diabetes UK Primary Care National Award Winner 2011; short-listed for award 2013 (as co-author only)
- South Asian Health Foundation Award Winner 2011
- Sanofi Aventis Excellence in Diabetes Runner up prize 2010.

Media appearances:

- ITV Central News, video link: http://vimeo.com/46370680 (July 2012)
- BBC Radio Leicester live broadcast: http://www.bbc.co.uk/radio/player/p00vhff7 (July 2012)
- Radio Kohi-Noor FM (local Leicester station): Vitamin D Deficiency talk: (October 2012)
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