The contribution of impaired glucose metabolism to cardiovascular disease and mortality in Australians

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Abstract

Diabetes and cardiovascular disease (CVD) together represent one of the greatest public health and economic burdens for the Australian population. There is strong evidence that diabetes increases the risk of CVD and mortality, and emerging evidence from overseas populations indicates that the risk of CVD and mortality extends below the threshold to diagnose diabetes. By analysing data from the Australian Diabetes, Obesity and Lifestyle (AusDiab) study, this thesis explored the relationships between hyperglycaemia and (i) all-cause mortality, (ii) CVD mortality, and (iii) CVD events, including myocardial infarction, stroke, percutaneous transluminal coronary artery angioplasty and coronary artery bypass surgery, and the extent to which these relationships were independent of other traditional CVD risk factors, including insulin sensitivity. The AusDiab study is a national prospective cohort study of 11,247 men and women aged 25 years and over which incorporated a 75 gm oral glucose tolerance test and three indices of blood glucose: fasting plasma glucose (FPG), two-hour post-load plasma glucose (2hPG) and haemoglobin A_{1c} (HbA_{1c}). The updated homeostasis model assessment was also used to calculate an index of insulin sensitivity (HOMA-%S). Data on all-cause and CVD mortality were obtained through data linkage with the National Death Index, and self-reported non-fatal CVD events were verified through medical record adjudication; the methods of which were validated by linking a cohort of AusDiab participants from Western Australia to the Western Australian Hospital Morbidity Database. Participants were followed for a median of five to six years, and the association between glucose metabolism, analysed as both continuous and categorical variables, and mortality and CVD were analysed with Cox proportional regression. The findings confirmed the strong association between previously diagnosed diabetes and all-cause mortality, CVD mortality and coronary heart disease, which was independent of traditional CVD risk factors. The risk for all-cause mortality and fatal or non-fatal myocardial infarction was also significantly increased among those with intermediate hyperglycaemia, although for CVD mortality, stronger associations were observed for impairments in FPG than 2hPG. In those without diagnosed diabetes, a continuous relationship between 2hPG and HbA_{1c} and both all-cause and CVD

mortality was observed. For FPG, the relationships were J-shaped, with the risks being increased at both high and low glucose concentrations. FPG was a better predictor of CVD mortality compared with 2hPG or HbA_{1c}, but none of the glucose measures improved CVD risk prediction above that provided by traditional CVD risk factors. There was a continuous relationship between HOMA-%S and fatal or non-fatal CVD events, but this was mainly explained by its association with high-density lipoprotein cholesterol. FPG, but not HOMA-%S, significantly improved the prediction of CVD beyond that of other risk factors. Finally, a stronger relationship was observed between abnormal glucose metabolism and fatal or non-fatal myocardial infarction than with coronary artery revascularisation or stroke. Taken together, the findings of this thesis indicate that abnormal glucose metabolism, including both diabetes and intermediate hyperglycaemia, play an important role in the development of CVD, and suggest that CVD prevention strategies should not only target diabetes, but all levels of hyperglycaemia.

General declaration

In accordance with Monash University Doctorate Regulation 17 / Doctor of Philosophy and Master of Philosophy (MPhil) regulations, the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes three original papers published in peer-reviewed journals, one published book chapter and two unpublished papers which have been submitted. The core theme of the thesis is the contribution of impaired glucose metabolism to cardiovascular disease and mortality in Australians. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Epidemiology and Preventive Medicine, Monash University under the supervision of Professor Andrew Tonkin and Associate Professor Jonathan Shaw.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

Thesis Chapter	Publication title	Publication status	Nature and extent of candidate's contribution
2	Mortality and life expectancy associated with diabetes	Published	Principal author, critically reviewed the literature, interpreted and synthesised the findings of other studies and drafted the manuscript. Responsible author who accepts overall responsibility for the publication.
4	Validity of self-reported cardiovascular disease events in comparison to medical record adjudication and a statewide hospital morbidity database – the AusDiab study	Published	Principal author, critically reviewed the literature and developed the research question, cleaned the self-reported CVD events data, coordinated the medical record abstraction and adjudication of over 600 self-reported CVD events from hospitals and general practices around Australia, collected medical record data in four of the six states and the Northern Territory, created a database for linkage to the Western Australian Hospital Morbidity Database, conducted the statistical analysis, interpreted the results, drafted the manuscript, responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.
5	Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: The Australian, Diabetes, Obesity, and Lifestyle Study (AusDiab)	Published	Principal author, critically reviewed the literature and developed the research question, collected mortality outcome data, planned and conducted the statistical analysis, interpreted the findings, drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.

6	Continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality: the Australian Diabetes, Obesity and Lifestyle (AusDiab) study	Published	Principal author, critically reviewed the literature and developed the research question, planned and conducted the statistical analysis, interpreted the findings, structured and drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.
7	Elevated blood glucose may differentially influence the five-year risk of myocardial infarction, stroke and coronary revascularisation – the AusDiab study	Submitted	Principal author, critically reviewed the literature and developed the research question, planned and conducted the statistical analysis, interpreted the findings, structured and drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.
8	HOMA insulin sensitivity index, hyperglycaemia and the risk of all-cause mortality, and cardiovascular disease events in the general population – the AusDiab study	Submitted	Principal author, critically reviewed the literature and developed the research question, planned and conducted the statistical analysis, interpreted the findings, structured and drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.

Signed: [redacted] ……

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Publications and awards

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Publications by the candidate relevant to the thesis

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Health Behaviors, and Incidence of Diabetes in Australia: The Australian Diabetes, Obesity and Lifestyle Study. *Diabetes Care* 2008;31(2):267-272.

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Shaw J, Barr E, Magliano D. AusDiab 5 years on – the first data from a national Australian follow-up study. Invited oral presentation at the *Australian Diabetes Society-Australian Diabetes Educators Association Annual Scientific Meeting*, Gold Coast, 23rd to 25th August 2006. Abstract published in *Book of Abstracts from the Australian Diabetes Society-Australian Diabetes Educators Association Annual Scientific Meeting*, 2006;A027, p.58.

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Barr ELM, Tonkin AM, Zimmet PZ and Shaw JE. Diabetes and intermediate hyperglycaemia increase the risk of five-year fatal or non-fatal cardiovascular disease – AusDiab. Poster presentation at the *Australian Diabetes Society-Australian Diabetes Educators Association Annual Scientific Meeting*, Melbourne, 23rd to 25th August 2008. Abstract published in the *Book of Abstracts from the Australian Diabetes Society-Australian Diabetes Educators Association Annual Scientific Meeting*, 2008; A279, p.135.

Abbreviations

2hPG	Two-hour post-load plasma glucose
AusDiab	The Australian Diabetes, Obesity and Lifestyle study
BMI	Body mass index
CABG	Coronary artery bypass surgery
CHD	Coronary heart disease
CI	Confidence intervals
CVD	Cardiovascular disease
DECODA	Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Asia
DECODE	Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe
FPG	Fasting plasma glucose
GHb	Glycosylated haemoglobin
HbA_{1c}	Haemoglobin A_{1c}
HDL-C	High density lipoprotein cholesterol
HOMA-%S	Homeostasis model assessment of insulin sensitivity
HOMA-IR	Homeostasis model assessment of insulin resistance
HR	Hazard ratio
ICD-10	2006 International Classification of Diseases 10^{th} revision
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
KDM	Previously known (diagnosed) diabetes
NDM	Newly diagnosed (or screen detected) diabetes
NDI	National Death Index of Australia
OGTT	Oral glucose tolerance test
PTCA	Percutaneous transluminal coronary artery angioplasty
SD	Standard deviation
WHO	World Health Organization

Thesis outline

Chapter 1 outlines the background to the problem being addressed and provides a rationale for the study.

Chapter 2 provides a critical review of the relevant literature which incorporates a published book chapter.

Chapter 3 provides an overview of the methods employed to evaluate the contribution of impaired glucose metabolism to cardiovascular disease and mortality, and discusses the mortality and morbidity data that were collected as part of this thesis.

Chapter 4 presents and discusses findings from a study that assessed the validity of self-reported cardiovascular disease events in comparison to medical record adjudication and a statewide hospital morbidity database.

Chapter 5 presents and discusses findings from a study that assessed the risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance.

Chapter 6 presents and discusses findings from a study that assessed the continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality.

Chapter 7 presents and discusses findings from a study which found that elevated blood glucose may differentially influence the five-year risk of myocardial infarction, stroke and coronary revascularisation.

Chapter 8 presents and discusses findings from a study that assessed the HOMA-%S insulin sensitivity index, hyperglycaemia and the risk of all-cause mortality, and cardiovascular disease events in the general population.

Chapter 9 contains the main discussion and presents the conclusions with reference to the initial aims and hypotheses, limitations and strengths of the study and areas for further research.

CHAPTER 1

Introduction

1.1 Background to the problem

The metabolism and regulation of glucose is fundamental to life, as glucose is the primary source of energy for the body's cells. Glucose metabolism involves several precisely orchestrated physiological processes that control the transfer of glucose from the digestive tract and the liver to the circulation, making it available for uptake by muscle and fat cells to be used as energy. Insulin, a hormone secreted from the β-cells in the pancreas, is responsible for the regulation of glucose metabolism. After a meal, insulin is released into the circulation and helps to maintain glucose homeostasis by suppressing glycogen production from the liver and sensitising muscle and fat cells to take up the circulating blood glucose. Diabetes mellitus develops when there is a disruption in glucose homeostasis, which occurs when there is a reduction in the production of insulin from the pancreas, and/or muscle and fat cells no longer respond sufficiently to the actions of insulin [1].

Diabetes is associated with cardiovascular disease (CVD) and increases the likelihood of premature mortality. Findings from experimental studies suggest that independent of other factors such as elevated blood pressure and dyslipidaemia, abnormal glucose metabolism increases the likelihood of macrovascular disease because it disrupts normal endothelial function, accelerates atherosclerotic plaque formation, and contributes to plaque rupture and subsequent thrombosis [2-4]. In addition, impaired glucose metabolism is often associated with hyperinsulinaemia and insulin resistance, which in themselves may be risk factors for macrovascular disease. The risk attributed to other CVD risk factors such as abnormal blood pressure and lipid levels may also be compounded by the presence of abnormal glucose metabolism [2].

Diabetes and CVD together represent one of the greatest public health and economic burdens for the Australian population. The prevalence of diabetes has more than doubled over a 20 year period between 1981 and 2000 [5], and recent data from the Australian Diabetes, Obesity and Lifestyle study (AusDiab) indicate that nearly one million Australians aged 25 years and over have diabetes (7.4%), with a further 10.6% having impaired glucose tolerance (IGT) and 5.8% having impaired fasting glucose (IFG). IGT and IFG represent conditions of intermediate hyperglycaemia. It is estimated that in Australia by 2025, the prevalence of diabetes will rise to 11.4% and that more than one third of individuals aged 25 years and over will develop diabetes during their lifetime [6]. Despite the fact that age-adjusted mortality rates from CVD have been declining in the general population since the 1960s, CVD continues to have a significant impact on the health of Australians. It remains the single greatest cause of death, being responsible for over one third of all deaths [7, 8]. Therefore, improving our understanding of the impact of abnormal glucose metabolism on CVD and mortality has the potential to improve the health of a significant proportion of the Australian population.

Australian epidemiological data on diabetes and the incidence of CVD and mortality are limited to the Busselton Health Studies [9-11], the National Heart Foundation Risk Factor Prevalence Studies [12], the Dubbo Study [13] and the National Health Surveys [14], all of which have significant limitations. The Busselton Health Studies and the Dubbo study only recruited participants from a single rural town, and the Dubbo study only included people aged 60 years and over, which limits the generalisability of these studies to the wider Australian population. Furthermore, although the National Heart Foundation Risk Factor Prevalence Studies and the National Health Surveys included a broader group of Australians and evaluated the CVD and mortality consequences of diagnosed diabetes, these studies were unable to assess the impact of different levels of abnormal glucose metabolism, as they did not employ an oral glucose tolerance test (OGTT). The OGTT is the gold standard measure for evaluating deficiencies in glucose metabolism because it is based on two measures; fasting blood glucose and post-challenge blood glucose [15, 16]. This is an important limitation, as one in two cases of diabetes in Australia are not diagnosed [5], and other studies have indicated that the risk for CVD and mortality may develop

before the diagnosis of diabetes, when there are intermediate elevations in blood glucose [17-21].

The AusDiab study is a national population-based longitudinal survey of 11,247 Australian adults aged 25 years and over, and currently represents the largest study in the developed world to employ an OGTT. This thesis uses existing data from the AusDiab cohort, combined with mortality and CVD morbidity data specifically collected during the period of candidature, to provide the first national Australian data on the CVD and mortality risks associated with both diabetes and intermediate levels of hyperglycaemia. Findings from the studies outlined in this thesis have significantly contributed to the international body of work in this area, providing an opportunity to improve our broader understanding of the extent to which hyperglycaemia, compared to other traditional risk factors such as elevated blood pressure and dyslipidaemia, increase the risk of CVD and premature mortality. Such information may be used to develop strategies that will help to prevent CVD and improve the health of the Australian population.

1.2 Aims of the thesis

This research project aimed to investigate the extent to which impaired glucose metabolism contributes to the burden of CVD morbidity, CVD mortality and all-cause mortality in the Australian population. To achieve this, the following were undertaken:

- a review of the literature to investigate: (i) the methodological considerations of evaluating diabetes mortality data, (ii) the epidemiology and burden of diabetes, IFG, and IGT in relation to CVD and premature mortality, (iii) the nature and strength of the association between different measures of blood glucose and CVD and all-cause mortality, (iv) the specific role of abnormal glucose metabolism in the risk of myocardial infarction, stroke and coronary revascularisation, (v) the extent to which plasma glucose compared to measures of insulin resistance and other traditional risk factors increases the risk of CVD morbidity, CVD mortality

and all-cause mortality, and (vi) whether the associations between abnormal glucose metabolism and CVD and premature mortality varies in populations of different ages, sex, and baseline CVD risk (chapter 2)

- ascertainment of vital status and cause of death data on the AusDiab cohort by linking the dataset to the National Death Index (NDI) (chapter 3)

- ascertainment of CVD morbidity data on the AusDiab cohort by collecting medical record information to adjudicate self-reported non-fatal CVD events (myocardial infarction, stroke, percutaneous transluminal coronary artery angioplasty and coronary artery bypass surgery) (chapter 3)

- evaluation of the accuracy of self-reported non-fatal CVD events, as compared to adjudication of medical records and a hospital morbidity database (chapter 4)

- investigation of the association between diabetes, IGT, IFG and CVD mortality and all-cause mortality using multivariate Cox proportional hazards methods (chapter 5)

- exploration of the nature of the associations between continuous measures of fasting plasma glucose (FPG), two-hour post-load glucose (2hPG), and glycosylated haemoglobin (HbA_{1c}) and CVD mortality and all-cause mortality (chapter 6)

- analysis of whether hyperglycaemia improves the prediction of CVD and all-cause mortality over and above the risks attributed by other traditional risk factors such as elevated blood pressure and dyslipidemia (chapter 6)

- assessment of the specific relationships between IFG, IGT and diabetes with different CVD outcomes, including myocardial infarction, stroke and coronary artery revascularisation (chapter 7)

- exploration of the relative contribution of insulin sensitivity, as measured with HOMA-%S, and plasma glucose to the risk of CVD events and all-cause mortality (chapter 8)

- determination of whether the relationship between plasma glucose and CVD is different in men compared to women, in older compared to younger people and in populations with a high versus a low baseline risk for CVD (chapters 5 to 8)

1.3 Hypotheses

- the use of self-reported CVD events in epidemiological surveys is a valid measure of CVD outcomes

- elevated plasma glucose is an important risk factor for CVD mortality and all-cause mortality

- the association between elevated plasma glucose and CVD mortality and all-cause mortality is independent of other traditional risk factors, such as elevated blood pressure and dyslipidemia

- IFG and IGT are associated with an increased risk of CVD mortality and all-cause mortality

- elevated 2hPG is a better predictor of all-cause and CVD mortality compared to FPG or HbA_{1c}

- the nature of the relationships between 2hPG, FPG and HbA_{1c} and all-cause and CVD mortality is linear

- hyperglycaemia differentially influences the risk of myocardial infarction, stroke, and coronary artery interventions

- insulin sensitivity, as measured with HOMA-%S, is an important risk factor for all-cause mortality and CVD events

- the risk associated with elevated plasma glucose does not differ in women compared to men, in younger compared to older people, or in people with and without a previous history of CVD

1.4 Summary of research activities

Prior to enrolling in the Monash University doctoral program in March 2006, I commenced full-time employment as an epidemiologist at the International Diabetes Institute in March 2004. In this role, I assisted in data collection for the 2004–2005 follow-up AusDiab study. This involved logistical support to field staff, as well as collating, filing and cleaning data collected from over 8,000 participants during the five-year follow-up study. In addition, I analysed data from the baseline AusDiab study and authored three peer-reviewed manuscripts based on this data.

In 2005, having developed a detailed understanding of the AusDiab study, I generated a series of research questions relating to the role of hyperglycaemia in CVD and mortality which formed the basis of my PhD proposal. I was awarded a National Health and Medical Research Council / National Heart Foundation of Australia Public Health Postgraduate Scholarship to undertake my PhD at Monash University, and was the highest National Heart Foundation ranked scholarship recipient in Victoria in 2006.

In order to examine the role of hyperglycaemia in CVD and mortality in the AusDiab cohort, I completed three stages of data collection. Firstly, the vital status and causes of death of the 11,247 AusDiab participants was obtained by linkage to the NDI on an annual basis. Data linkage involved compiling a database of personal identifiers for each AusDiab participant and sending this to the Australian Institute of Health and Welfare. There, a computer program that searches for possible matches on the NDI was used, and a list of potential matches was returned for review. I conducted

the review process which was extensive and thorough to minimise misclassification of death status. This involved checking each potential match manually, and in cases where personal information was missing or inaccurate, contact with the next-of-kin and or the participant's local medical officer was undertaken to verify the death. Secondly, I cleaned the self-reported data on over 8,000 individuals who completed the 2004–2005 AusDiab existing health conditions questionnaire that included questions on previous CVD events. This was necessary to ascertain: (i) which self-reported events occurred between the baseline and follow-up AusDiab studies, and (ii) the hospital or medical facility at which the participant had received medical treatment. I coordinated the medical record abstraction and validation of over 600 self-reported CVD events from 154 hospital and primary care medical clinics across Australia. While my colleague, Shirley Murray, collected medical record data in Tasmania and Queensland, I conducted the medical record abstraction in Victoria, New South Wales, Western Australia, South Australia and the Northern Territory. I also planned and coordinated a series of sessions in which these records were adjudicated by a cardiologist and diabetologist, and entered all information into databases for analysis. Finally, the self-reported and physician-adjudicated CVD events for a cohort of AusDiab participants from Western Australia were linked to the Western Australian Hospital Morbidity Database to validate both positive and negative responses to self-reported CVD, and to compare the adjudicated results with the hospital discharge diagnoses listed on the Western Australian Hospital Morbidity Database.

In addition to performing the statistical analyses and drafting the manuscripts outlined in this thesis, these findings were presented at 2 national and 1 international conference, the details of which are outlined on page xv.

CHAPTER 2

Literature review

This chapter comprises five sections. The first section provides a brief overview of the measures of glucose metabolism and the definitions used to characterise abnormal glucose metabolism. The second section, which was published as a book chapter, examines the mortality, CVD risks and life expectancy associated with diabetes, as well as the methodological issues related to the collection and evaluation of mortality data on diabetes. The third section then focuses on the mortality and CVD risks associated with intermediate hyperglycaemia, and reviews the evidence on the nature of the continuous relationship between hyperglycaemia and all-cause mortality and CVD. The fourth section discusses the impact of hyperglycaemia specifically in relation to myocardial infarction, stroke and coronary artery revascularisation. The final section examines the relationship between insulin sensitivity and CVD and the extent to which this is independent of hyperglycaemia and other metabolic CVD risk factors.

2.1 Measurement and definition of abnormal glucose metabolism

Several factors are involved in maintaining glucose homeostasis, but glucose metabolism can be assessed with three commonly used measures: FPG, 2hPG, and HbA_{1c}. Both FPG and 2hPG are measured during a 75 gm OGTT, which is the gold standard for determining glucose tolerance [15, 16]. HbA_{1c} is a form of haemoglobin to which glucose is irreversibly bound. The percentage of haemoglobin molecules that have been glycated in this manner is an integrated measure of the exposure of red blood cells to glucose over their life span (two to three months), and hence HbA_{1c} is a valuable indicator of recent average glycaemia [22].

The terms IGT and IFG were developed to describe non-diabetic elevations in blood glucose associated with an elevated risk of developing diabetes [15, 16]. IGT characterises abnormally high 2hPG, and IFG characterises abnormally elevated FPG. Although different criteria have been used to classify IGT and IFG, in 1999, the World Health Organization (WHO) recommended that both fasting blood glucose, and two-hour blood glucose following a 75 gm OGTT be used in the classification of IFG, IGT and diabetes [15]. This was further endorsed by a subsequent joint report by the WHO and the International Diabetes Federation in 2006 [16]. Table 1 summarises the criteria are used to define known diabetes mellitus (KDM), newly diagnosed diabetes (NDM), IGT and IFG throughout this thesis.

Table 1. Criteria for diagnosing diabetes, impaired glucose tolerance and impaired fasting glucose in this thesis

Glucose tolerance category	Fasting plasma glucose (FPG)		Two-hour plasma glucose (2hPG)
Known diabetes mellitus (KDM)*	≥ 7.0 mmol/l	or	≥ 11.1 mmol/l
Newly diagnosed diabetes (NDM)†	≥ 7.0 mmol/l	or	≥ 11.1 mmol/l
Impaired glucose tolerance (IGT)	< 7.0 mmol/l	and	≥ 7.8 and < 11.1 mmol/l
Impaired fasting glucose (IFG)	6.1–6.9 mmol/l	and	< 7.8 mmol/l
Normal glucose tolerance (NGT)	< 6.1 mmol/l	and	< 7.8 mmol/l

These criteria are based on a joint report by the World Health Organization and the International Diabetes Federation [16].
*Participants were classified as having known diabetes mellitus (KDM) if they reported having physician-diagnosed diabetes and were (i) either taking insulin or oral hypoglycemic medication, or (ii) had FPG ≥ 7.0 mmol/l or 2hPG ≥ 11.1 mmol/l.
†Participants with newly diagnosed diabetes had not reported having physician-diagnosed diabetes and did not report taking any hypoglycaemic medication.

2.2 Mortality and life expectancy associated with diabetes

This section of the literature review comprises a book chapter titled *Mortality and life expectancy associated with diabetes* which was published in 2008 in the second edition of *The epidemiology of diabetes mellitus* (John Wiley & Sons, Ltd, London). This section starts with a discussion on the methodological issues related to the collection and evaluation of mortality data associated with diabetes. It highlights the inherent limitations of solely using death certificate data to evaluate the risk of

premature mortality and CVD among individuals with diabetes, and the need to conduct national longitudinal population-based cohort studies, like AusDiab, to assess the burden of disease. The chapter then covers several topics, focusing on type 1 and type 2 diabetes separately. The topics include: (i) mortality trends, (ii) the excess risk of premature mortality, CVD and non-CVD mortality and whether risks differ according to age, sex, and disease duration, (iii) causes of death, and (iv) the extent to which risk factors for CVD and premature death are modifiable.

Monash University
Declaration for thesis chapter 2

Declaration by candidate

In the case of chapter 2, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Principal author, critically reviewed the literature, interpreted and synthesised the findings of other studies and drafted the manuscript. Responsible author who accepts overall responsibility for the publication.	80%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Zimmet PZ	Critically evaluated the literature review and edited the book chapter
Shaw JE	Critically evaluated the literature review and edited the book chapter

Candidate's signature [redacted] **Date:** 25th June 2009

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the following location and will be held for at least five years from the date indicated below:

Location:	Department of Epidemiology and Clinical Diabetes, Baker IDI Heart and Diabetes Institute, Commercial Rd Melbourne, 3004, Victoria, Australia.
Date:	17th June 2009

Zimmet PZ [redacted] Date: 6th May 2009

Shaw JE [redacted] Date: 6th May 2009

This section highlights that although there has been a dramatic reduction in the mortality from diabetes during the last 100 years, largely due to the introduction of insulin, people with diabetes still experience an excess risk of both CVD and premature mortality. There is now a growing body of evidence from several countries that suggests that these risks are also elevated among individuals with intermediate hyperglycaemia, however, data are equivocal. The following section of this literature review will summarise the findings from these studies, and how they relate to the Australian context.

2.3 Risk of cardiovascular disease and all-cause mortality associated with non-diabetic hyperglycaemia

2.3.1 Excess risk of CVD and total mortality associated with non-diabetic hyperglycaemia

The diagnostic cut-points for diabetes are based on blood glucose thresholds that optimise the prediction of microvascular disease, in particular retinopathy [16]. However, there is now a growing body of evidence to suggest that macrovascular disease may develop at much lower levels of abnormal glucose metabolism. In 1979, several studies were published on the relationship between intermediate hyperglycaemia and CVD, but findings were inconsistent and no consensus could be reached as to whether or not intermediate hyperglycaemia was an important predictor of CVD [23]. However, the various limitations of these studies, which included small sample sizes, cross-sectional study designs and the assessment of surrogate outcomes instead of clinical CVD end-points, made it difficult to draw any substantive conclusions on intermediate hyperglycaemia and CVD.

Since then, several other studies have evaluated the relationship between intermediate hyperglycaemia and CVD and premature mortality [24-29], with the most important evidence coming from large meta-analyses that combined results from a number of diverse occupation- and population-based cohorts [17-21]. Table 2 summarises the findings from these studies.

Table 2. Summary of findings from the principal meta-analyses that have investigated the association between intermediate hyperglycaemia and all-cause mortality and CVD

Study	N (% women)	Pooled estimates (95% CI)			
		All-cause mortality		Cardiovascular disease	
		IFG	IGT	IFG	IGT
Coutinho et al. [17]	95,783 (6 %)	-	-	1.33 (1.06–1.67)[*]	1.58 (1.19–2.10)[*]
DECODE [18]	22,514 (32 %)	1.11 (1.00–1.23)[†]	1.40 (1.27–1.54)[†]	1.09 (0.90–1.30)[†]	1.34 (1.14–1.57)[†]
DECODA [20]	6,817 (54 %)	1.19 (0.87–1.64)[†]	1.34 (1.03–1.74)[†]	1.35 (0.86–2.08)[†]	1.27 (0.86–1.88)[†]
Levitan et al. [19]	33,430 (17 %)	-	-	1.27 (1.13–1.43)[‡§]	1.27 (1.09–1.48)[‡‖]
Asia Pacific Cohort Studies Collaboration [21]	237,468 (43 %)	-	-	1.30 (1.09–1.60)[‡¶]	-

[*]Relative risk estimates are not adjusted for covariates
[†]Adjusted for age, cohort, sex, systolic blood pressure, total cholesterol, body mass index, systolic blood pressure and smoking
[‡]Pooled relative risk estimates are based on several studies, some of which did not control for important covariates
[§]Relative risk is based on a comparison of the top category (mid-point ranged from 5.4 to 7.2 mmol/l) to the bottom category (mid-point ranged from 3.7 to 5.0 mmol/l)
[‖]Relative risk is based on a comparison of the top category (mid-point ranged from 8.3 to 10.8 mmol/l) to the bottom category of blood glucose (mid-point ranged from 3.8 to 5.9 mmol/l)
[¶]Hazard ratio and 95% CI estimated from Figure 3 in Lawes et al. [21]. IFG defined as fasting blood glucose ≥ 6.0 to < 7.0 mmol/l and compared to fasting blood glucose ≥ 5.0 to < 6.0 mmol/l
CI – confidence interval; DECODE – Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe; DECODA – Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Asia; IFG – impaired fasting glucose; IGT – impaired glucose tolerance

Only the Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe (DECODE) and Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Asia (DECODA) reported all-cause mortality [18, 20], and these studies showed that the risk for this outcome was increased by approximately 10 to 20% for IFG, and by 35 to 40% for IGT. However, IFG did not reach statistical significance in the DECODA study, probably because of the smaller sample size. All studies reported estimates for CVD, although the specific end-points differed between the studies, with some only reporting CVD mortality [18, 20] and others combining both morbidity and mortality outcomes [17, 19, 21]. Overall, the risk for CVD was increased by approximately 30% for both IFG and IGT, although the increased risk for IFG and IGT did not reach statistical significance in the DECODA study, and the estimate for IFG was much lower (9%) in the DECODE study. Moreover, Coutinho et al. [17] reported a higher relative risk for IGT of 58%.

Some of these analyses investigated whether the association between intermediate hyperglycaemia and all-cause mortality and CVD varied according to specific population subgroups. The DECODE study showed that the relative risk for CVD outcomes and all-cause mortality tended to be greater in women compared to men for IFG, but not for IGT [18]. Levitan et al. [19] also found that the pooled relative risk for blood glucose and CVD was greater in studies that included women, compared to studies that only included men ($p=0.01$). However, the relationship between fasting blood glucose and CVD did not differ according to sex in the Asia Pacific Cohort Studies Collaboration [21]. Only Levitan et al. [19] and the Asia Pacific Cohort Studies Collaboration [21] assessed whether age modified the relationship between intermediate hyperglycaemia and CVD, and no significant effect was reported. Furthermore, DECODA [20], which included data from Asian populations, and the Asia Pacific Studies Collaboration [21], which included data from Asia, Pacific Islands, Australia and New Zealand, found no evidence that the relationship between intermediate hyperglycaemia and all-cause mortality and CVD differed according to ethnicity.

However, several limitations from these meta-analyses need to be considered when interpreting these findings. Firstly, some studies predominantly consisted of men, which may limit the generalisability of these findings to women [17-19]. Secondly,

the component studies used different blood sample types (e.g. whole blood and plasma), and/or different glucose assays which may have influenced the precision of these results [17-21]. Thirdly, as some of these analyses did not have access to individual level data, they were not able to fully adjust for concomitant CVD risk factors, which may have led to some residual confounding of the estimate between intermediate hyperglycaemia and CVD [17, 19, 21]. Levitan et al. [19] showed in a sub-group analysis that the pooled relative risk for CVD was significantly lower for studies that adjusted for CVD covariates, compared with studies that did not adjust for potential confounders (RR = 1.19 vs. 1.64, $p< 0.001$). Finally, these analyses may have limited relevance to contemporary populations as baseline data were collected up to 20 years ago [17-21].

2.3.2 Continuous dose-response relationships between measures of glycaemia and all-cause mortality and CVD

Even though recent meta-analyses have shown that the risk of premature mortality and CVD is elevated at glucose levels below the threshold to diagnose diabetes, data on the nature of this association are inconsistent, with threshold [30, 31], continuous [21, 32] and J-shaped [33, 34] relationships being described. Understanding the nature of the continuous relationship between blood glucose and disease is important as it helps to inform management decisions. If a linear relationship exists between a risk factor and the disease, then treatment that aims to lower the risk factor as much as possible is warranted to improve health outcomes. However, if a threshold relationship is observed, then lowering of the risk factor would not provide any benefit in risk reduction below the threshold point, and in the case of a J-shaped relationship, excessive lowering may actually increase the risk of disease.

Only one meta-analysis has reported on the progressive relationship between blood glucose and all-cause mortality, and in this study a J-shaped relationship was shown for fasting and post-challenge blood glucose [35]. For CVD risk, several meta-analyses report a graded continuous relationship for post-challenge blood glucose [17, 19, 35]. In contrast, for fasting blood glucose, a graded continuous relationship [17], a J-shaped relationship [35] and a threshold relationship [19] have been reported. However, as mentioned in section 2.3.1, these meta-analyses have several limitations, relating to: (i) combining data from component studies that used different

methods and assays to collect blood glucose [17, 19, 35], (ii) failure to fully adjust for concomitant CVD risk factors [17, 19], (iii) lack of data on women [17, 35], and (iv) baseline data from the component studies being collected up to 20 years ago [17, 19, 35]. Furthermore, none of these meta-analyses reported on the nature of the relationship between HbA_{1c} and all-cause mortality and CVD. Although several studies have reported a graded relationship for all-cause mortality [36-39], the data are mixed for CVD, with both a graded continuous [37, 39] and threshold relationship being described [31].

2.3.3 Relative associations of FPG, 2hPG and HbA_{1c} to all-cause mortality and CVD events

Fasting blood glucose reflects the steady basal state which is maintained primarily by the liver, which stores glucose as glycogen and breaks it down for release into the circulation. In contrast, post-load blood glucose levels fluctuate in response to the consumption of carbohydrates and are dependent on the rate at which glucose is taken up by muscle and fat cells, and the degree to which hepatic glucose output is suppressed. These processes are modulated by the secretion of insulin from the pancreas and the sensitivity of muscle and fat cells to the available insulin, as well as being affected by exercise. HbA_{1c} is a measure of glycosylated haemoglobin and reflects ambient blood glucose over a two to three month period, the average lifespan of a red blood cell. Red blood cells are freely permeable to blood glucose, and therefore the higher the blood glucose levels, the greater the formation of glycosylated haemoglobin [22]. Therefore, as each of these measures reflects different physiological mechanisms, it is quite possible each may have different associations with all-cause mortality and CVD.

Several studies have evaluated the associations between different measures of blood glucose and all-cause mortality and CVD in people without diabetes. Assessing these relationships might not only help to improve our understanding of the mechanisms involved in the development of CVD in the general population, but also help ascertain whether any of these measures could contribute to individual risk stratification. Some studies report that fasting blood glucose is associated with all-cause and CVD mortality [19, 21, 40], whereas others report that post-load blood glucose is more strongly related to these outcomes than fasting blood glucose [17,

35, 41]. The risk of all-cause mortality and subsequent CVD has also been linked with HbA_{1c} concentrations [31, 37, 39, 42]. Most studies have compared the predictive capabilities of fasting blood glucose to two-hour blood glucose [18, 20], but few have compared all three measures in the same study population [43-46]. In the Rancho Bernardo Study [43], total glycosylated haemoglobin (GHb) was found to be a better predictor of CVD and ischaemic heart disease mortality than was FPG or 2hPG in women only. In contrast, other studies found that post-load blood glucose was a better predictor of mortality and/or CVD outcomes than was FPG or HbA_{1c} [44-46]. However, studies that compared all three blood glucose measures were undertaken in men only [46], in middle- to older-aged adults [43, 44, 46] or analysed GHb rather than HbA_{1c} [43], and hence their generalisability may be limited. Furthermore, these studies did not comprehensively assess the nature of the relationships between each glucose measure and the outcomes of interest or the contribution of the different glucose measures to individual risk discrimination for total and CVD mortality risk [43-46].

2.3.4 Individual CVD risk stratification according to measures of glycaemia

Blood glucose has often been shown to be significantly associated with CVD independent of other CVD risk factors, such as smoking, blood pressure and dyslipidaemia [18, 25, 26, 30, 44-47], signifying that at a population level, glucose may be important in characterising mortality risk even among individuals without diagnosed diabetes, and suggesting that intermediate hyperglycaemia may play a role in the development of CVD. However, the extent to which blood glucose is able to discriminate, or identify individuals who will develop CVD from those who will not develop CVD, has not been extensively evaluated in relation to the predictive capabilities of other traditional CVD risk factors among people without diabetes. Data from the DECODE group [48] did show that glucose significantly improved CVD risk prediction, whereas in the Framingham Offspring Study glucose only marginally improved CVD prediction beyond that explained by other risk factors [45]. The DECODE study only used log-likelihood ratio tests to evaluate the benefit of adding glucose to a multivariate model, which does not specifically assess the discriminatory abilities of glucose. In contrast, the analysis of the Framingham

Offspring Study [45] was based on comparing the *c*-statistics (analogous to the area under the receiver operating characteristic curve) from a model with only the traditional CVD risk factors to a model that also incorporated blood glucose. However, it has been shown that very large independent associations between the risk factor and outcome are required to achieve meaningful changes in the *c*-statistic [49, 50].

Recently, Pencina et al. [51] have proposed two new methods for evaluating the predictive capabilities of a new risk marker. These are referred to as the Integrated Discrimination Improvement and the Net Reclassification Improvement which take into account that any increases in predicted probabilities for an event due to an improved model for risk are only beneficial among those who experienced an event, whereas any decreases in predicted probabilities due to the improved model are only beneficial for risk prediction among those who did not experience an event. One study has applied the Net Reclassification Improvement methods to evaluate whether the addition of HbA_{1c} to the Framingham Risk Score improved prediction of coronary heart disease (CHD) [52]. This study found that although the addition of HbA_{1c} to the Framingham Risk Score did significantly improve the area under the receiver operating characteristic curve (0.72 vs. 0.73, p=0.005), this did not translate to a significant change in individual reclassification of CHD risk.

Therefore, uncertainty remains regarding the relationship between glycaemia and each of mortality and CVD, especially below the diagnostic threshold for diabetes. Developing a better understanding of the magnitude and nature of these relationships, as well as evaluating the extent to which glucose contributes to individual risk stratification would elucidate the potential benefit of glucose control among persons without diabetes and indicate which, if any, measure of glycaemia is the most appropriate for prediction of mortality in the general population.

2.4 The differential relationships between hyperglycaemia and myocardial infarction, stroke and coronary artery revascularisation

CVD outcomes can include CHD events and stroke, as well as procedural end-points such as percutaneous transluminal coronary artery angioplasty (PTCA) and coronary artery bypass surgery (CABG). Hyperglycaemia may play a different role in the pathophysiological processes responsible for each of these outcomes, and therefore it is important to compare the relationship between hyperglycaemia and different CVD outcomes.

2.4.1 The impact of hyperglycaemia on myocardial infarction compared with stroke

Even though myocardial infarction and stroke share similar risk factors, there are some quantitative differences between the effects of risk factors on these outcomes. Firstly, age increases the risk for both disorders, but myocardial infarction is more common in middle-age, whereas stroke is more common in older age. Secondly, although the relative risk for myocardial infarction tends to be greater in women than in men, these risk differentials are not as great for stroke. Thirdly, while both total cholesterol and elevated blood pressure are important risk factors for both outcomes, total cholesterol is more important for myocardial infarction and elevated blood pressure is more important for stroke [53]. Hyperglycaemia is thought to contribute to myocardial infarction by triggering inflammation, dyslipidaemia and fibrinolysis [2], while stroke is more strongly related to endothelial dysfunction and elevated blood pressure [53]. Therefore, it is quite possible that hyperglycaemia may also demonstrate different relationships with myocardial infarction and stroke.

Diabetes increases the risk of stroke [18, 53-55], but few studies have specifically investigated whether the risk for CHD differs to that observed for stroke. Some studies show that, in people with diabetes, the relative risk of CHD is greater than that of stroke [56], whereas others show the risk to be greater for stroke [57], or the relative risks to be similar [58, 59]. Only two meta-analyses have reported stroke and CHD outcomes separately. In the DECODE study, there were small differences in

the relative risk for stroke compared with CHD mortality. The HR (95% CI) for stroke mortality in those with NDM was 1.92 (1.16–3.17) as determined by fasting blood glucose and 1.74 (1.01–2.99) as determined by two-hour blood glucose, whereas the HR for CHD mortality was 1.43 (1.02–2.02) as determined by fasting blood glucose and 1.64 (1.18–2.28) as determined by two-hour blood glucose. In those with KDM, the HR (95% CI) for CHD was 1.94 (1.51–2.50) and the HR for stroke was 1.72 (1.11–2.66) [18]. The Asia Pacific Cohort Studies Collaboration showed the relative risks for CHD and stroke to be similar for people with diabetes [21].

It is unclear as to whether the risk of stroke is also increased at intermediate levels of hyperglycaemia, and whether the relative risk of stroke differs to that observed for CHD. Although some studies have reported an association between IFG and stroke [18, 21, 60, 61], and between IGT and stroke [56, 62], other studies have reported no association for intermediate hyperglycaemia [63, 64]. Furthermore, two studies reported a stronger relationship between IGT and stroke than between IGT and CHD [56, 62]. In the DECODE study [18], IFG was significantly associated with stroke, but only in women. Overall, neither IFG nor IGT were significantly associated with stroke, whereas IGT was significantly associated with CHD. In the Asia Pacific Cohort Studies Collaboration [21], however, IFG was significantly associated with both stroke and ischaemic heart disease, and the magnitude of these associations were similar.

2.4.2 The impact of hyperglycaemia on acute coronary events compared with non-acute events

There are insufficient data on the association between hyperglycaemia and risk of coronary artery revascularisation, as studies that do measure coronary revascularisation outcomes tend to merge these end-points with other CVD outcomes, and only evaluate the risks associated with the composite CVD measure. Consequently, the association between hyperglycaemia and revascularisation is masked.

Histological and physiological studies have shown that atherosclerotic disease may be more severe in people with diabetes [3, 4, 65]. This occurs through a complex set

of mechanisms that increase the rate and extent of development of atherosclerosis [4]. Specifically, hyperglycaemia and associated diabetic dyslipidaemia are thought to accelerate inflammatory, microangiopathic and thrombotic processes which increase the likelihood of plaque rupture and thrombosis [3]. Hyperglycaemia may therefore have a greater impact on the likelihood of having an acute coronary event than it does on the likelihood of undergoing coronary revascularisation for stable CHD with angina. Indeed, one study has demonstrated that in older individuals, diabetes conferred a greater risk for fatal than for non-fatal CVD outcomes [66]. Therefore, it is important to specifically assess the role of hyperglycaemia in relation to myocardial infarction compared to coronary revascularisation.

2.5 Insulin sensitivity, non-diabetic hyperglycaemia and all-cause mortality and CVD

2.5.1 Overview and measurement of insulin resistance

Insulin resistance occurs when cells (predominantly muscle and fat cells) are no longer sensitive to the effects of insulin, a hormone released from pancreatic β-cells. When this occurs, both hyperinsulinaemia and hyperglycaemia ensue. In some individuals, the pancreas is able to compensate for the insulin resistance by secreting more insulin, and therefore maintaining glucose homeostasis. However, in other individuals, after prolonged periods of insulin resistance, pancreatic β-cells fail to secrete adequate amounts of insulin, which leads to hyperglycaemia and diabetes [1]. It is important to explore the relative associations of these indices of glucose homeostasis with premature mortality and CVD in the general population, as both hyperglycaemia and insulin resistance have been implicated as risk factors. Furthermore, as some evidence suggests that insulin resistance varies according to the degree of glucose tolerance [67], it is important to ascertain whether the presence of both conditions can assist in the identification of a particularly high risk group.

The hyperinsulinaemic-euglycaemic clamp is currently considered the gold standard method for measuring insulin resistance [68], however, it is invasive and time consuming, and therefore unsuitable for large epidemiological studies. Consequently,

surrogate markers of insulin resistance have been used. Hyperinsulinaemia is the simplest of these markers, and can be measured in the fasting or post-glucose challenge states. However, the role of hyperinsulinaemia as a risk factor for the development of atherosclerosis has been debated [69-71]. Other markers take into consideration both glucose and insulin levels, and may therefore provide a better approximation of clamp-derived insulin resistance. These including the Gutt insulin sensitivity index [72], the homeostasis model assessment of insulin resistance (HOMA-IR) [73] or the updated computer model of the homeostasis model assessment of insulin sensitivity (HOMA-%S) [74], and the Quantitative Insulin Sensitivity Check Index [75]. Indeed, several studies have shown HOMA-IR or HOMA-%S to have moderate to high correlations (r=0.5 to 0.8) with clamp measured insulin sensitivity [74, 76].

2.5.2 Association between insulin resistance and CVD

Insulin resistance has been shown to be correlated with several other CVD risk factors including hyperglycaemia, adiposity, dyslipidaemia and elevated blood pressure [77], which tend to cluster together and are collectively identified as the metabolic syndrome [78]. However, uncertainty remains as to whether insulin resistance is independently related to CVD or whether it is merely associated with CVD through its correlation with other CVD risk factors. Few large epidemiological studies have assessed the association between directly measured insulin resistance and CVD. In a cross-sectional analysis of the Insulin Resistance Atherosclerosis Study, decreasing insulin sensitivity, as determined from a frequently sampled intravenous glucose tolerance test, was significantly associated with increasing carotid artery intima media thickness. The relationship, although attenuated after adjusting for age, sex, and factors related to the metabolic syndrome, remained significant [79]. However, as this was a cross-sectional analysis that only used a surrogate measure of CVD it was not possible to determine causality. In another prospective study of older men, after adjusting for CVD risk factors, decreasing insulin sensitivity was significantly associated with an increased risk of CHD events, but residual confounding may have influenced these findings as this study did not adjust for triglycerides or high density lipoprotein cholesterol (HDL-C) [80].

Epidemiological data are also available on the relationship between surrogate measures of insulin resistance and CVD, but findings are inconsistent. Two meta-analyses have reported a statistically significant but weak association between hyperinsulinaemia and CVD [81, 82]. However, the results of one meta-analysis may have been influenced by some residual confounding as the component studies did not adequately adjust for all metabolic syndrome components [81]. Furthermore, although adjustment for covariates was better in the other meta-analysis (as individual level data was pooled), results from the component studies were based on data that was collected using different protocols and insulin assays (some specific and some not specific for insulin), which may have introduced a degree of measurement imprecision [82]. Since the publication of these meta-analyses [81, 82], subsequent studies on hyperinsulinaemia and CVD have adjusted for several CVD risk factors, including the components of the metabolic syndrome, but findings remain inconclusive. Some studies have reported a significant independent association between fasting [83-85], post-glucose challenge [86] or non-fasting [87] hyperinsulinaemia and CVD, but others have found no association with fasting [86, 88] or post-challenge hyperinsulinaemia [88]. However, the various limitations of these studies are: (i) small sample size [84], (ii) including men only [85-87], (iii) adjustment for body mass index (BMI) instead of central obesity [84, 86, 87] which has been shown to be a better predictor of CVD [89], or (iv) including a high risk population [85]. Additionally, most studies measured plasma insulin with a non-specific insulin assay which cross-reacts with proinsulin [83-86]. As some studies have shown that proinsulin may be a better predictor of CVD than fasting insulin or insulin resistance [80, 90, 91], the association between true insulin and CVD may have been concealed in these studies. Findings from large prospective population-based studies on HOMA-IR or HOMA-%S are also inconsistent, with several [92-98], but not all [88, 99-103] reporting significantly associations between HOMA and CVD. However, the limitations of these studies are: (i) not adjusting for each individual component of the metabolic syndrome [92, 94-96, 98], (ii) using a non-specific insulin assay which cross-reacts with proinsulin [92, 94, 95, 97], or (iii) only including populations at high-risk of CVD [92, 95, 96].

2.5.3 Relative associations of insulin resistance and hyperglycaemia with risk of CVD

As outlined in sections 2.3 and 2.5.2, both hyperglycaemia and insulin resistance, respectively, have been shown to be risk factors for premature mortality and subsequent CVD events. However, few prospective cohort studies have compared the associations of these conditions of glucose homeostasis with CVD and premature mortality in the same study population. Findings from the Paris Prospective Study [104, 105], the Helsinki Policemen Study [106, 107] and the Busselton Studies [108, 109] showed that in men, hyperinsulinaemia remained significantly associated with CVD despite adjustment for other CVD risk factors including glucose. Moreover, a meta-analysis of European studies conducted by the DECODE group also found that hyperinsulinaemia was significantly associated with CVD mortality after adjusting for glucose [82]. However, these studies did not adjusted for HDL-C [104-109] or waist circumference [82, 104-109], factors known to coexist with insulin resistance which also increase the likelihood of CVD [78]. It is therefore possible that the significant associations observed for hyperinsulinaemia in these studies were the result of residual confounding.

Despite comprehensive adjustment for important CVD covariates, findings on the independent associations of insulin resistance and hyperglycaemia with CVD from more recent studies remain equivocal. Data obtained from a Taiwanese population [88] and from a United States population [110] both indicated that glucose is significantly associated with CVD independent of the indices of insulin resistance, whereas other studies did not [84, 85, 87, 111]. However, both the Taiwanese [88] and United States [110] studies represented a broad cross-section of the population, whereas studies reporting a significant association between indices of insulin resistance and CVD independent of glucose were conducted in select populations, including older women [111], patients at high risk for developing CVD [85], and middle-aged men [87], which may limit the generalisability of the findings. Furthermore, Perry et al. [87] and Fujiwara et al. [84] only found a significant association between post-challenge insulin and CVD, which may reflect different physiological mechanisms to those represented by fasting insulin or other indices of basal insulin resistance. Therefore, further investigation of the relationship between

insulin resistance and all-cause mortality and CVD, and the extent to which this is attenuated by other CVD risk factors (including hyperglycaemia) is warranted.

2.6 Summary

The findings of this review indicate that there is strong evidence that diabetes contributes significantly to the burden of CVD, and although data from overseas populations now indicate that the risk of CVD and mortality is elevated at lower levels of hyperglycaemia, this has not been previously evaluated in Australia at a national level. Moreover, several aspects on the relationship between abnormal glucose metabolism and mortality and CVD are not clearly understood. Firstly, there are inconsistent data on the nature of the relationship between blood glucose and mortality. Secondly, very few studies have compared the mortality and CVD risks associated with all three measures of blood glucose – FPG, 2hPG and HbA_{1c} – which is important because each represent different metabolic processes and may therefore be differentially linked to CVD. Thirdly, there are limited data on whether glucose measures contribute to individual CVD risk prediction over and above that conferred by traditional CVD risk factors. Fourthly, hyperglycaemia may increase the likelihood of more severe atherosclerotic disease, however, there are insufficient data on the impact of abnormal glucose metabolism on acute and non-acute CVD. Fifthly, the detrimental impact of insulin resistance in relation to hyperglycaemia has not been examined fully with reference to other CVD risk factors related to the metabolic syndrome. Finally, uncertainty remains as to whether the relationship between hyperglycaemia and mortality and CVD varies according to important characteristics such as age and sex. Therefore, the aims of this thesis are to provide the first national data on the effects of all levels of hyperglycaemia on CVD and premature mortality in Australia, which will also help to address these gaps in the literature and improve current understanding on the contribution of abnormal glucose metabolism to CVD and mortality.

CHAPTER 3

Methods

This chapter provides a general overview of the methods used in this thesis, as specific details on each sub-study are described in chapters 4 to 8. Firstly, it introduces the theoretical and conceptual framework upon which this thesis is based. Secondly, it provides an overview of the AusDiab study, including its aims and methods, and how this thesis utilises data from this cohort. Finally, it describes the methods that were undertaken as part of this thesis to ascertain mortality and CVD outcomes.

3.1 Theoretical and conceptual framework

This thesis in based on the study of epidemiology, which is the evaluation of patterns of disease in defined populations. It is one discipline that, together with other scientific approaches such as physiology and genetics, assists in our understanding of the mechanisms of disease. Broadly, epidemiology aims to (i) measure the prevalence and incidence of disease in the population, which is often referred to as *descriptive epidemiology*, and (ii) identify the relationships between specific risk factors and disease, defined as *analytical epidemiology* [112]. Cross-sectional epidemiological analyses investigate the association between risk factors and disease at one particular point in time. However, the main limitation with this approach is that it is difficult to ascertain whether the postulated risk factors preceded the development of the disease or whether the disease influenced the risk factors. Longitudinal studies are more useful for investigating the temporal relationships between risk factors detected at baseline screening and outcomes that develop over the course of follow-up [112].

Longitudinal population-based cohort studies that incorporate an OGTT are required to investigate the associations between abnormal glucose metabolism and CVD and

mortality outcomes. It is not possible to gather comprehensive data on abnormal glucose metabolism from medical records, as the early stages of these conditions are often asymptomatic. This is exemplified by the finding from the AusDiab study which revealed that half of all people classified as having diabetes according to FPG and 2hPG values were actually undiagnosed [5]. Furthermore, as diabetes has been shown to be poorly reported on death certificates [113-116], and IFG and IGT may not be mentioned at all, it is difficult to ascertain from death certificate data the extent that abnormal glucose metabolism contributed to the CVD death. Therefore, this thesis will primarily use analytical epidemiology principles to explore the relationships between measures of abnormal glucose metabolism and the development of CVD and death over a five year period.

3.2 The AusDiab Study

The AusDiab study is recognised as one of the world's leading epidemiological studies into the impact of diabetes and its related conditions. It is the first national Australian study to investigate the prevalence of diabetes, its risk factors and related conditions, such as obesity, kidney disease and CVD. Notably, as this study employed an OGTT, the gold standard test for ascertaining glucose tolerance status, it has provided vital information on the prevalence of undiagnosed diabetes and intermediate hyperglycaemia. Between 1999 and 2000, findings from the AusDiab study indicated that 7.4% of Australian adults aged 25 years and over had diabetes, half of whom were undiagnosed. In addition, another 10.6% had IGT and 5.8% had IFG. Taken together, these findings indicated that one in four adult Australians had some form of abnormal glucose metabolism [5].

The AusDiab study is therefore a valuable resource, and key findings have informed Government health policy planning and service provision. Specifically, this has included funding through the *National Diabetes Strategy* and the *National Integrated Diabetes Program* and *Support for Diabetes Research* policies. Large well-designed cohort studies, such as the AusDiab study, are central to the field of epidemiology, because they have the capacity to collect an extensive amount of information from many individuals. There have now been over 80 publications based on the data from

the AusDiab study, covering several areas including diabetes and its complications [5, 117-120], obesity [121, 122], hypertension [123, 124], kidney disease [125, 126] and physical activity and sedentary behaviours [127-129].

3.2.1 Study design, sample selection and response rates

The AusDiab study began in 1999 and a five year follow-up survey was undertaken between 2004 and 2005. Methods and response rates for the baseline [130] (see Appendix 1) and follow-up [117] studies have been previously described in detail. Briefly, non-institutionalised men and women aged 25 years and over were recruited from 42 urban and non-urban Census Collector Districts (CCDs are the smallest geographic unit defined by the Australian Bureau of Statistics at each census, and have an average of 225 dwellings in each) using a stratified clustered sampling method. The sample size was chosen so as to provide estimates of a national diabetes prevalence of 7%. Sampling involved randomly selecting six CCDs from seven strata (each of the States and the Northern Territory) with the selection probability proportional to the population size of the CCD. The following CCDs were excluded so as to meet the logistic and economic constraints of the survey, and to avoid including a large number of groups likely to have a high prevalence of diabetes which would have biased the prevalence estimates: (i) CCDs containing fewer than 100 persons aged 25 years and over, (ii) CCDs that were was classified as 100% rural according to the 1996 Australian census, and (iii) CCDs that comprised more than 10% indigenous people.

Recruitment of participants at baseline was conducted in two phases: (i) a household survey, and (ii) a full physical examination at a local testing site. The household survey involved a brief questionnaire on demographic information. Following the completion of the questionnaire, participants were invited to attend a local testing site for a full physical examination. From the 17,129 eligible households, 20,347 individuals completed a household interview and 11,247 (55%) agreed to participate in the physical examination, giving an estimated baseline response rate of 37%. Participants were more likely to be older and female when compared to the 1998 Australian Bureau of Statistics census data. Moreover, after adjusting for this age and sex imbalance, and for the stratified, clustered study design, responders to the

physical examination were more likely to have been born in the United Kingdom, speak English at home, complete more than 12 years of education, and suspect they had diabetes. However, there were no significant differences with respect to being born in Australia, being married, or reporting ever being told that they had diabetes [130] (see Appendix 1).

Between 2004 and 2005, all eligible participants were invited to return for another examination. The following participants were not eligible: (i) those who refused further contact (n=128), (ii) those who had died (n=310), and (iii) those who had moved overseas or into a nursing facility classified for high care, or had a terminal illness (n=21). Of the 10,788 participants eligible for testing in 2004–2005, 6,400 (59%) attended the full physical examination. A further 137 (1%) participants had blood and urine tests only, and another 2,261 (21%) completed a telephone questionnaire only. Comparison between attendees (n=6,537) and non-attendees (n=4,710) to the follow-up survey showed that attendees were significantly less likely to be hypertensive, to have a lower level of education attainment or to be smokers, and had lower 2hPG, HbA_{1c}, and smaller waist circumferences at baseline [117].

3.2.2 Baseline measures

The protocol for the AusDiab physical examination was based on the WHO recommended model for diabetes and other non-communicable disease field surveys [131-133]. The physical examination took place at a local centre, and involved fasting (at least nine hours overnight) blood tests by venepuncture, a 75 gm OGTT, anthropological measurements, including waist circumference, height, weight and blood pressure, as well as interviewer-administered questionnaires which enquired about a range of health and lifestyle issues, including demographic characteristics, family history, smoking, alcohol intake, medical history and physical activity. All blood samples were centrifuged on-site to separate plasma from serum. These samples were transported on a daily basis to a central laboratory for analysis. A brief description of the baseline measures included in the studies outlined in chapters 4 to 8 is provided below.

Three indices of blood glucose were measured: FPG, 2hPG and HbA_{1c}. All participants, except those on insulin or oral hypoglycaemic drugs, those who were pregnant or those who failed to fast were asked to undergo a 75g OGTT in order to measure both FPG and 2hPG. After blood collection, plasma and serum were placed in separate fluoride/oxalate tubes and FPG and 2hPG levels were determined by a glucose oxidase method using an Olympus AU600 automated analyser (Olympus Optical Co. Ltd, Tokyo, Japan). GHb was measured from frozen samples of whole blood collected in EDTA tubes and stored at -70° Celsius for 2 to 36 months, using high performance liquid chromatography (Bio-Rad Variant Hemoglobin Testing System, Bio-Rad, Hercules, CA, USA) with standardised conversion to HbA_{1c} (normal range 4.2–6.3%).

Categories of abnormal glucose metabolism were determined according to the 2006 WHO criteria [16], as previously outlined in Table 1, chapter 2. Participants were classified as having KDM if they reported having physician-diagnosed diabetes and were (i) either taking insulin or oral hypoglycemic medication, or (ii) had FPG ≥ 7.0 mmol/l or 2hPG ≥ 11.1 mmol/l. Participants not reporting having diabetes, but who had FPG ≥ 7.0 mmol/l or 2hPG ≥ 11.1 mmol/l were classified as NDM. For the purposes of this thesis, results were combined for type 1 and type 2 diabetes, as 96% of participants with diabetes were classified as having type 2 diabetes. Participants determined not to have diabetes were classified as having either IFG (FPG ≥ 6.1 and < 7.0 mmol/l with 2hPG < 7.8 mmol/l), IGT (2hPG ≥ 7.8 and < 11.1 mmol/l with FPG < 7.0 mmol/l) or NGT (FPG < 6.1 mmol/l and 2hPG < 7.8).

Fasting blood samples were also collected to measure insulin, total cholesterol, HDL-C and triglycerides. Serum and plasma were placed into separate plain tubes prior to transfer to the laboratory. All lipid measures were determined by enzymatic methods using an Olympus AU600 analyser (Olympus Optical Co. Ltd, Tokyo, Japan). Serum insulin was measured using a human insulin-specific radioimmunoassay kit (Linco Research, Inc., St Charles, MO, USA). Insulin assays were only conducted in participants aged older than 35 years. The insulin sensitivity index HOMA-%S was estimated from FPG and fasting insulin concentrations, and was calculated with the HOMA-2 computer program [74].

Blood pressure was measured with the appropriate cuff size in a seated position after at least five minutes rest using the arm not used for blood testing. A standard mercury sphygmomanometer was used in one state (Victoria) with blood pressure recorded within 2 mmHg of the first and fifth Korotkoff sounds. For all other states, a Dinamap semi-automatic oscillometric recorder was used (Critikon, Tampa, FL, USA). A comparison study (conducted on every 20th person in the last six states surveyed [n=469]) was undertaken to compare manual and automatic blood pressure readings. This study revealed differences for the diastolic blood pressure readings, and therefore adjustments was made to all diastolic blood pressure readings recorded in the state using the sphygmomanometer as previously described [123]. Three measurements were taken at one minute intervals. The final measure of blood pressure was defined as the mean of the first two readings, unless the difference between these readings was greater than 10 mmHg, in which case the mean of the two closest of three measurements was used. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or self-reported use of anti-hypertensive medication.

Height, weight, waist and hip circumference was measured in all participants except for pregnant women and people using a wheelchair. Height was measured to the nearest 0.5 cm without shoes using a stadiometer. Weight was measured without shoes and excess clothing to the nearest 0.1 kg using a mechanical beam balance, and BMI (kg/m^2) was calculated. Waist circumference was measured halfway between the lower border of the ribs and the iliac crest on a horizontal plane, and hip circumference was measured at the widest point over the buttocks. For waist and hip circumference measurement, two measurements to the nearest 0.5 cm were recorded. If the measurements varied by more than 2 cm, a third measurement was taken. The mean of the two closest measurements was calculated.

3.3 Ethical approval

The AusDiab study was approved by the International Diabetes Institute Ethics Committee (3/99, 3/2002). The Australian Institute of Health and Welfare Ethics Committee approved the matching of the AusDiab cohort to the NDI (EC358).

Ethical approval for this PhD research project was obtained from Monash University's Standing Committee on Ethics in Research Involving Humans (2006/986MC). Additionally, approval was also obtained from the Confidentiality of Heath Information Committee (CHIC) in Western Australia for linking the AusDiab cohort residing in Western Australia to the Western Australian Hospital Morbidity Database, as well as the Sir Charles Gairdner Hospital in Perth, so as to obtain medical record information from that facility (see Appendix 2 for copies of the approval letters). All participants provided written informed consent to participate in both the 1999–2000 and 2004–2005 surveys. Furthermore, participants who reported CVD events were also asked for their consent to access their medical records (see Appendix 3).

3.4 Fatal and non-fatal outcomes and data collection

The candidate was responsible for the collection of all mortality data and non-fatal CVD events data for the AusDiab study. The following sections provide an overview of the methods used to obtain these data.

3.4.1 Vital status and cause of death

In Australia, all deaths are registered with State and Territory authorities. Death certificate information from each State and Territory is forwarded on an annual basis to the Australian Bureau of Statistics to be coded according to the 2006 International Classification of Diseases 10th revision (ICD-10). Vital status, as well as the codes for the underlying and contributory causes of death, are then sent to the Australian Institute of Health and Welfare for uploading to the NDI. This database contains individual level data, however, as Australians do not have a unique identification number, personal identifiers such as date of birth, name and date of death are used to identify individuals on this database.

Mortality status and underlying and contributory causes of death were obtained through data linkage of the AusDiab cohort to the NDI. Data linkage to the NDI occurred on an annual basis to provide mortality data for a median follow-up of five

to six years. Names, date of birth, sex, date of last contact, date of death and geographic code were used to match AusDiab participants to the NDI. Data matching involved two phases. Firstly, a database of all AusDiab participants and their personal identifiers was sent to the Australian Institute of Health and Welfare for linkage to the NDI. This was undertaken with a computer program designed to search the NDI for possible matches to participants in the AusDiab cohort. Secondly, a list of potential matches was then sent to the candidate for clerical review, which involved reviewing each potential match to determine whether or not it actually identified the AusDiab individual. Only high level matches (defined as correct matches for full name and date of birth) between the NDI database and the AusDiab participants were accepted as confirmed deaths, and where possible these deaths were verified by contacting the next-of-kin and/or the local medical officer. People who were not matched to the NDI were assumed to be alive. A recent study by Magliano et al. [134] found that the NDI was very accurate in identifying both vital status and CVD deaths in the Australian population. Verification of vital status in AusDiab was greatly assisted by the fact that up-to-date contact details of both the participants and their next-of-kin were maintained through annual letters and telephone calls.

Deaths were attributed to CVD if the underlying cause of death was coded I10-I25, I46.1, I48, I50-I99 or R96 according to the ICD-10. In addition, participants with uncomplicated diabetes (E109, E119 or E149) or unspecified hyperlipidemia (E785) as an underlying cause of death on the death certificate were attributed a CVD death if any of the CVD codes (I10-I25, I46.1, I48, I50-I99 or R96) were recorded in the first position on the death certificate.

3.4.2 Non-fatal CVD events

All non-fatal CVD events, which included myocardial infarction, stroke, PTCA and CABG that occurred between the baseline and 2004–2005 follow-up AusDiab surveys were ascertained by adjudicating self-reported events, the methods of which are reported in chapter 4. Briefly, during the 2004–2005 follow-up survey, 8,802 (82%) of the 10,788 eligible individuals completed questions from an interviewer-administered survey on whether they had experienced a CVD event (see Appendix

4). The interviewer-administered questionnaire asked “have you ever been told by a doctor or nurse that you have had a heart attack (including a ‘coronary’, coronary occlusion, coronary thrombosis or myocardial infarction), a stroke, a heart bypass operation (including ‘coronary bypass’) or an angioplasty or stent for your heart (including ‘coronary angioplasty’, ‘coronary stent’ or ‘balloon’)?”. Participants who answered ‘yes’ to any of these questions were asked to provide the date and hospital admission details for each event [135]. The candidate then used this self-reported information to gather copies of relevant medical record information for each of the CVD events (including multiple events) reported by the participants from 154 health care facilities around Australia. This information was de-identified and reviewed independently by two physicians (diabetologist and cardiologist) according to the following pre-specified criteria: (i) WHO / MONICA criteria for myocardial infarction [136], (ii) WHO criteria for stroke [137], and (iii) operation reports for CABG and PTCA. The level of initial agreement between the two adjudicators was: (i) 85.5% for any non-fatal CVD event (myocardial infarction – myocardial infarction, stroke, CABG or PTCA), (ii) 77.3% for MI only, (iii) 84.0% for stroke only, (iv) 97.5% for CABG only, and (v) 89.8% for PTCA only. All disagreements were resolved by consensus.

The methods used for the ascertainment of non-fatal CVD events in this thesis were validated by comparing both self-reported CVD events and adjudicated outcomes to a hospital morbidity database, as outlined in the study in chapter 4. As there is no national hospital morbidity database in Australia, validation was undertaken in the Western Australian Hospital Morbidity Database. This database covers all hospital admissions, both private and public, in the state of Western Australia, and therefore it was possible to ascertain the number of unreported CVD events to gauge the potential loss-to-follow-up for these non-fatal outcomes. This study found that self-reported events were unlikely to have been missed, with only 0.2% of those denying any CVD event being recorded as having had an event on the Western Australian Hospital Morbidity Database. Furthermore, the agreement between adjudication and linkage to the Western Australian Hospital Morbidity Database was very good, with 88% of the total CVD events being identified with both methods.

3.5 Statistical analysis

The statistical methods used in this thesis are fully explained in each of the studies in chapters 4 to 8. Unadjusted mortality and morbidity rates were reported for the WHO categories of abnormal glucose metabolism and for categories of FPG, 2hPG and HbA_{1c}. To assess the excess all-cause mortality and CVD risk associated with abnormal glucose metabolism, a wide range of inferential statistics were employed, with survival analysis using Cox proportional hazards regression being the principal method. Both univariate and multivariate models were developed to assess the relationships between baseline covariates and the outcome measures, as well as to assess the impact of covariates on the relationships between abnormal glucose metabolism and all-cause mortality and CVD. All models were adjusted for known risk factors, including age, sex, smoking, previous history of CVD (angina, myocardial infarction or stroke), hypertension (defined as systolic blood pressure ≥ 140 mmHG or diastolic blood pressure ≥ 90 mmHg or self-reported use of anti-hypertensive medication), total cholesterol, lipid-lowering medication use, triglycerides or HDL-C and body composition (waist circumference, waist-to-hip ratio or BMI).

In addition, more novel and complex statistical methods were used in chapter 6. Firstly, higher order polynominals and piecewise linear splines were used to explore the non-linear relationships between the continuous glucose measures of FPG, 2hPG and HbA_{1c} and all-cause and CVD mortality. Secondly, the Net Reclassification Improvement and the Integrated Discrimination Improvement, two new methods outlined by Pencina et al. [51], were used to evaluate the discriminative ability of FPG, 2hPG and HbA_{1c} to identify individuals at higher risk of all-cause and CVD mortality. These measures take into consideration that any increases in predicted probabilities for an event due to an improved model for risk are only beneficial among those who experienced an event, and any decreases in predicted probabilities due to the improved model are only beneficial for risk prediction among those who did not experience an event. Finally, a program was written to run bootstrap methods, based on 1,000 replications to derive 95% CIs for the Net Reclassification Improvement and the Integrated Discrimination Improvement estimates.

The assumptions required for each statistical test were thoroughly examined. The distributions of all continuous measures were explored, and in cases where there was a marked deviation from the normal distribution, the variable was transformed as appropriate. Additionally, the assumptions required for proportional hazards were investigated with graphs of log-log plots of the relative hazards by time and scaled Schoenfeld residuals. Analyses were conducted in SPSS version 15.0 (SPSS, Chicago, Illinois, USA) and Stata Statistical Software version 9.2 and 10 (StataCorp, College Station, Texas, USA).

CHAPTER 4

Validity of self-reported cardiovascular disease events in comparison to medical record adjudication and a statewide hospital morbidity database – the AusDiab study

4.1 Overview

In order to evaluate the role of hyperglycaemia in the development of CVD in the AusDiab study, it was essential to obtain accurate information on individual CVD events. As outlined in chapter 3, this was achieved by reviewing medical record information to adjudicate all self-reported CVD events (myocardial infarction, stroke, PTCA and CABG) that occurred over a five year period from baseline testing. However, while this approach was useful for validating self-reported CVD, it was clearly not feasible to assess all medical records nationwide to verify negative responses. Ideally, linkage to a national hospital morbidity database would have enabled the verification of both positive and negative responses, but this was not possible, as such databases are currently not available on a national level in Australia. Population-based hospital morbidity information, however, is currently collected in the state of Western Australia. Linkage to the Western Australian Hospital Morbidity Database provided both an opportunity to verify positive and negative responses to the CVD questionnaire, and also an avenue to validate the methods used for adjudication.

Therefore, the aims of the study presented in this chapter were to: (i) compare self-reported CVD events for the whole AusDiab cohort to physician adjudication of medical records, (ii) compare self-reported CVD events from a cohort of AusDiab participants residing in Western Australia to discharge diagnoses from the Western

Australian Hospital Morbidity Database, and (iii) compare medical record adjudication to Western Australian Hospital Morbidity Database discharge diagnoses. The findings showed that self-reported CVD events tended to be overestimated, particularly for stroke and myocardial infarction. However, misclassifications were mainly the result of participants confusing stroke with transient ischaemic attacks, myocardial infarction with angina, and PTCA with angiography, which suggests that self-reported stroke, myocardial infarction, and coronary revascularisation were a reasonably valid measure of composite CVD outcomes. Interestingly, linkage to the Western Australian Hospital Morbidity Database showed that only 0.2% of those denying any CVD event were detected on the Western Australian Hospital Morbidity Database to have experienced an event, showing that events are unlikely to go unreported. Although agreement between the adjudication of medical records and record linkage to the Western Australian Hospital Morbidity Database was high, some misclassification occurred for myocardial infarction and stroke. These findings indicate therefore that verification of self-reported CVD with medical record adjudication and if possible, a hospital morbidity database, may be required when data is specifically sought for myocardial infarction or stroke outcomes. As such, the study provides evidence to support the combined use of self-report and medical record adjudication to determine specific CVD outcomes in the AusDiab study. This approach was therefore adopted for the studies presented in chapters 7 and 8 of this thesis.

This study was published in the peer-reviewed *Internal Medicine Journal* in January 2009, along with an accompanying editorial by Joshi and Turnbull (also reproduced on the following pages).

Monash University
Declaration for thesis chapter 4

Declaration by candidate

In the case of chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Principal author, critically reviewed the literature and developed the research question, cleaned the self-reported CVD events data, coordinated the medical record abstraction and adjudication of over 600 self-reported CVD events from hospitals and general practices around Australia, collected medical record data in four of the six states and the Northern Territory, created a database for linkage to the Western Australian Hospital Morbidity Database, conducted the statistical analysis, interpreted the results, drafted the manuscript, and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.	80%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Tonkin AM	Adjudicated the self-reported CVD events, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Welborn TA	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Shaw JE	Adjudicated the self-reported CVD events, conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript

Candidate's signature [redacted] **Date:** 25th June 2009

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the following location and will be held for at least five years from the date indicated below:

Location:	Department of Epidemiology and Clinical Diabetes, Baker IDI Heart and Diabetes Institute, Commercial Rd Melbourne, 3004, Victoria, Australia.
Date:	17th June 2009

Tonkin AM [redacted] Date: 7th May 2009

Welborn TA [redacted] Date: 7th May 2009

Shaw JE [redacted] Date: 6th May 2009

CHAPTER 5

Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance – the Australian Diabetes, Obesity and Lifestyle Study (AusDiab)

5.1 Overview

As outlined in chapter 2, there is increasing recognition that non-diabetic levels of hyperglycemia, as observed in IFG and IGT, may be associated with an elevated risk of CVD and premature mortality. However, much of this evidence is derived from meta-analyses of relatively old studies that used different blood sample types and assays, and did not adequately adjust for potential confounding variables. Therefore, the aim of the study presented in this chapter was to investigate whether IFG and IGT, as well as diabetes, increase the risk of all-cause and CVD mortality in the AusDiab study population.

The results indicated that those with KDM had twice the risk of mortality than those with NGT, and those with IGT and IFG had a 50 to 60% greater mortality risk after adjustment for age, sex, previous history of CVD, smoking, blood pressure, total cholesterol, lipid-lowering medication use, dyslipidaemia and central obesity. CVD mortality risk was also significantly greater for those with KDM or IFG, but not for those with IGT, compared to those with NGT. Additionally, the findings showed that 65% of all fatal CVD events occurred among those with IFG, IGT, NDM or KDM, thus suggesting that abnormal glucose metabolism contributes to a substantial number of CVD deaths in Australia. As such, CVD prevention may need to be

targeted not only to people with diabetes mellitus, but also toward people with milder forms of abnormal glucose metabolism.

This paper was published in the peer-reviewed journal *Circulation* in June 2007. The paper attracted considerable media coverage, some of which is presented in Appendix 5.

Monash University
Declaration for thesis chapter 5

Declaration by candidate

In the case of chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Principal author, critically reviewed the literature and developed the research question, collected mortality outcome data, planned and conducted the statistical analysis, interpreted the findings, drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.	80%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Zimmet PZ	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Welborn TA	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Jolley D	Evaluated the statistical analyses and provided statistical advice
Magliano DJ	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Dunstan DW	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Cameron AJ (Monash PhD Student)	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript Extent of contribution: 5%
Dwyer T	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Taylor HR	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript

Tonkin AM	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Wong TY	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
McNeil J	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Shaw JE	Conceptualisation of the AusDiab study, facilitated the development of the research question, critically evaluated the analysis and interpretation of the findings, and edited the manuscript

Candidate's signature [redacted] **Date:** 25th June 2009

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the International Diabetes Institute and will be held for at least five years from the date indicated below:

Location:	Department of Epidemiology and Clinical Diabetes, Baker IDI Heart and Diabetes Institute, Commercial Rd Melbourne, 3004, Victoria, Australia.
Date:	17th June 2009

Zimmet PZ	[redacted]	Date: 6th May 2009
Welborn TA	[redacted]	Date: 7th May 2009
Jolley D	[redacted]	Date: 6th May 2009
Magliano DJ	[redacted]	Date: 6th May 2009
Dunstan DW	[redacted]	Date: 6th May 2009
Cameron AJ	[redacted]	Date: 5th May 2009
Dwyer T	[redacted]	Date: 22nd May 2009
Taylor HR	[redacted]	Date: 11th May 2009
Tonkin AM	[redacted]	Date: 7th May 2009
Wong TY	[redacted]	Date: 6th May 2009

McNeil J [redacted] Date: 12th May 2009

Shaw JE [redacted] Date: 6th May 2009

CHAPTER 6

Continuous relationships between non-diabetic hyperglycaemia and both cardiovascular disease and all-cause mortality – the Australian Diabetes, Obesity and Lifestyle (AusDiab) study

6.1 Overview

In chapter 5, it was shown IFG and IGT were associated with an increased risk of all-cause mortality, and that IFG was associated with an increased risk of CVD mortality. Although grouping individuals into these categories might have practical value, dividing a continuous variable into groups can mask the true relationship between a risk factor and outcome. This is because: (i) the risk relationship is often continuous according to the severity of the particular risk factor, (ii) people in the one group are considered to have the same risk for disease, even though their risk might vary according to their specific measurement of the risk factor, (iii) people on either side of a cut-point are artificially classified as having very different risks for the disease, when in fact their risks might be similar because they have very similar values of the risk factor, and (iv) categorising the risk factor conceals any potential non-linear relationship between the risk factor and disease [138].

However, considerable uncertainty remains regarding the nature of the continuous relationship between glycaemia and mortality, with previous studies variously reporting threshold, continuous, and J-shaped relationships between glucose measures and both CVD and all-cause mortality. Furthermore, as outlined in chapter 2, few studies have compared the relative associations of FPG, 2hPG and HbA_{1c} to all-cause and CVD mortality in the same population, or evaluated whether blood glucose can improve individual CVD risk stratification over that provided by

traditional CVD risk factors. Therefore, the aims of the study outlined in this chapter were to explore: (i) the nature and strength of the relationships between each of FPG, 2hPG and HbA_{1c}, and CVD and all-cause mortality, and (ii) whether any of the glucose measures improved prediction of CVD and all-cause mortality beyond that achieved by traditional risk factors.

The results indicated that FPG and 2hPG, but not $HbA_{1c,}$ were significantly associated with an increased risk of all-cause mortality, and all three measures were significantly associated with an increased risk of CVD mortality. The risks of all-cause and CVD mortality progressively increased throughout the range of 2hPG and HbA_{1c}, but J-shaped relationships were observed for FPG. The discriminative ability of each measure was similar, and their addition to mortality prediction models did not substantially improve individual risk identification. Nevertheless, the findings highlight the potentially important role of glycaemia in the development of CVD and all-cause mortality in the general population.

This paper was published in the peer-reviewed journal *Diabetologia* in January 2009.

Monash University
Declaration for thesis chapter 6

Declaration by candidate

In the case of chapter 6, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Principal author, critically reviewed the literature and developed the research question, planned and conducted the statistical analysis, interpreted the findings, structured and drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.	80%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Boyko, EJ	Provided statistical advice, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Zimmet, PZ	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Wolfe, R	Provided statistical advice, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Tonkin, AM	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Shaw, JE	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript

Candidate's signature 

Date: 25th June 2009

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the International Diabetes Institute and will be held for at least five years from the date indicated below:

Location:	Department of Epidemiology and Clinical Diabetes, Baker IDI Heart and Diabetes Institute, Commercial Rd Melbourne, 3004, Victoria, Australia.
Date:	17th June 2009

Boyko, EJ	[redacted]	Date: 6th May 2009
Zimmet, PZ	[redacted]	Date: 6th May 2009
Wolfe, R	[redacted]	Date: 7th May 2009
Tonkin, AM	[redacted]	Date: 7th May 2009
Shaw, JE	[redacted]	Date: 6th May 2009

CHAPTER 7

Elevated blood glucose may differentially influence the five-year risk of myocardial infarction, stroke and coronary revascularisation – the AusDiab study

7.1 Overview

In chapters 5 and 6, it was shown that hyperglycaemia is significantly associated with CVD mortality. However, as noted in chapter 2, CVD encompasses several different outcomes, including myocardial infarction and stroke, as well as procedural end-points such as PTCA and CABG. It is well recognised that there are some quantitative differences between the effects of risk factors for myocardial infarction and stroke. Furthermore, hyperglycaemia may increase the likelihood of having an acute coronary event because hyperglycaemia may specifically increase the vulnerability of coronary atherosclerotic plaques. Therefore, it is important to assess the specific role of hyperglycaemia in relation to a variety of CVD outcomes.

The study presented in this chapter examined the impact of diabetes, IFG and IGT in comparison to NGT on myocardial infarction, stroke, coronary revascularisation, as well as the composite outcome of fatal or non-fatal CVD. The results showed that diabetes and IFG were independently associated with an increased risk of fatal or non-fatal CVD events. However, when CVD outcomes were considered separately, the risk of myocardial infarction was significantly increased for KDM, IFG, and IGT, but not NDM, whereas the risk of coronary revascularisation was only significantly increased for KDM, and no significant associations were observed for stroke.

These findings are novel. While previous studies have shown that hyperglycaemia is associated with subsequent CVD events, the results from this study suggest that abnormal glucose metabolism may be more strongly associated with myocardial infarction than stroke or coronary revascularisation. It is acknowledged, however, that the study may not have been sufficiently powered to detect relationships between hypoglycaemia and revascularisation and stroke. Despite this, in addition to providing further insights into the role of hyperglycaemia in CVD outcomes, the findings of this study also have implications for both clinical practice and the design of future epidemiological studies and clinical trials. Patients with hyperglycaemia may benefit from earlier, more aggressive treatment, prior to the development of angina, and it may be worthwhile to further evaluate such interventions. Furthermore, in future studies it may not be appropriate to use a composite CVD end-point in isolation to describe the association between hyperglycaemia and CVD, as this may miss the elevated risk of very important CVD outcomes in individuals with IFG, IGT or diabetes.

The study was submitted for publication on 19th June, 2009.

Monash University
Declaration for thesis chapter 7

Declaration by candidate

In the case of chapter 7, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Principal author, critically reviewed the literature and developed the research question, planned and conducted the statistical analysis, interpreted the findings, structured and drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.	80%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Tonkin, AM	Facilitated the development of the research questions, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Zimmet, PZ	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Balkau, B	Provided statistical advice, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Welborn, TA	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Shaw, JE	Conceptualisation of the AusDiab study, facilitated the development of the research questions, critically evaluated the analysis and interpretation of the findings, and edited the manuscript

Candidate's signature [redacted]

Date: 25th June 2009

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the International Diabetes Institute and will be held for at least five years from the date indicated below:

Location:	Department of Epidemiology and Clinical Diabetes, Baker IDI Heart and Diabetes Institute, Commercial Rd Melbourne, 3004, Victoria, Australia.
Date:	17th June 2009

Tonkin, AM	[redacted]	Date: 7th May 2009
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Shaw, JE	[redacted]	Date: 6th May 2009

Elevated blood glucose may differentially influence the five-year risk of myocardial infarction, stroke and coronary revascularisation – the AusDiab study

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Abstract

Objectives: To assess the contribution of hyperglycaemia to the risk of myocardial infarction, stroke and coronary revascularisation.

Design: Prospective cohort study. In 1999-2000, baseline testing involved fasting plasma samples, an oral glucose tolerance test, anthropometrics and questionnaires.

Setting and participants: 8357 adults aged ≥ 25 years from the Australian Diabetes, Obesity and Lifestyle Study (AusDiab).

Main outcome measures: Five year fatal and non-fatal CVD events were ascertained by linkage to the National Death Index, and by medical record review, respectively.

Results: After a median (25^{th}, 75^{th} percentiles) of 5.0 (4.9, 5.1) years, there were 318 CVD events (103 fatal and 175 non-fatal). Compared to those with normal glucose tolerance, the multivariate-adjusted hazard ratios and 95% confidence intervals for total CVD among those with impaired fasting glucose (IFG), impaired glucose tolerance (IGT), newly diagnosed diabetes mellitus (NDM) and known diabetes mellitus (KDM) were 1.6 (1.0–2.4), 1.1 (0.8–1.6), 1.5 (1.0–2.3), and 2.3 (1.6–3.2), respectively. Myocardial infarction risk was significantly increased for IFG (2.4, 1.2–5.0), IGT (1.9, 1.1–3.4) and KDM (3.9, 2.1–7.3), but not for NDM (1.6, 0.7–3.5). Coronary revascularisation risk was significantly increased for KDM (2.1, 1.2–3.6), but not for IFG (1.5, 0.8–2.7), IGT (1.1, 0.7–1.9), or NDM (1.5, 0.8–2.9). No significant associations were seen with stroke (IFG: 2.0, 0.9–4.7; IGT: 0.7, 0.3–1.7; NDM: 1.5, 0.6–3.6; KDM: 2.1, 1.0–4.6).

Conclusions: Hyperglycaemia may be associated with a greater risk for myocardial infarction than for coronary revascularisation or stroke.

Introduction

Diabetes mellitus is a major risk factor for cardiovascular disease (CVD) and mortality [1, 2, 3]. Although age-adjusted mortality rates from coronary heart disease and stroke are declining in the general population, similar improvements have usually not been observed in people with diabetes [4, 5]. The increased risk for CVD is not restricted to diabetic hyperglycaemia, as we [3] and several meta-analyses have also shown that milder forms of abnormal glucose regulation increase this risk [1, 6, 7, 8].

Most studies assessing the relationship between hyperglycaemia and CVD have used composite outcomes [6, 8] or have focused on the risk of CVD death rather than non-fatal outcomes [1, 6]. However, CVD encompasses several different outcomes, including coronary heart disease events and stroke, as well as procedural end-points such as percutaneous transluminal coronary artery angioplasty (PTCA) and coronary artery bypass surgery (CABG). It is well recognised that there are some quantitative differences between the effects of risk factors for myocardial infarction and stroke [9]. Furthermore, hyperglycaemia may have a greater impact on the likelihood of having an acute coronary event than it does on the likelihood of undergoing coronary revascularisation for stable coronary heart disease, because hyperglycaemia may specifically increase the vulnerability of coronary atherosclerotic plaques [10]. Therefore, it is useful to assess the specific role of hyperglycaemia in relation to a variety of CVD outcomes.

The Australian, Diabetes Obesity and Lifestyle (AusDiab) study is a large national population-based prospective cohort study that used an oral glucose tolerance test (OGTT) and therefore provides the opportunity to examine the impact of hyperglycaemia, including impaired fasting glucose and impaired glucose tolerance, on myocardial infarction, stroke, coronary revascularisation, and fatal CVD.

Methods

Study design and population

Baseline measurements were collected between 1999 and 2000 on 11,247 non-institutionalised men and women aged ≥ 25 years, and the five-year follow-up was undertaken between 2004 and 2005. As previously described, individuals were recruited from 42 randomly selected census districts, six in each state and in the Northern Territory of Australia. Of the 17,129 eligible households, 20,347 individuals completed a household interview, and 11,247 (55%) had a biomedical examination, yielding an estimated overall response rate at baseline of 37% [11, 12].

Baseline measurements

Data on age, sex, use of anti-hypertensive and lipid-lowering medications, history of CVD (angina, myocardial infarction or stroke) and smoking (never, ex- or current smoker) were collected by interview. Physical measurements included blood pressure [13], anthropometrics [14], and a 75g OGTT. FPG, 2hPG, fasting serum total cholesterol, triglycerides and high density lipoprotein cholesterol (HDL-C) were measured using an Olympus AU600 analyser (Olympus Optical, Tokyo, Japan) at a central laboratory. Glucose tolerance status was classified according to the 1999 World Health Organization criteria [15]. Participants who reported having physician-diagnosed diabetes and were (i) taking hypoglycaemic medication, or (ii) had FPG ≥ 7.0 mmol/l or 2h-PG ≥ 11.1 mmol/l were classified as having known diabetes mellitus (KDM). Those not reporting having diabetes, but who had FPG ≥ 7.0 mmol/l or 2h-PG ≥ 11.1 mmol/l were classified as having newly diagnosed diabetes mellitus (NDM). Those without diabetes were classified as having either impaired fasting glucose (IFG: FPG ≥ 6.1 and < 7.0 mmol/l with 2h-PG < 7.8 mmol/l), impaired glucose tolerance (IGT: 2h-PG ≥ 7.8 and < 11.1 mmol/l with FPG < 7.0 mmol/l) or normal glucose tolerance (NGT: FPG < 6.1 mmol/l and 2h-PG < 7.8 mmol/l).

Follow-up and outcomes

Between 2004 and 2005, 10,788 participants were eligible for re-testing and 8,802 (82%) completed an interviewer-administered questionnaire about previous CVD

events, including myocardial infarction, stroke, CABG and PTCA. Self-reported CVD events that occurred between baseline and follow-up were adjudicated by reviewing medical records according to: (i) World Health Organization/MONICA criteria [16] for myocardial infarction, (ii) World Health Organization criteria for stroke [17], and (iii) operation reports for coronary artery bypass and angioplasty. Full details on the methods for adjudication of CVD outcomes in the AusDiab study have been previously reported [18].

CVD mortality was determined by linking the AusDiab cohort to the Australian National Death Index (NDI) using methods previously described [3]. The accuracy of the NDI for ascertainment of vital status and CVD deaths has been previously established [19]. People who could not be matched to the NDI were assumed to be alive, except for five participants who were excluded because they were known to be deceased. The underlying cause of death was classified according to 2006 International Classification of Diseases 10th revision (ICD-10). Fatal outcomes included: (i) any CVD (I10–I25, I46.1, I48, I50–I99 or R96), (ii) myocardial infarction (I21–I23 or R96) or (iii) stroke (I60–I64). In cases where the underlying cause of death was uncomplicated diabetes (E109, E119 or E149) or unspecified hyperlipidaemia (E785), CVD was considered to be the cause of death (n=4) if any of the CVD codes (I10–I25, I46.1, I48, I50–I99 or R96) were recorded in the first position on the death certificate. Fatal and non-fatal CVD events were combined, and four outcome measures were considered: (i) any fatal or non-fatal CVD event, (ii) fatal or non-fatal myocardial infarction only, (iii) fatal or non-fatal stroke only and (iv) coronary revascularisation (CABG or PTCA). Coronary revascularisation procedures were also classified as acute or non-acute, with acute revascularisation being within 14 days of a myocardial infarction.

Of the 8,802 (82%) participants who completed the questionnaire, 330 reported having at least one CVD event and the records of 276 were reviewed. In addition, 124 participants died from CVD. Participants who reported an event were excluded from adjudication if they: (i) did not provide consent to have their medical records reviewed (n=36), (ii) had records that could not be obtained (n=13) or (iii) had events that occurred in a hospital outside of Australia (n=5). Other exclusions included: pregnancy (n=43), inadequate fasting (n=19), unclassified diabetes status (n=86) and

missing data (n=367). Participants were included irrespective of whether or not they reported having a prior CVD event. Thus, these analyses include 8,357 participants.

Statistical analysis

Analyses were conducted with Stata Statistical Software version 10.0 (StataCorp, College Station, Texas, USA). Differences in means and proportions for baseline characteristics between the normal glucose tolerance, impaired fasting glucose, impaired glucose tolerance, newly diagnosed diabetes and known diabetes groups were tested with one-way analysis of variance and chi-square analyses, respectively. The distribution for triglycerides was skewed and therefore values were logarithmically transformed prior to analysis.

The follow-up period for fatal and non-fatal CVD events was up to the date of the event or up to the 2004–2005 testing date (ranged from 1-6-2004 to 7-4-2006), whichever occurred first. Analyses were based on the first CVD event that occurred after baseline testing. Incidence of CVD events were plotted using Kaplan-Meier failure function. Cox proportional hazards regression was used to estimate unadjusted and adjusted CVD event hazard ratios (HR) and 95% confidence intervals (CI) for known diabetes, newly diagnosed diabetes, impaired glucose tolerance and impaired fasting glucose compared to normal glucose tolerance. Age, sex, history of CVD, smoking, hypertension (blood pressure ≥ 140/90 mmHg or use of anti-hypertensive medications), waist-to-hip ratio, lipid-lowering medication use, total cholesterol and triglycerides were considered to be important confounders and were entered into the multivariate models. The individual covariate variance inflation factors (VIF) were used to examine multicollinearity between covariates [20]. None of the individual covariate VIFs were greater than 3. Further analyses were also conducted to investigate whether adding (i) systolic and/or diastolic blood pressure instead of hypertension, (ii) total cholesterol to HDL-C ratio instead of total cholesterol, and (iii) adding waist circumference or BMI instead of waist-to-hip ratio altered the estimates for the glucose tolerance categories in multivariate models. As estimates for CVD outcomes remained largely unchanged (data not shown), results from the primary multivariate model were reported. Interactions between abnormal glucose metabolism categories with sex, age (< 64 and ≥ 65 years), and history of CVD events (yes or no) were tested using log-likelihood ratio tests of models

containing the two variables nested within models additionally including the first-order interactions. The assumptions required for proportional hazards were met, and these were assessed with graphs of log-log plots of the relative hazards by time, and scaled Schoenfeld residuals.

Results

The cohort comprised 8,357 individuals (55% women) who had a mean age of 52 years (range=25–90, standard deviation=14). Compared to individuals with normal glucose tolerance, those with abnormal glucose metabolism had more unfavourable risk factor profiles at baseline (Table 1).

After a median of 5.0 years, 318 CVD events had occurred. Of the 103 fatal events (66 in men), causes of death were: myocardial infarction (n=36), stroke (n=29) and other CVD (n=38). Of the 215 non-fatal events (150 in men) causes were: myocardial infarction (n=54), stroke (n=29), CABG (n=51) and PTCA (n=81). Figure 1 shows that the unadjusted cumulative incidence rates of any CVD event, myocardial infarction, stroke or coronary revascularisation for impaired fasting glucose and impaired glucose tolerance were intermediate between those observed in people with diabetes and normal glucose metabolism.

The risk (HR and 95% CI) of fatal or non-fatal CVD without and with adjustment for age and sex, history of CVD, smoking, hypertension, waist-to-hip ratio, total cholesterol, triglycerides and lipid-lowering medication use is shown in Table 2. Compared to those with normal glucose metabolism, those with known diabetes, newly diagnosed diabetes and impaired fasting glucose were significantly more likely to experience any CVD event, and those with known diabetes, impaired fasting glucose, and impaired glucose tolerance, but not newly diagnosed diabetes were significantly more likely to have had a myocardial infarction. The risk of coronary revascularisation was only significantly increased for known diabetes. Moreover, the hazard ratios for fatal or non-fatal myocardial infarction were larger than those observed for coronary revascularisation for each of the abnormal glucose metabolism categories. Larger differences between the risk for myocardial infarction and revascularisation were particularly noted for known diabetes, as neither set of

confidence intervals for each of myocardial infarction and revascularisation overlapped with the other point estimates. After acute coronary revascularisations were excluded (n=26), the HR for known diabetes reduced and was no longer significant for coronary revascularisation (HR=1.7; 95% CI 0.9–3.1). No significant associations were observed for stroke.

Testing the interactions between abnormal glucose metabolism categories and each of sex (p=0.81), history of CVD (p=0.06) and age (p=0.04) for any fatal or non-fatal CVD event revealed that the interactions with age and history of CVD were of borderline significance.

Discussion

In this large prospective population-based cohort, diabetes and impaired fasting glucose were independently associated with an increased risk of fatal or non-fatal CVD events after a median of 5.0 years follow-up. When CVD outcomes were considered separately, the risk of myocardial infarction was significantly increased for known diabetes, impaired fasting glucose, and impaired glucose tolerance, but not newly diagnosed diabetes, whereas the risk of coronary revascularisation was only significantly increased for known diabetes, and no significant associations were observed for stroke. These findings are novel in that while previous studies have shown that hyperglycaemia is associated with subsequent CVD events, our results indicate that abnormal glucose metabolism may be more strongly associated with myocardial infarction than stroke or coronary revascularisation.

Evidence from meta-analyses now indicate that the risk of coronary heart disease increases according to worsening hyperglycaemia [1, 7]. Our findings support this, showing that known diabetes was associated with a four-fold increase in risk for fatal or non-fatal myocardial infarction and a two-fold increase in risk for coronary revascularisation, and that both impaired fasting glucose and impaired glucose tolerance were significantly associated with a two-fold increased risk for myocardial infarction.

Histological and physiological studies have shown that in people with diabetes, atherosclerotic disease may be more severe [10], and as a result of autonomic neuropathy the usual symptoms of coronary heart disease may go unrecognised. Hyperglycaemia and associated diabetic dyslipidaemia are thought to accelerate inflammatory, microangiopathic and thrombotic processes which increase the likelihood of plaque rupture and thrombosis [10]. Diabetes may therefore have a greater impact on the risk of having an acute coronary event, than it does on the risk of developing stable coronary heart disease with angina. Indeed, one study has demonstrated that in older individuals, diabetes conferred a greater risk for fatal than for non-fatal CVD outcomes [21]. Our data support this as we have shown that the risk for myocardial infarction in individuals with impaired fasting glucose, impaired glucose tolerance and known diabetes was up to two times greater than the risk for coronary revascularistion, and the risk differential widened once acute coronary revascularisation for the management of acute coronary syndrome was excluded in a sub-analysis. Should further work confirm this, it would strengthen the need to identify people with diabetes who have no symptoms associated with atherosclerosis but who might potentially benefit from coronary interventions.

An alternative explanation for the stronger association between diabetes and coronary events than is seen between diabetes and revascularisation may relate to selective provision of care. Clinicians may be less likely to intervene in those with diabetes, either because they are considered to be unsuitable for revascularisation, or because they do not adequately recognise the increased coronary risk associated with diabetes and the need for an aggressive approach to treatment and intervention because of this higher risk. However, further research on clinical management practices would be required to test this hypothesis.

Diabetes increases the risk of stroke [1, 9]. However, it is unclear as to whether the risk of stroke is also increased at intermediate levels of hyperglycaemia, independent of other risk factors. Although some studies have reported an association between impaired fasting glucose and stroke [1, 7, 22], and between impaired glucose tolerance and stroke [23, 24], other studies have reported no association [25, 26]. Differences in study findings could be due to several factors, including the definition of stroke, and participant characteristics. In our study, known diabetes was associated

with a two-fold risk of stroke of borderline significance, and neither newly diagnosed diabetes, impaired fasting glucose nor impaired glucose tolerance were significantly associated with stroke. However, as the number of strokes was limited, this study lacks power for detecting significant differences. However, any differences in risk for stroke and myocardial infarction in those with impaired fasting glucose and impaired glucose tolerance could be explained by the different pathophysiological processes responsible for these outcomes. While hyperglycaemia is thought to contribute to myocardial infarction by triggering inflammation, dyslipidaemia and fibrinolysis [27], stroke is more strongly related to endothelial dysfunction and hypertension [9]. It is therefore possible that hyperglycaemia may need to be more severe before it has a significant impact on the underlying processes that lead to stroke.

The following limitations need to be considered. Firstly, the generalisability of this study to the Australian population may have been affected by the baseline response rate of 37%. However, differences between responders and non-responders are unlikely to have impacted on the associations between abnormal glucose metabolic status and CVD. Secondly, as adjudicated non-fatal CVD events were derived from questionnaire data, they may have been influenced by survivor bias. Furthermore, events that were not reported would not have been adjudicated, and potentially missed. However, a previous validation study in which we compared self-reported data to a statewide hospital morbidity database demonstrated that only 0.2% of those denying an event, were found to actually have had such an event [18]. Thirdly, we may have underestimated the relative risk of a CVD event, as a single OGTT was used to determine glucose tolerance status. This may have introduced some imprecision and regression dilution bias, as these measures are subject to within-person variability [28]. In our study impaired fasting glucose is consistently more predictive than impaired glucose tolerance and thus suggests that impaired fasting glucose is a more stable measure of hyperglycaemia than the response to an OGTT. Fourthly, our finding of a lack of a significant association between abnormal glucose metabolism and stroke may have related to the heterogeneous nature of stroke [9], Diabetes may be more strongly associated with certain stroke sub-types, particularly ischaemic stroke [29]. We did not have the power to consider different stroke outcomes in separate analyses. Finally, this analysis is based on five-year data.

Longer follow-up is required to provide sufficient power to ascertain whether the impact of age, sex and history of CVD was different for each of the CVD outcomes examined in this study, and to more clearly delineate the risks for different CVD outcomes.

In addition to providing further insights into the relationships between abnormal glucose metabolism and CVD outcomes, the findings of this study also have implications for the design of future epidemiological studies and clinical trials. CVD manifests as several different clinical outcomes, and our results indicate that the risk conferred by abnormal glucose metabolism for myocardial infarction, stroke and coronary revascularisation may differ. Therefore, it may not be appropriate to use a broad composite CVD end-point in isolation to describe the association between hyperglycaemia and CVD, as this may miss the elevated risk of very important CVD outcomes in individuals with impaired fasting glucose, impaired glucose tolerance or diabetes. This may be an important consideration when designing clinical trials in populations with abnormal glucose metabolism, as valuable treatment effects could be missed.

In conclusion, in our study the risk of five-year myocardial infarction was significantly elevated in individuals with IFG, IGT and previously diagnosed diabetes, but the risk for coronary revascularisation was only significantly increased among those with previously diagnosed diabetes, and no associations were observed for stroke. Further research into the impact of hyperglycaemia on cerebrovascular disease, and stable and unstable coronary heart disease is warranted.

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Competing interests

None

Ethics approval

Ethics approval was obtained from the ethics committees of the International Diabetes Institute, Monash University, the Australian Institute of Health and Welfare and the Sir Charles Gairdner Hospital, Nedlands (WA). Participants provided

informed consent to participate in the study and to have their medical records reviewed.

The sponsors had no influence on the design, analysis, or interpretation of results and took no part in the writing of this paper.

Table 1. Baseline characteristics according to abnormal glucose tolerance status – the AusDiab study

	NGT	IFG	IGT	NDM	KDM	Total
N (%)	6193 (74)	498 (6)	1012 (12)	326 (4)	328 (4)	8357
Age, years	49.5 (13.1)	53.9 (12.3)	58.1 (13.1)	61.4 (12.2)	63.1 (11.5)	51.8 (13.6)
Male	2653 (43)	350 (70)	406 (40)	170 (52)	180 (55)	3759 (45)
Waist-to-hip ratio	0.85 (0.09)	0.92 (0.08)	0.89 (0.09)	0.92 (0.09)	0.93 (0.09)	0.86 (0.09)
Prior CVD*	336 (5)	54 (11)	98 (10)	47 (14)	82 (25)	617 (7)
Smoking (%)						
- Past	1705 (28)	186 (37)	329 (33)	103 (32)	139 (42)	2462 (29)
- Current	878 (14)	79 (16)	99 (10)	49 (15)	44 (13)	1149 (14)
Total cholesterol, mmol/l	5.6 (1.1)	5.9 (1.1)	5.9 (1.1)	5.9 (1.1)	5.4 (0.9)	5.7 (1.1)
Triglycerides, mmol/l†	1.2 (0.8, 1.7)	1.5 (1.1, 2.2)	1.6 (1.1, 2.3)	1.9 (1.3, 2.9)	1.7 (1.2, 2.5)	1.3 (0.9, 1.9)
Lipid-lowering medication use	373 (6)	62 (12)	130 (13)	54 (17)	123 (38)	742 (9)
Hypertension‡	1511 (24)	203 (41)	528 (52)	221 (68)	232 (71)	2695 (32)
Systolic blood pressure, mmHg	126.4 (17.2)	133.6 (16.9)	137.1 (18.8)	144.5 (19.7)	143.2 (20.2)	129.5 (18.5)
Diastolic blood pressure, mmHg	69.2 (11.4)	73.9 (11.2)	72.3 (12.1)	75.9 (12.4)	73.3 (11.6)	70.2 (11.7)

Data are mean (SD) or n (%)

*History of CVD defined as self-reported angina, myocardial infarction or stroke

†Data are median (25^{th}, 75^{th} percentile)

‡Hypertension defined as blood pressure > 140/90 mmHg or reported anti-hypertensive medication use

Significant differences ($P< 0.001$) between categories of abnormal glucose metabolism were observed for all baseline characteristics

NGT – normal glucose tolerance; IFG – impaired fasting glucose; IGT – impaired glucose tolerance; NDM – newly diagnosed diabetes at baseline; KDM – previously diagnosed diabetes at baseline; CVD – cardiovascular disease.

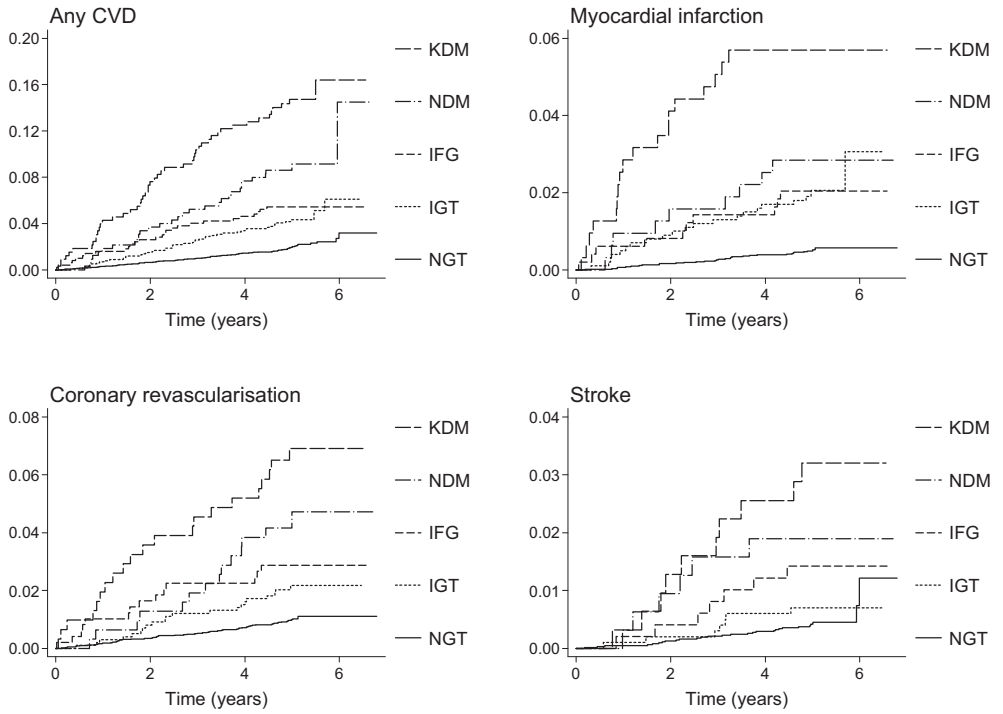

Figure 1. Unadjusted cumulative incidence of any fatal or non-fatal CVD event, fatal or non-fatal myocardial infarction, coronary revascularisation (percutaneous transluminal coronary artery angioplasty or coronary artery bypass surgery) and fatal or non-fatal stroke according to baseline glucose tolerance status – the AusDiab study.

CVD – cardiovascular disease; KDM – known diabetes mellitus; NDM – newly diagnosed diabetes; IFG – impaired fasting glucose; IGT – impaired glucose tolerance; NGT – normal glucose tolerance.

Table 2. Five-year risk of fatal or non-fatal cardiovascular disease events according to baseline abnormal glucose tolerance status – the AusDiab study.

Outcomes[*]	**NGT**	**IFG**			**IGT**			**NDM**			**KDM**		
Fatal or non-fatal CVD (n=8357)													
n (%)	127 (2.1)	27 (5.4)			45 (4.5)			30 (9.2)			49 (14.9)		
	Reference	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Unadjusted	1.0	2.7	1.8–4.0	< 0.001	2.2	1.6–3.1	< 0.001	4.6	3.1–6.9	< 0.001	7.8	5.6–10.9	< 0.001
Age and sex adjusted	1.0	1.8	1.2–2.7	0.006	1.2	0.8–1.6	0.396	1.9	1.3–2.9	0.002	2.9	2.0–4.0	< 0.001
Multivariate adjusted[†]	1.0	1.6	1.0–2.4	0.040	1.1	0.8–1.6	0.599	1.5	1.0–2.3	0.048	2.3	1.6–3.2	< 0.001
Fatal or non-fatal MI only (n=8290) [‡]													
n (%)	32 (0.5)	10 (2.0)			21 (2.1)			9 (2.8)			18 (5.5)		
	Reference	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Unadjusted	1.0	3.9	1.9–8.0	< 0.001	4.1	2.4–7.1	< 0.001	5.5	2.6–11.6	< 0.001	11.3	6.4–20.2	< 0.001
Age and sex adjusted	1.0	2.8	1.4–5.8	0.004	2.1	1.2–3.7	0.008	2.3	1.1–4.9	0.030	4.4	2.4–7.9	< 0.001
Multivariate adjusted[†]	1.0	2.4	1.2–5.0	0.016	1.9	1.1–3.4	0.021	1.6	0.7–3.5	0.267	3.9	2.1–7.3	< 0.001
Fatal or non-fatal stroke only (n=8283) [‡]													
n (%)	28 (0.5)	7 (1.4)			7 (0.7)			6 (1.8)			10 (3.0)		
	Reference	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Unadjusted	1.0	3.1	1.3–7.0	0.008	1.6	0.7–3.4	0.292	4.2	1.7–10.1	0.002	7.1	3.4–14.6	< 0.001
Age and sex adjusted	1.0	2.2	1.0–5.1	0.062	0.8	0.3–1.8	0.591	1.7	0.7–4.1	0.254	2.5	1.2–5.2	0.015
Multivariate adjusted[†]	1.0	2.0	0.9–4.7	0.100	0.7	0.3–1.7	0.472	1.5	0.6–3.6	0.422	2.1	1.0–4.6	0.054
CABG or PTCA (n=8254)[§]													
n (%)	62 (1.0)	14 (2.9)			21 (2.1)			14 (4.5)			21 (6.8)		
	Reference	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Unadjusted	1.0	2.9	1.6–5.1	< 0.001	2.1	1.3–3.5	0.003	4.5	2.5–8.0	< 0.001	7.0	4.3–11.4	< 0.001
Age and sex adjusted	1.0	1.9	1.0–3.3	0.036	1.3	0.8–2.1	0.326	2.2	1.2–4.0	0.009	3.0	1.8–5.1	0.000
Multivariate adjusted[†]	1.0	1.5	0.8–2.7	0.195	1.1	0.7–1.9	0.602	1.5	0.8–2.9	0.175	2.1	1.2–3.6	0.006

[*]Outcomes are first adjudicated non-fatal CVD event (myocardial infarction, stroke, CABG or PTCA) or CVD death, and therefore numbers of MI, stroke and CABG or PTCA events do not add up to the numbers included in the composite fatal or non-fatal CVD outcome

[†]adjusted for baseline age, sex, previous self-reported CVD (angina, MI or stroke), smoking (current or ex-smoker), hypertension (BP≤ 140/90 mmHg or reported anti-hypertensive medication use), waist to hip ratio, total cholesterol, triglycerides and self-reported lipid-lowering medication use

[‡]Total n differs to whole cohort (n = 8357). Participants with CVD deaths unrelated to MI or stroke were excluded from these analyses because non-fatal events that occurred prior to the fatal event could not be ascertained

[§]Participants with fatal CVD events were excluded from this analysis

CABG – coronary artery bypass graft; CVD – cardiovascular disease; IFG – impaired fasting glucose; IGT – impaired glucose tolerance; KDM – previously diagnosed diabetes at baseline; MI – myocardial infarction; NDM – newly diagnosed diabetes at baseline; NGT – normal glucose tolerance; PTCA – percutaneous transluminal coronary artery angioplasty.

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CHAPTER 8

HOMA insulin sensitivity index, hyperglycaemia and the risk of all-cause mortality, and cardiovascular disease events in the general population – the AusDiab study

8.1 Overview

The studies presented in chapters 5 to 7 examined the contribution of hyperglycaemia to CVD and mortality. However, as discussed in chapter 2, glucose metabolism involves several precisely orchestrated physiological processes that control the transfer of glucose from the circulation. Glucose homeostasis can be disrupted by a reduction in the production of insulin from the pancreas, and/or by an insufficient response to insulin by muscle and fat cells. The latter mechanism, referred to as insulin resistance, is thought to be an independent risk factor for premature mortality and CVD, however, findings from previous epidemiological studies have been equivocal. Furthermore, few studies have compared the relative associations of hyperglycaemia and insulin resistance with CVD in the same study population, and findings are inconsistent. Therefore, the aim of the study presented in this chapter was to determine whether the insulin sensitivity index, HOMA-%S, was associated with all-cause mortality and fatal or non-fatal CVD events independent of other CVD risk factors including hyperglycaemia, in individuals without clinically diagnosed diabetes.

The results revealed that HOMA-%S was not associated with all-cause mortality. However, compared to those who were most insulin sensitive, the CVD event hazard ratios increased across decreasing quintiles of HOMA-%S, adjusting for age and sex. Smoking, previous history of CVD, hypertension, lipid-lowering medication and

total cholesterol moderately attenuated this relationship; however, the association was rendered non-significant by adding HDL-C. Furthermore, FPG, was a better predictor of CVD than HOMA-%S.

The findings of this study, the largest prospective population-based study of men and women to evaluate the association between HOMA-%S and CVD after adjusting for a wide range of CVD risk factors, indicate that insulin sensitivity, as determined from HOMA-%S, is not associated with all-cause mortality, and is only modestly associated with CVD, largely due to its relationship with HDL-C. As such, data from this study suggest that insulin resistance can only be considered to play an indirect role in the development of CVD in the general population.

The study was submitted for publication on 2nd June, 2009.

Monash University
Declaration for thesis chapter 8

Declaration by candidate

In the case of chapter 8, the nature and extent of my contribution to the work was the following:

Nature of contribution	**Extent of contribution**
Principal author, critically reviewed the literature and developed the research question, planned and conducted the statistical analysis, interpreted the findings, structured and drafted the manuscript and responded to journal reviewer comments. Responsible author who accepts overall responsibility for the publication.	80%

The following co-authors contributed to the work.

Name	**Nature of contribution**
Cameron, AJ (Monash PhD Student)	Calculated HOMA-%S, critically evaluated the analysis and interpretation of the findings, and edited the manuscript Extent of contribution: 10%
Balkau, B	Provided statistical advice, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Zimmet, PZ	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Welborn, TA	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Tonkin, AM	Critically evaluated the analysis and interpretation of the findings, and edited the manuscript
Shaw, JE	Conceptualisation of the AusDiab study, critically evaluated the analysis and interpretation of the findings, and edited the manuscript

Candidate's signature 

Date: 25th June 2009

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;

(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and

(6) the original data are stored at the International Diabetes Institute and will be held for at least five years from the date indicated below:

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HOMA insulin sensitivity index, hyperglycaemia and the risk of all-cause mortality and cardiovascular disease events in the general population – the AusDiab study

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Abstract

Objective: To assess the independent associations of insulin sensitivity, fasting plasma glucose (FPG), 2 h plasma glucose (2hPG) and HbA_{1c} with all-cause mortality and fatal or non-fatal cardiovascular disease (CVD) events in individuals without diagnosed diabetes.

Research Design and Methods: Between 1999 and 2000, baseline fasting insulin, glucose and lipids, 2hPG, $HbA_{1c,}$ anthropometrics, blood pressure, medication use, smoking and history of CVD were collected from 8,533 adults aged > 35 years from the population-based Australian Diabetes, Obesity and Lifestyle study. Insulin sensitivity was estimated by the homeostasis model assessment (HOMA-%S). All deaths and fatal or non-fatal CVD events were ascertained through linkage to the National Death Index and medical records adjudication.

Results: After a median of 5.0 years there were 277 deaths and 225 CVD events. HOMA-%S was not associated with all-cause mortality. Compared to those who were most insulin sensitive, the combined fatal or non-fatal CVD HR (95% CI) for quintiles of decreasing HOMA-%S were 1.1 (0.6–1.9), 1.4 (0.9–2.3), 1.6 (1.0–2.5), 2.0 (1.3–3.1), adjusting for age and sex. Smoking, CVD history, hypertension, lipid-lowering medication, total cholesterol and waist-to-hip ratio only moderately attenuated this relationship; however, the association was rendered non-significant by adding HDL. FPG, but not HOMA-%S, significantly improved the prediction of CVD, beyond that seen with other risk factors.

Conclusions: In this cohort, HOMA-%S showed no association with all-cause mortality; and a modest association with CVD events, largely explained by its association with HDL. FPG was a better predictor of CVD than was HOMA-%S.

Introduction

Insulin resistance is a precursor to the development of hyperglycaemia and subsequent diabetes [1], which are both considered to be important risk factors for the development of cardiovascular disease (CVD) and premature mortality [2-4]. Exploring the relative associations of hyperglycaemia and insulin resistance (or its reciprocal, insulin sensitivity) among those without diagnosed diabetes may help to elucidate possible mechanisms underlying premature mortality and CVD in the general population. Furthermore, as there is evidence of considerable heterogeneity in insulin resistance among individuals with different degrees of glucose tolerance [5], it is important to ascertain whether the presence of hyperglycaemia and insulin resistance can assist in the identification of a particularly high risk group.

In addition to hyperglycaemia, insulin resistance is also associated with several other CVD risk factors, including adiposity, dyslipidaemia and hypertension [6], which are collectively identified as the metabolic syndrome [7]. However, uncertainty remains as to whether insulin resistance is itself an independent risk factor for premature mortality and CVD. Part of the problem relates to the measurement of insulin resistance, as epidemiological studies have used a variety of different indices. Although the hyperinsulinaemic-euglycaemic clamp is currently considered the gold standard method for measuring insulin resistance [8], few large studies have used this to evaluate the association between insulin resistance and CVD [9-11], as it is invasive and time-consuming. Consequently, surrogate markers of insulin resistance have been developed.

The simplest of these is plasma insulin; typically measured in the fasting state, although post-challenge insulin has also been used. The role of hyperinsulinaemia as a risk factor for the development of atherosclerosis has been debated [12-14], as findings from epidemiological studies are equivocal [15, 16]. Two meta-analyses have reported a weak but statistically significant association between hyperinsulinaemia and CVD [17, 18]. However, one analysis combined results from several studies that did not adequately adjust for all components of the metabolic syndrome [17], and the other potentially introduced measurement error by combining individual level data from studies that used different protocols and insulin assays (some specific and others not specific for insulin) [18].

Other markers of insulin resistance are based on both plasma glucose and insulin values [19, 20], and may therefore provide a better approximation of clamp-derived insulin resistance. The homeostasis model assessment of insulin resistance (HOMA-IR) [21] or insulin sensitivity (HOMA-%S) [22] are the most commonly used, and several [23-26], but not all [27-29] large prospective population-based studies have found it to be significantly associated with incident CVD. However, the various limitations of these studies include: (i) not adjusting for each individual component of the metabolic syndrome [23, 25], (ii) using a non-specific insulin assay which cross-reacts with proinsulin [23, 25, 26], in itself a possible independent risk factor for CVD [10, 30] or (iii) only including select populations [23].

The Australian Diabetes, Obesity and Lifestyle study (AusDiab) is a national, prospective, population-based study of adult men and women, and represents the largest single study to assess the relationship between insulin resistance (as measured with the insulin sensitivity index, HOMA-%S) and CVD after adjusting for components of the metabolic syndrome including blood glucose and other important CVD risk factors. In this study, we used insulin data from 8,533 men and women aged > 35 years without diagnosed diabetes, as assessed with an insulin-specific assay, to investigate (i) the associations between HOMA-%S and five-year all-cause mortality and combined fatal or non-fatal CVD events, and (ii) whether these relationships were independent of blood glucose, HbA_{1c} and other metabolic factors.

Research design and methods

Study design

The AusDiab study includes 11,247 men and women aged ≥ 25 years. Methods and response rates have been reported [31, 32]. Between 1999 and 2000, a stratified clustered sample was drawn from 42 randomly selected census districts, six in each of the states and the Northern Territory of Australia. The sampling frame consisted of 17,129 eligible households, from which 20,347 individuals completed a household interview, and 11,247 (55%) of these participants attended a biomedical examination, yielding an estimated response rate at baseline of 37%. A five-year follow-up study which included the assessment of non-fatal CVD outcomes was undertaken between 2004 and 2005, and 8,802 (82%) of the 10,788 eligible individuals completed a

questionnaire which included history of previous CVD events. The ethics committees of the International Diabetes Institute, Monash University, the Australian Institute of Health and Welfare and the Sir Charles Gairdner Hospital, Western Australia approved the study, and informed consent was obtained from all participants.

Baseline measures

Baseline data on age, sex, use of anti-hypertensive and lipid-lowering medications, history of CVD (angina, myocardial infarction or stroke) and smoking were collected by interviewer-administered questionnaires. Measurements included blood pressure [33], anthropometrics [34] and a fasting (≥ 9 hours) blood sample. All participants, except for pregnant women and people taking hypoglycaemic medication, underwent a 75g OGTT. Fasting plasma glucose (FPG), 2 h plasma glucose (2hPG), fasting serum total cholesterol, triacylglycerol and HDL were measured using an Olympus AU600 analyser (Olympus Optical, Tokyo, Japan). Serum samples for insulin were stored at -80°C until assayed. Serum insulin was measured using a human insulin-specific radioimmunoassay kit (Linco Research, Inc., St Charles, MO). Insulin assays were only conducted in participants aged older than 35 years. All specimens were analysed at a central laboratory. Insulin sensitivity was estimated from FPG and fasting insulin concentrations, and HOMA-%S was calculated with the HOMA-2 program [22].

Categories of abnormal glucose metabolism were determined according to the 1999 World Health Organization (WHO) criteria [35]. Participants were classified as having known diabetes mellitus if they reported having physician-diagnosed diabetes and were (i) either taking hypoglycemic medication, or (ii) had FPG ≥ 7.0 mmol/l or 2hPG ≥ 11.1 mmol/l. Participants not reporting having diabetes, but who had FPG ≥ 7.0 mmol/l or 2h-PG ≥ 11.1 mmol/l were classified as having newly diagnosed diabetes mellitus (NDM). Participants determined not to have diabetes were classified as having either impaired fasting glucose (IFG: FPG ≥ 6.1 and < 7.0 mmol/l with 2hPG < 7.8 mmol/l), impaired glucose tolerance (IGT: 2h-PG ≥ 7.8 and < 11.1 mmol/l with FPG < 7.0 mmol/l) or normal glucose tolerance (NGT: FPG < 6.1 mmol/l and 2hPG < 7.8 mmol/l).

Outcome measures

Mortality status, and underlying and contributory causes of death were ascertained by linking all participants to the Australian National Death Index (NDI), using methods previously described [4]. People not matched to the NDI were assumed to be alive. The accuracy of the NDI for ascertainment of CVD deaths and vital status has been previously established [36]. Deaths were attributed to CVD if the underlying cause of death was coded I10-I25, I46.1, I48, I50-I99 or R96 according to the 2006 International Classification of Diseases 10th revision. In addition, participants with uncomplicated diabetes (E109, E119 or E149) or unspecified hyperlipidemia (E785) as an underlying cause of death on the death certificate were attributed a CVD death (n=3) if any of the CVD codes (I10-I25, I46.1, I48, I50-I99 or R96) were recorded in the first position on the death certificate.

During the five-year follow-up study, 8,802 participants answered an interview-administered questionnaire on CVD events, including myocardial infarction, stroke, percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft surgery (CABG). Self-reported CVD events that occurred between baseline and follow-up were adjudicated by two physicians (J. E. S – diabetologist and A. M. T – cardiologist) using medical records. This method has been described in detail, and validated against a statewide hospital morbidity database [37]. A composite CVD outcome was defined as fatal or non-fatal myocardial infarction, fatal or non-fatal stroke, PTCA, CABG or other deaths associated with CVD.

Participants were excluded if at baseline they were aged ≤ 35 years (n=1603), had previously known diabetes (n=470), had not fasted for ≥ 9 hours (n=16), were pregnant (n=17), had FPG or 2hPG values < 2.5 mmol/l (n=37), had missing HOMA-%S values (n=40), or had missing values for other covariates included in the analyses (n=527). Four participants who died but could not be matched to the NDI were also excluded. Additionally, a further 1,591 participants could not be included in the follow-up for fatal or non-fatal CVD events, as they did not complete the 2004-2005 non-fatal CVD questionnaire. Thus, for all-cause mortality the analyses were undertaken in 8,533 participants, and for fatal or non-fatal CVD follow-up the analyses were based on 6,942 participants.

The follow-up period for all-cause mortality was up to the date of death or 7th April 2006, whichever came first, and follow-up of the composite fatal or non-fatal CVD events was up to the date of event or death or the individual's 2004–2005 testing date (ranged from 9th June 2004 to 7th April 2006), whichever occurred first. CVD follow-up analyses were based on the first CVD event that occurred after baseline testing. For people who died from CVD, non-fatal CVD events could not be obtained and therefore, their CVD death was considered the CVD end-point.

Statistical analysis

HOMA-%S was analysed as both a continuous and categorical variable. For the continuous analysis, HOMA-%S had a positively skewed distribution and was therefore log transformed. Categories were based on quintiles of HOMA-%S, and were determined separately for all-cause mortality and CVD events follow-up. The first quintile represented individuals with the highest insulin sensitivity and the fifth quintile represented those with the lowest insulin sensitivity. Unadjusted mortality rates (95% CI) per 1,000 person-years were calculated for each quintile of HOMA-%S. To test differences in means and proportions for baseline characteristics between the quintiles of HOMA-%S, one-way analysis of variance and χ^2 tests were used, respectively. FPG, 2hPG and triacylglycerol were not normally distributed and were therefore logarithmically transformed prior to analysis.

Cox proportional hazards regression was used to examine the unadjusted and adjusted association between HOMA-%S and all-cause mortality and CVD events (fatal or non-fatal). A linear or non-linear relationship between HOMA-%S and each of the outcomes was tested by calculating the log-likelihood ratio tests of a Cox model, adjusted for age and sex, with and without the squared term for HOMA-%S. Multivariate models were adjusted for age, sex, previous history of CVD (yes or no), smoking (never, ex-smoker or current smoker), hypertension (blood pressure ≥ 140/90 mmHg or self-reported antihypertensive medication use or blood pressure < 140/90 mmHg and no medication use), systolic and diastolic blood pressure, total cholesterol, HDL, non-HDL (total cholesterol minus HDL), triacylglycerol, self-reported lipid-lowering medication use (yes or no), waist-to-hip ratio and body mass index (BMI kg/m^2). Age, systolic and diastolic blood pressure, total cholesterol, HDL, non-HDL, triacylglycerol, waist-to-hip ratio and BMI were modelled as

continuous variables. The influence of CVD risk factors on the relationship between HOMA-%S and all-cause mortality and CVD was evaluated by entering each covariate on a one-by-one basis. To assess whether the relationships between HOMA-%S and the outcome measures were independent of abnormal glucose metabolism, HOMA-%S was modelled with FPG, 2hPG, HbA_{1c} and a categorical variable of abnormal glucose metabolism based on WHO criteria. Models were adjusted for age, sex and the CVD covariates listed above. The predictive ability of HOMA-%S was compared to FPG, 2hPG and HbA_{1c} using log-likelihood ratio statistics, where a model containing HOMA-%S, age, sex, and CVD covariates was nested within models also containing FPG, 2hPG or HbA_{1c}. For these models, 2hPG and HbA_{1c} were modelled as linear variables and FPG was modelled as a linear spline with one knot at 5.1 mmol/l [38].

Interactions between quintiles of HOMA-%S and sex, age (< 65 and ≥ 65 years), previous history of CVD (no or yes), categories of abnormal glucose metabolism (NGT or IFG, IGT and NDM), BMI (< 25 or ≥ 25 to < 30 or ≥ 30 kg/m^2) and waist-to-hip ratio (men: ≤ 0.95 or > 0.95; and women: ≤ 0.80 or > 0.80) were tested by using log-likelihood ratio tests of models containing the variables as single terms nested within models including the first-order interactions. Interactions were considered significant at $p< 0.01$.

All analyses were repeated using (i) fasting insulin (pmol/l) as the surrogate measure of insulin sensitivity instead of HOMA-%S, (ii) using a cohort that excluded individuals with NDM at baseline and (iii) using a cohort that excluded individuals with self-reported myocardial infarction or stroke at baseline. Multicollinearity between covariates was tested with the variance inflation factor, which was found to be < 3 for all independent variables [33]. Proportional hazards assumptions were satisfied as assessed with graphs of log-log plots of the relative hazards by time for discrete variables and by scaled Schoenfeld residuals. Analyses were conducted with Stata Statistical Software version 9.2 (StataCorp, College Station, TX, USA).

Results

For the total cohort of 8,533 individuals the mean (range) age was 54 (36 to 91) years, 4,700 (55%) were women and 7,448 (87%) reported being born in Australia,

New Zealand, or the United Kingdom. In unadjusted analyses (Table 1), declining insulin sensitivity, as measured by HOMA-%S, was significantly associated with other risk factors for premature mortality and CVD events.

There were 277 deaths (117 in women) after a median (inter-quartile range - IQR) of 5.0 (0.5) years, and 225 fatal or non-fatal CVD events (74 in women) (69 fatal or non-fatal myocardial infarctions, 45 fatal or non-fatal strokes, 45 PTCAs and 35 CABGs and 31 deaths due to other types of atherosclerotic CVD) after a median (IQR) of 5.0 (0.2) years.

In unadjusted analyses, there was no relationship between HOMA-%S and all-cause mortality (Figure 1a). For CVD a linear association with HOMA-%S was observed (Figure 1b). Prediction of CVD was not improved by adding a squared term for HOMA-%S ($p > 0.5$ in log-likelihood ratio test).

For all-cause mortality, no association was observed with quintiles of declining insulin sensitivity in either unadjusted (quintile HR [95%CI]: 1.2 [0.8–1.8], 1.4 [1.0–2.1], 1.2 [0.8–1.8] and 1.3 [0.9–1.9]), or age- and sex-adjusted (quintile HR [95%CI]: 1.1 [0.8–1.7], 1.0 [0.7–1.5], 0.9 [0.6–1.4] and 1.1 [0.7–1.6]) models. After adjustment for other covariates, and reanalysing HOMA-%S as a continuous variable, there was still no relationship with all-cause mortality (data not shown). Age, sex, waist-to-hip ratio, history of CVD and abnormal glucose tolerance status did not significantly modify the relationship between HOMA-%S and all-cause mortality (all $p> 0.03$).

In unadjusted analysis, individuals in the least insulin sensitive quintile of HOMA-%S were more than twice as likely to experience a CVD event as those in the most insulin sensitive quintile (Table 2). Adjustment for age, sex, glucose and other metabolic covariates only moderately influenced this relationship (Tables 2 and 3). Marked attenuation between HOMA-%S and CVD was observed after adjusting for HDL (age- and sex-adjusted p for trend = 0.04 [Table 2]; multivariate adjusted p for trend = 0.14 [Table 3]).

Similar results were found when HOMA-%S was modelled as a continuous variable. The fatal or non-fatal CVD HRs (95% CI, p-value) per SD decrease of log-HOMA-%S were 1.36 (1.20–1.54, $p< 0.01$) in an unadjusted model and 1.30 (1.14–1.49, p<

0.01) after adjusting for age and sex. Although attenuated, the relationship remained significant after adjusting for previous history of CVD, smoking, hypertension (blood pressure ≥ 140/90 mmHg or self-reported antihypertensive medication use), total cholesterol, self-reported lipid-lowering medication use, and waist-to-hip ratio (1.24 [1.08–1.43, p< 0.01]), but the relationship became non-significant after further adjustment for HDL (1.15 [1.00–1.34, p=0.06]).

FPG (p< 0.01), but not 2hPG (p= 0.43) nor HbA_{1c} (p= 0.13), significantly improved the prediction of CVD over and above HOMA-%S and covariates (Table 4). Adding quintiles of HOMA-%S to multivariate models with FPG, 2hPG or HbA_{1c} did not improve the prediction of CVD (p > 0.6 for all). Multivariate models which included both HOMA-%S and plasma glucose measures showed that the association between CVD and FPG was of borderline significance (HR=3.6 [95% CI 1.5–8.5, p< 0.01] per SD [0.7mmol/l] decrease for FPG < 5.1 mmol/l and 1.1 [1.0–1.2, p=0.05] per SD increase for FPG ≥ 5.1 mmol/l), whereas 2hPG and HbA_{1c} were not significantly associated with CVD when modelled with HOMA-%S (HR=1.0 [95% CI 0.9–1.2, p=0.42] per SD [2.2 mmol/l] increase for 2hPG; and HR=1.1 [95% CI 1.0–1.2, p=0.10] per SD [0.4 mmol/l] increase for HbA_{1c}).

No significant interactions were observed between HOMA-%S (in quintiles) and age, sex, BMI, waist-to-hip ratio, history of CVD or abnormal glucose tolerance status for CVD (all p> 0.05). Results were essentially unchanged when we repeated these analyses excluding people with newly diagnosed diabetes, or by replacing HOMA-%S with fasting insulin or after excluding individuals with self-reported myocardial infarction or stroke at baseline. Adding education level or physical activity did not alter the association between HOMA-%S and CVD (data not shown).

Discussion

To the best of our knowledge, this is the largest population-based prospective study of men and women to examine the relationship between HOMA-%S and CVD and all-cause mortality, accounting for a broad range of CVD risk factors including previous history of CVD, smoking, hypertension, total cholesterol, lipid-lowering medication use, dyslipidaemia, and obesity. We found that declining insulin sensitivity was associated with five-year fatal or non-fatal CVD events, but not with

all-cause mortality. The relationship between HOMA-%S and CVD events was largely explained by other CVD risk factors, in particular HDL, which significantly attenuated the association between HOMA-%S and CVD, rendering it non-significant. Furthermore, only FPG significantly improved the prediction of CVD beyond that achieved by HOMA-%S and other CVD risk factors. There were no significant interactions between HOMA-%S and age, sex, glucose tolerance categories, history of CVD or obesity.

Whether insulin resistance directly leads to CVD, or whether it has an indirect deleterious effect via other metabolic abnormalities is the subject of debate. Findings from insulin clamp studies are limited, as although decreasing insulin sensitivity has been shown to be independently associated with an increased risk of CVD [9, 10], one study only used a surrogate CVD outcome (carotid artery intimal-medial thickness) [9], and the other study was based on older men [10]. Several prospective studies using surrogate measures of insulin resistance have shown that the metabolic syndrome significantly attenuates the relationship between HOMA-IR and CVD [27-29], supporting the notion of an indirect link between insulin resistance and CVD through other metabolic pathways [39]. However, it is not possible to determine from these studies which metabolic factor had the strongest influence, as the metabolic syndrome was only considered in its entirety. Our study is one of the few epidemiological investigations [24, 26] to assess the relative impact of several metabolic risk factors on the association between insulin resistance as measured with HOMA, and CVD. Our findings extend the work of previous studies [24, 26] because we have specifically examined the individual impact of hyperglycaemia, hypertension, dyslipidaemia, and obesity on the association between insulin resistance and CVD, whereas previous studies adjusted for more than one factor simultaneously [24, 26].

The findings from this study suggest that HDL may be an important confounder of the association between insulin resistance and CVD. Although insulin resistance and dyslipidaemia (high triacylglycerol and low HDL) are characteristic of type 2 diabetes [40], individuals with diagnosed diabetes were excluded in this analysis, suggesting that insulin resistance and dyslipidaemia may also play a role in the development of CVD in the general population. However, we do not have sufficient evidence to conclude that insulin resistance causes a deterioration in HDL or whether

HDL has a detrimental impact on insulin resistance. It is possible that other underlying factors are responsible for the development of both insulin resistance and abnormal HDL metabolism. One theory suggests that obesity may be a common antecedent, as it leads to an increase in circulating free fatty acids, which in turn have a deleterious effect on insulin resistance and associated hepatic lipoprotein abnormalities, including a reduction in HDL [41]. Indeed, central obesity (as measured by waist circumference) has been shown to be more strongly associated with and precede the development of metabolic abnormalities, than is insulin resistance measured by HOMA-%S [42].

Nevertheless, other studies have shown HOMA [24, 26] and hyperinsulinaemia [18, 43, 44] to be significantly associated with CVD, despite adjusting for HDL and other metabolic factors. However, all but two [43, 44] of these studies used a non-specific insulin assay which cross-reacts with proinsulin [18, 24, 26]. As proinsulin may be a better predictor of CVD than fasting insulin or HOMA [10, 30], the association between true insulin and CVD may have been concealed in these previous studies. Moreover, the findings from the two studies that did use a specific insulin assay may not be generalisable to the general population as one was conducted in older women [44] and the other only included men and measured non-fasting insulin [43].

Many studies have reported on the CVD risks associated with non-diabetic hyperglycaemia and insulin resistance, but few have compared the relative associations of these conditions with CVD in the same study population, and results are inconsistent. Several studies [45-47], including a meta-analysis [18], have reported a significant association between hyperinsulinaemia and CVD events after adjusting for glucose. However, these associations may have been the result of residual confounding, as none adjusted for HDL or waist circumference, factors known to coexist with insulin resistance which also increase the likelihood of CVD [7]. Furthermore, although some studies have found blood glucose to be a better predictor of CVD than hyperinsulinaemia [48] or HOMA [29], others have not [43, 44]. However, these two studies were conducted in particular populations: older women [44] and middle-aged men [43]. In our study, prediction of CVD events significantly improved with the addition of FPG, but not HOMA-%S to models adjusted for age, sex and other CVD risk factors. This suggests that in the general

population, FPG and other CVD risk factors, rather than HOMA-%S, may be the important targets for treatment to prevent the development of CVD.

The following limitations need to be considered when interpreting these results. First, as this is a large population-based study, it was not feasible to measure insulin sensitivity directly with an insulin clamp. Instead, insulin sensitivity was estimated with HOMA-%S. Consequently, the non-significant findings in this study may be attributed to HOMA-%S not precisely measuring true insulin sensitivity, as HOMA has been shown to be only moderately correlated (r=0.5 to 0.8) with clamp-measured insulin sensitivity [22, 49] (personal communication: B Balkau). Thus, further research using the insulin clamp is required to confirm our findings. Second, we have not been able to account for the influence of intra-individual variation in HOMA-%S measurements, as they were based on only one fasting glucose and insulin result from each individual. Although findings from large datasets have shown that measurements based on a single sample yield similar results to those based on multiple samples [22]. Third, this study examined the risk of CVD and mortality over a five year period. Several studies have demonstrated that the relationship between insulin resistance (as estimated with hyperinsulinaemia) and CVD becomes weaker with time [50], and therefore we need to wait for longer follow-up to ascertain whether these findings are sustained. Finally, the generalisability of this study to the Australian population may have been affected by the estimated baseline response rate of 37%.

In conclusion, this is the largest prospective population-based study of men and women to evaluate the association between HOMA-%S and CVD after adjusting for a wide range of CVD risk factors, including hyperglycaemia and several metabolic covariates. It is also one of the few studies to use a specific insulin assay to measure serum insulin levels. We found FPG to be a better predictor of CVD than HOMA-%S. Over a five year period there was no association between HOMA-%S and all-cause mortality, and only a modest relationship between HOMA-%S and fatal or non-fatal CVD events which was mainly explained by the clustering of other risk factors, in particular HDL. These findings are consistent with insulin resistance playing an indirect role in the development of CVD, although whether insulin resistance is the cause of other metabolic abnormalities is debatable, and requires further research.

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Duality of Interest

The authors declare that there is no duality of interest associated with this manuscript.

Table 1. Baseline characteristics according to quintiles of HOMA-%S in individuals without diagnosed diabetes (n=8533) – the AusDiab study

Baseline characteristics	Quintiles of HOMA-%S					Total
	1	2	3	4	5	
HOMA-%S	> 74.4	74.4–59.1	59.0–48.5	48.4–36.9	< 36.8	53 (30)[a]
Age, years	52 (12)	54 (12)	56 (13)	56 (12)	55 (12)	54 (12)
Male	691 (41)	694 (41)	772 (46)	829 (48)	847 (50)	3833 (45)
Smoking						
-ex-smoker	478 (28)	493 (29)	500 (30)	550 (32)	583 (34)	2604 (31)
-current smoker	323 (19)	256 (15)	231 (14)	206 (12)	220 (13)	1236 (15)
History of CVD[b]	110 (6)	113 (7)	153 (9)	165 (10)	177 (10)	718 (8)
Hypertension[c]	373 (22)	457 (27)	586 (35)	708 (41)	883 (52)	3007 (35)
Systolic blood pressure, mmHg	124 (18)	127 (18)	131 (18)	133 (19)	137 (18)	131 (19)
Diastolic blood pressure, mmHg	67 (12)	69 (12)	71 (11)	72 (12)	75 (12)	71 (12)
Total cholesterol, mmol/l	5.5 (1.1)	5.7 (1.0)	5.8 (1.0)	5.9 (1.1)	5.9 (1.1)	5.8 (1.1)
HDL, mmol/l	1.6 (0.4)	1.6 (0.4)	1.5 (0.4)	1.4 (0.3)	1.2 (0.3)	1.4 (0.4)
Triacylglycerol, mmol/l[a]	1.0 (0.6)	1.1 (0.8)	1.3 (0.9)	1.5 (1.1)	1.9 (1.3)	1.3 (1.0)
Lipid-lowering medication use	74 (4)	122 (7)	166 (10)	174 (10)	214 (13)	750 (9)
Waist-to-hip ratio	0.83 (0.8)	0.84 (0.8)	0.90 (0.8)	0.90 (0.9)	0.92 (0.8)	0.90 (0.9)
BMI, kg/m^2	24 (4)	25 (4)	27 (4)	28 (4)	31 (5)	27 (5)
FPG, mmol/l[a]	5.1 (0.5)	5.3 (0.6)	5.4 (0.6)	5.5 (0.6)	5.8 (0.9)	5.4 (0.7)
2hPG, mmol/l[a]	5.4 (1.9)	5.6 (1.9)	5.8 (2.0)	6.2 (2.1)	7.0 (2.9)	5.9 (2.2)
HbA_{1c}%[a]	5.0 (0.3)	5.0 (0.3)	5.1 (0.3)	5.2 (0.4)	5.3 (0.4)	5.1 (0.4)
NGT	1480 (87)	1442 (84)	1331 (79)	1211 (70)	842 (49)	6306 (74)
IFG	38 (2)	64 (4)	107 (6)	151 (9)	212 (12)	572 (7)
IGT	154 (9)	175 (10)	205 (12)	294 (17)	415 (24)	1243 (15)
NDM	29 (2)	31 (2)	45 (3)	67 (4)	240 (14)	412 (5)

Differences across quintiles of HOMA-%S were significant for all variables ($p< 0.01$).
Data are n (%) or mean (SD)
[a]Data are median (inter-quartile range)
[b]History of cardiovascular disease (CVD) includes self-reported angina, myocardial infarction or stroke
[c]Hypertension defined as blood pressure ≥ 140/90 mmHg or self-reported antihypertensive medication use

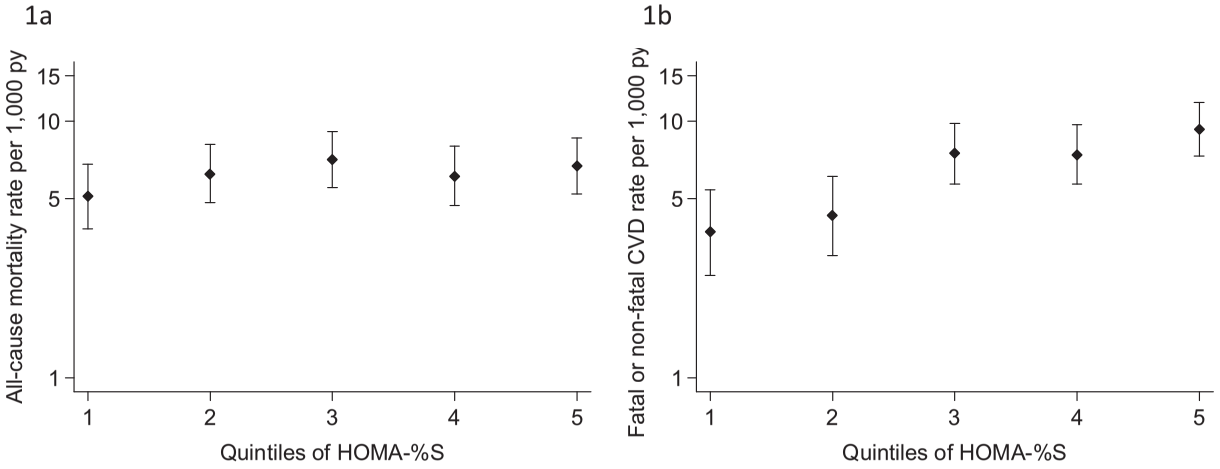

Figure 1 – Unadjusted rates for all-cause mortality and fatal or non-fatal cardiovascular disease (CVD) events according to quintiles of homeostasis model assessment of insulin sensitivity (HOMA-%S) in individuals without diagnosed diabetes – the AusDiab study

Table 2. Unadjusted and age- and sex-adjusted risk (HR and 95% CI) of fatal or non-fatal CVD events according to quintiles of HOMA-%S in individuals without diagnosed diabetes (n=6942) – the AusDiab study

	Quintiles of HOMA-%S					P for trend
	1	2	3	4	5	
HOMA-%S	> 74.6	74.6–59.3	59.2–48.7	48.6–37.2	< 37.1	
CVD events n (%)	26 (2.0)	30 (2.2)	52 (3.8)	52 (3.7)	65 (4.7)	225 (3.2)
Unadjusted	1.0	1.2 (0.7–2.0)	2.0 (1.3–3.2)*	2.0 (1.3–3.2)*	2.5 (1.6–3.9)*	< 0.01
Age + sex	1.0	1.1 (0.6–1.9)	1.4 (0.9–2.3)	1.6 (1.0–2.5)	2.0 (1.3–3.1)*	< 0.01
+ FPG[a]	1.0	1.1 (0.7–2.0)	1.5 (0.9–2.4)	1.6 (1.0–2.7)*	2.0 (1.2–3.2)*	< 0.01
+ 2hPG[a, b]	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.9 (1.2–2.9)*	< 0.01
+ HbA_{1c}[a]	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.2)	1.5 (0.9–2.4)	1.8 (1.1–2.8)*	< 0.01
+ glucose metabolism status[c]	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.7 (1.1–2.8)	0.01
+ history of CVD	1.0	1.2 (0.7–2.0)	1.6 (1.0–2.5)	1.6 (1.0–2.6)*	2.1 (1.3–3.3)*	< 0.01
+ smoking	1.0	1.1 (0.7–1.9)	1.5 (0.9–2.3)	1.6 (1.0–2.5)*	2.0 (1.3–3.2)*	< 0.01
+ hypertension	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.2)	1.5 (0.9–2.3)	1.7 (1.1–2.8)*	0.01
+ SBP and DBP	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.2)	1.5 (0.9–2.4)	1.8 (1.1–2.8)*	< 0.01
+ lipid-lowering therapy and TC	1.0	1.1 (0.6–1.8)	1.4 (0.8–2.2)	1.5 (0.9–2.4)	1.9 (1.2–2.9)*	< 0.01
+ triacylglycerol	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.2)	1.4 (0.9–2.3)	1.7 (1.1–2.8)*	0.01
+ non-HDL	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.9 (1.2–3.0)*	< 0.01
+ HDL	1.0	1.0 (0.6–1.8)	1.3 (0.8–2.1)	1.3 (0.8–2.1)	1.5 (1.0–2.5)	0.04
+ WHR	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.9 (1.2–3.0)*	< 0.01
+ BMI	1.0	1.1 (0.6–1.8)	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.9 (1.1–3.1)*	0.01

[a]FPG (modelled as a linear spline with a knot at 5.1 mmol/l) and HbA_{1c} and 2hPG modelled as linear terms
[b]Models based on 6933 participants with non-missing 2hPG values. Quintiles based on this sub-cohort
[c]Abnormal glucose metabolism status based on 1999 World Health Organisation criteria [35]
FPG – fasting plasma glucose; 2hPG – 2 h plasma glucose, HbA_{1c} – Haemoglobin A_{1c}; SBP – systolic blood pressure; DPB – diastolic blood pressure; TC – total cholesterol; HDL – high density lipoprotein cholesterol; WHR – waist-to-hip ratio; BMI – body mass index

Table 3. Multivariate adjusted risk (HR and 95% CI) of fatal or non-fatal CVD events according to quintiles of HOMA-%S in individuals without diagnosed diabetes (n=6942) – the AusDiab study

									Quintiles of HOMA-%S					P for trend
									1	2	3	4	5	
		HOMA-%S							> 74.6	74.6–59.3	59.2–48.7	48.6–37.2	< 37.1	
		CVD events n (%)							26 (2.0)	30 (2.2)	52 (3.8)	52 (3.7)	65 (4.7)	225 (3.2)
Age, sex, history of CVD[a] and smoking									1.0	1.2 (0.7–2.0)	1.6 (1.0–2.5)	1.6 (1.0–2.6)*	2.1 (1.3–3.3)*	< 0.01
	+	SBP and DBP							1.0	1.2 (0.7–2.0)	1.5 (0.9–2.3)	1.5 (1.0–2.5)	1.9 (1.2–3.0)*	< 0.01
	+	Hypertension							1.0	1.2 (0.7–2.1)	1.5 (0.9–2.4)	1.6 (1.0–2.5)	1.9 (1.2–3.0)*	< 0.01
			+	WHR					1.0	1.2 (0.7–2.1)	1.5 (0.9–2.4)	1.5 (1.0–2.5)	1.9 (1.2–3.0)*	0.01
			+	BMI					1.0	1.2 (0.7–2.1)	1.5 (0.9–2.4)	1.5 (1.0–2.5)	1.9 (1.1–3.1)*	0.01
			+	LLT and TC					1.0	1.2 (0.7–2.0)	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.8 (1.1–2.9)*	0.01
	+	Hypertension	+	LLT and TC	+	Triacylglycerol			1.0	1.2 (0.7–2.0)	1.4 (0.9–2.3)	1.5 (0.9–2.4)	1.7 (1.1–2.8)*	0.01
					+	Non-HDL[b]			1.0	1.2 (0.7–2.0)	1.4 (0.9–2.2)	1.5 (0.9–2.3)	1.7 (1.1–2.7)*	0.01
					+	HDL			1.0	1.1 (0.7–1.9)	1.3 (0.8–2.1)	1.3 (0.8–2.1)	1.4 (0.9–2.3)	0.14
		Hypertension	+	LLT and TC	+	HDL	+	WHR	1.0	1.2 (0.7–2.0)	1.3 (0.8–2.1)	1.3 (0.8–2.2)	1.5 (0.9–2.4)	0.12
	+						+	Glucose tolerance status[c]	1.0	1.1 (0.7–1.9)	1.3 (0.8–2.0)	1.3 (0.8–2.1)	1.3 (0.8–2.1)	0.32

*p< 0.05

[a]History of previous cardiovascular disease (CVD) includes self-reported angina, myocardial infarction or stroke

[b]Model does not include total cholesterol as non-HDL and total cholesterol are highly correlated (r=0.93)

[c]Abnormal glucose metabolism status based on 1999 World Health Organisation criteria [35]

SBP – systolic blood pressure; DBP – diastolic blood pressure; WHR – waist-to-hip ratio; BMI – body mass index; LLT – lipid-lowering medication use; TC – total cholesterol; HDL – high density lipoprotein cholesterol..

Table 4. Risk (HR and 95% CI) of fatal or non-fatal CVD events according to quintiles of HOMA-%S and adjusted for FPG, 2hPG and HbA_{1c} in individuals without diagnosed diabetes (n=6942) – the AusDiab study

HOMA-%S quintiles	Multivariate-adjusted[a]	Additionally adjusted for FPG[a, b]			Additionally adjusted for 2hPG[a, c, d]			Additionally adjusted for HbA_{1c}[a, d]		
	HR (95% CI)	HR (95% CI)	Likelihood ratio test		HR (95% CI)	Likelihood ratio test		HR (95% CI)	Likelihood ratio test	
			χ^2	P-value		χ^2	P-value		χ^2	P-value
> 74.6	1.0	1.0	11.8	< 0.01	1.0	0.64	0.43	1.0	2.34	0.13
74.6–59.3	1.2 (0.7–2.0)	1.2 (0.7–2.0)			1.1 (0.7–1.9)			1.1 (0.7–1.9)		
59.2–48.7	1.3 (0.8–2.1)	1.4 (0.9–2.2)			1.3 (0.8–2.1)			1.3 (0.8–2.1)		
48.6–37.2	1.3 (0.8–2.2)	1.4 (0.9–2.3)			1.3 (0.8–2.1)			1.3 (0.8–2.1)		
< 37.1	1.5 (0.9–2.4)	1.5 (0.9–2.5)			1.4 (0.9–2.4)			1.4 (0.8–2.3)		
P for trend	0.12	0.11			0.15			0.21		

[a]Adjusted for age, sex, history of CVD (angina, myocardial infarction or stroke), smoking (never smoked, ex-smoker, current smoker), hypertension (blood pressure ≥ 140/90 mmHg or self-reported antihypertensive use), total cholesterol, high-density lipoprotein cholesterol, lipid-lowering medication use, and waist-to-hip ratio
[b]FPG added to model as a linear spline with one knot at 5.1 mmol/l
[c]Models based on 6933 participants with non-missing 2hPG values. Quintiles based on this sub-cohort
[d]2hPG and HbA_{1c} added to model as continuous variables
2hPG – two-hour post-load plasma glucose; CVD – cardiovascular disease; FPG – fasting plasma glucose; HbA_{1c} – Haemoglobin A_{1c}

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CHAPTER 9

Discussion and Conclusions

9.1 Strengths of the study

9.1.1 Sample size

The studies presented in this thesis are based on the AusDiab study, which comprises a random sample of 11,247 men and women. AusDiab is the largest national population-based longitudinal cohort study conducted in Australia to assess the impact of diabetes and its related risk factors and conditions. Therefore, this thesis provides the first large-scale Australian data to show that both diabetes and intermediate hyperglycaemia are important independent predictors of premature mortality and CVD events in men and women. Moreover, as data from the AusDiab study are based on a contemporary population, with baseline testing performed in 1999 to 2000, findings from this thesis substantially add to the international literature in this area, as several of the other important studies assessing the relationship between glucose and mortality and CVD have been conducted on populations where data collection occurred more than 20 years ago [17, 18, 20].

9.1.2 Longitudinal cohort

The longitudinal study design of the AusDiab study meant that all baseline risk factors, including blood glucose measurements, were determined before the occurrence of CVD events and death, which, with reference to the ascertainment of non-fatal CVD events, removed the influence of recall bias. Additionally, this study design also allowed for the assessment of the temporal relationship between baseline abnormal glucose tolerance and all-cause and CVD outcomes. Although this thesis is based on a median of five to six years of follow-up, the benefit of examining the relationship between abnormal glucose metabolism and premature mortality and CVD events over a relatively short period is that it reduces the likelihood that the

associations observed can be explained by individuals converting to diabetes during the follow-up period.

9.1.3 Oral glucose tolerance test

One of the most important strengths of this thesis is the use of an OGTT. This is the gold standard for determining glucose tolerance status, as it measures both FPG and 2hPG levels [15, 16], and it has been shown that individuals can have elevated glucose levels on one test but not the other, which may lead to misclassification of glucose tolerance status [139]. Moreover, several studies have indicated that the risks for CVD and premature mortality may be different for 2hPG and FPG. As the AusDiab study is the single largest study within the developed world (and the first national study in Australia) to assess the relationship between abnormal glucose metabolism and premature mortality and CVD using an OGTT, the findings reported in this thesis substantially add to the body of research in this area. Additionally, this thesis is one of the few studies [43-46] world-wide to report on the mortality and CVD risks associated with three different measures of blood glucose, namely FPG, 2hPG and HbA_{1c} within the same study population.

9.1.4 High quality mortality and CVD outcomes data

The mortality and CVD outcomes data in this thesis are of high quality because: (i) the mortality follow-up was almost complete (99%), (ii) the non-fatal CVD outcomes were independently adjudicated using medical record information, and (iii) the methods used for adjudication of the non-fatal CVD events were validated against a hospital morbidity database through record linkage. Furthermore, to minimise loss to follow-up and improve the accuracy of mortality classification, the following steps were taken. Firstly, staff members of the epidemiological team attempted to make annual contact with all AusDiab participants through postal surveys and telephone calls to maintain an up-to-date database of contact details of both participants and next-of-kin. Secondly, only high quality matches to the NDI were accepted, where the full name and date of birth for the AusDiab participant corresponded to the NDI match. Thirdly, all matches to the NDI were verified where possible with direct communication with the next-of-kin and/or local medical officer of the participant.

9.2 Limitations

The major findings of the studies presented in this thesis need to be interpreted within the context of several limitations which are outlined below. Although each of the papers included in this thesis discusses specific limitations related to each study, the purpose of the discussion below is to provide an overview of the key limitations, and how these could have possibly influenced the major findings presented in this thesis.

9.2.1 Response rates and loss to follow-up

The overall baseline participation rate for AusDiab was estimated to be 37% of those eligible for the study, and as explained in chapter 3, there were some differences between responders and non-responders. Although these differences indicate that the findings from AusDiab may not be fully representative of the whole Australian population, any selection bias would have been unlikely to affect the strength of the relationships between abnormal glucose metabolism and all-cause mortality and CVD events as reported in chapters 5 to 8.

Follow-up for all-cause and CVD mortality was almost complete, with only a small number (n=6) of participants known to have died not being matched to the NDI. However, as a unique identifying number is not available in Australia for data linkage, linkage to the NDI had to be based on personal identifiers such as name and date of birth, and therefore, misclassification of death status and cause of death was a possibility.

The ascertainment of non-fatal CVD events was less complete. Of the 11,247 AusDiab participants, 10,788 were eligible to participate in the 2004–2005 five-year follow-up AusDiab survey, and 8,802 (82%) completed questions on past history of CVD events. Furthermore, of the 330 participants who reported a CVD event, the events of 54 participants could not be adjudicated because: (i) the participant did not provide consent to have their medical records reviewed (n=36), (ii) medical records could not be obtained (n=13), or (iii) the events occurred in a hospital outside of Australia (n=5). As a consequence, 2,040 (19%) of those eligible to participate in the

CVD questionnaire were lost to follow-up. As outlined in chapter 4, compared to the non-responders, responders to the CVD questionnaire were significantly more likely at baseline to be female (55.6 vs. 55.4%), be older (51.5 vs. 48.1 years), have completed 12 or more years of school (57.6 vs. 52.4%), report higher income (A$485 vs. A$446 per week) and speak English at home (96.6 vs. 93.2%). It is unlikely however, that this source of selection bias resulted in an overestimate of the strength of the associations between abnormal glucose metabolism and all-cause and CVD mortality reported in this thesis. Indeed, the findings outlined in chapters 7 and 8 may have been influenced by survivor bias, as non-fatal CVD events could not be ascertained for people who died between the baseline and five-year follow up surveys. This source of selection bias may have *weakened* the associations between abnormal glucose metabolism and CVD events (chapter 7), or HOMA-%S and CVD events (chapter 8), as participants with more severe metabolic abnormalities may have died during the follow-up period. However, in order to minimise the impact of survivor bias, the outcome measures used in both these studies combined fatal and non-fatal CVD events.

9.2.2 Measurement error and misclassification

Blood glucose measurements in the studies outlined in chapters 5 to 8 were all based on a single OGTT, and as these measures are subject to intra-individual variation [140, 141], this may have introduced some imprecision and regression dilution bias, leading to misclassification of glucose tolerance status and therefore underestimation of the relative risk of mortality and CVD events in these studies. Moreover, it has been shown that 2hPG is more variable than FPG [141], which may explain the weaker associations that were observed between IGT and CVD events in the studies outlined in chapters 5 and 7, and between the continuous measure of 2hPG and CVD mortality in chapter 6. It is possible to account for measurement variability by taking the average of more than one measure. Although this was undertaken for other baseline measures in AusDiab, including blood pressure and waist circumference, this was not a practical option for glucose measurement, as repeat OGTTs need to be conducted on separate days making the procedure very time consuming and inconvenient to the participant. Nevertheless, the WHO does recognise that a single OGTT is suitable for evaluating glucose tolerance status in epidemiological surveys

[16]. Similarly, insulin measures are also subject to intra-individual variability, which may have affected the reliability of the HOMA-%S measurement. However, measurement imprecision may have been greater for HOMA-%S than for blood glucose, as one study has demonstrated the specific insulin variability is 1.5 to 2 times higher than 2hPG concentrations [140]. This may have explained the weaker association between HOMA-%S and CVD events, compared to FPG and CVD events.

Misclassification of vital status and CVD deaths could have occurred as these data were obtained from the NDI which is based on death certificate information. A previous Australian study has shown that the ascertainment of vital status and CVD deaths through the NDI is robust, with the sensitivity and specificity for the identification of deaths being 93.7% and 100%, respectively, and the sensitivity and specificity for CVD deaths being 92.5% and 89.6%, respectively [134]. However, this validation study was derived from a randomised controlled trial in which CVD deaths were closely monitored, and as such the results may slightly overstate the accuracy of the NDI when applied to an epidemiology study of the general population.

At follow-up, non-fatal CVD events were ascertained by adjudicating self-reported events with medical record review. Although this method is able to verify the accuracy of a reported event, it is not able to determine whether or not an event occurred when a participant reported not having an event. Thus, it was possible that misclassification of non-fatal CVD outcomes could have introduced some imprecision and regression dilution bias to the findings reported in chapters 7 and 8. However, the study in chapter 4 demonstrated that the influence of this type of misclassification on non-fatal CVD outcomes would have been minimal, because comparison of self-reported events to a statewide hospital morbidity database in Western Australia revealed that only 0.2% of those reporting that they had not had an event were found to actually have an event.

9.2.3 Insufficient control for confounding factors

All multivariate analyses in the studies reported in chapters 5 to 8 adjusted for baseline history of CVD (angina, myocardial infarction or stroke) as this variable was found to be a strong confounder of the relationship between abnormal glucose metabolism and CVD. However, misclassification of baseline CVD could have occurred because data were based on self-report. This may have increased the likelihood of residual confounding, potentially leading to an overestimation of the strength of the relationship between abnormal glucose metabolism and CVD events. However, it has been shown that self-reported myocardial infarction [142-144], stroke [142-145] and ischaemic heart disease [143] are reasonably accurate in determining disease status. Furthermore, validation of self-reported CVD events in AusDiab, as outlined in chapter 4, demonstrated that self-reported myocardial infarction and stroke events in this study provided a reasonable indicator of CVD. Nonetheless, to account for any potential measurement error related to self-reported history of CVD events at baseline, other covariates associated with CVD, such as age, sex, hypertension, lipids, lipid-lowering medication use, and body composition were also included in the multivariate models.

Despite adjustment for these CVD covariates, it is possible however, that the associations between abnormal glucose metabolism and CVD events as reported in the studies in chapters 5 to 8 may have still been influenced by residual confounding by unknown or unmeasured factors. Moreover, the findings from these studies were unable to ascertain whether or not abnormal glucose metabolism is directly associated with these outcomes, or whether it is simply a marker of another underlying pathological factor not measured in the AusDiab study. It has been suggested that both abnormal glucose metabolism and CVD could be associated with some common antecedents, such as inflammatory markers and genetic factors, that singularly or together give rise to both conditions simultaneously [146, 147]. However, as these factors were not measured in the AusDiab study, it was not possible to test this theory.

9.2.4 Relatively short follow-up period

The findings from this thesis are based on a median follow-up period of five to six years. Although longer follow-up of the AusDiab study is planned, for this thesis it was not possible to examine the associations between abnormal glucose metabolism and mortality and CVD events over a longer period because only a median of five to six year mortality and CVD follow-up was available at the time the research was conducted.

The limitation of analysing data over a shorter timeframe is that there are fewer outcomes with which to conduct stratified analyses. Although the studies in this thesis assessed whether the observed relationships between abnormal glucose metabolism and premature mortality and CVD varied according to sex, age and previous history of CVD, some of these analyses were based on small numbers of outcomes, which reduced the precision of the results. The ascertainment of more outcomes with longer follow-up of the AusDiab cohort will permit a more thorough investigation into the risks of CVD and mortality outcomes among specific population groups.

Another limitation that relates to both the short follow-up time and measurement of risk factors at one baseline time point is that this study design does not permit investigations into the impact of glucose homeostasis over a longer time period. This limitation may have important implications for the study in chapter 8, as some studies have demonstrated that the relationship between hyperinsulinaemia (a marker of insulin resistance) and CVD becomes weaker over time [86, 107], and thus, the associations observed between HOMA-%S and CVD events in this study may be further attenuated with longer follow-up.

9.2.5 Generalisability

As mentioned in section 3.2.1, the AusDiab study only included men and women aged 25 years and over, and excluded people residing in institutions. Additionally, CCDs containing fewer than 100 persons aged 25 years and over, CCDs classified as 100% rural according to the 1996 census, or CCDs that comprised more than 10% indigenous population were excluded from the AusDiab sampling frame. Moreover,

comparison between responders and non-responders to the baseline AusDiab survey revealed that responders were more likely to have been born in the United Kingdom and speak English at home. Consequently, the findings from this thesis are only generalisable to community-dwelling men and women aged 25 years and over, and the findings cannot be applied to Aboriginal and Torres Straight Islanders, Australians living in remote areas or to Australians from other ethnic backgrounds.

9.3 Overview of major findings

9.3.1 Self-reported CVD events tend to overestimate the actual CVD event rate in epidemiological surveys

Epidemiological surveys often rely on questionnaires to measure the incidence and prevalence of CVD, as this offers a simple convenient method with which to collect data on a large number of participants. In the AusDiab study, as outlined in chapters 3 and 4, information was collected on CVD events during the 2004–2005 follow-up examination in order to ascertain how many participants experienced a non-fatal CVD event over the five-year period between the baseline and follow-up examinations. Although some studies have shown good concordance between self-reported CVD events and medical records or hospital diagnostic codes [135, 142, 143, 148, 149], it could not be assumed that these findings would necessarily apply to the Australian setting, as it has been shown that the level of accuracy of self-reported CVD events can depend on the participant characteristics [135, 142, 145, 148-150] and questionnaire method employed [151].

The study described in chapter 4 outlined the results of a study that assessed the accuracy of self-reported myocardial infarction, stroke, PTCA and CABG in the AusDiab study. It showed that compared to either medical record adjudication or linkage to a hospital morbidity database, self-reported CVD events tended to be overestimated, with the level of misclassification being the highest for stroke, then followed by myocardial infarction and PTCA, with no misclassification for CABG. However, further examination of the misclassifications revealed that participants tended to confuse CVD events with related CVD conditions, such as myocardial

infarction with angina, stroke with transient ischaemic attack and PTCA with coronary angiography only, which is consistent with previous reports [135, 145, 152, 153]. This is an important finding because it means that if a questionnaire specifically asks about myocardial infarction or stroke occurrence, the outcome is likely to measure CHD or cerebrovascular disease, respectively, though some of the responses will have actually been angina not myocardial infarction, and transient ischaemic attack not stroke. This suggests that the interviewer-administered questionnaire utilised in the baseline AusDiab survey was a reasonably valid tool for obtaining information on CVD events, particularly when the CVD events are considered as a composite outcome. The findings of this study therefore support the use of self-reported CVD as a baseline covariate in the multivariate analyses conducted in chapters 5 to 8.

Another important finding from the study in chapter 4 is that there was a high level of concordance between the verification of self-reported CVD events using medical record abstraction and adjudication, and hospital discharge diagnoses stored on a statewide hospital morbidity database, particularly for PTCA and CABG. In addition, very few CVD events were detected on the hospital morbidity database that were not self-reported. These findings provide good evidence for the validity of the adjudicated CVD event data used in chapters 7 and 8, which combined both non-fatal and fatal CVD outcomes.

9.3.2 Impaired glucose metabolism is an important independent risk factor for CVD and all-cause mortality over five years

Some Australian studies have assessed the association between diabetes and CVD and premature mortality [9-14], but as outlined in chapter 1, most of these studies did not use an OGTT to evaluate the impact of all degrees of impaired glucose metabolism. It is important to extend the evaluation of CVD and premature mortality risk beyond that of diabetes, as recent Australian data shows that known diabetes only accounts for 16% of the prevalence of all forms of abnormal glucose metabolism [5]. In this thesis, glucose tolerance status was assessed with both FPG and 2hPG measures after a 75 gm OGTT, and therefore, data from the studies outlined in chapters 5 to 8 represent the first national Australian data on the impact of both previously known and screen detected diabetes, as well as intermediate

hyperglycaemia on CVD events and all-cause mortality. Overseas studies have shown that the risk for CVD and all-cause mortality is increased in people with intermediate hyperglycaemia, with the most important data being derived from five meta-analyses [17-21]. However, as discussed in chapter 2, the various limitations of these studies, which include the component studies using different blood sample types (e.g. whole blood or plasma) and different glucose assays [17-19], the failure of these studies to fully adjust for concomitant CVD risk factors [17, 19], the lack of data on women [17, 18], and the component studies using data collected up to 20 years ago [17-19] make it difficult to generalise these findings to a contemporary Australian population. Therefore, the findings from this thesis also have important implications for the wider body of work in this area.

In chapter 5, the risk of CVD and all-cause mortality was assessed in individuals with IFG, IGT, NDM and KDM compared to those with NGT, and it was found that independent of age, sex, smoking, previous CVD, hypertension, dyslipidaemia, lipid-lowering medication use and obesity, KDM, IFG and IGT were significantly associated with all-cause mortality, and KDM and IFG were significantly associated with CVD mortality. Furthermore, 65% of all CVD deaths occurred in people classified as having KDM, NDM, IFG or IGT at baseline, highlighting the importance of abnormal glucose metabolism to the risk of CVD mortality in Australians. KDM was associated with a two-fold risk for all-cause and CVD mortality, consistent with previous findings [24, 154-159]. However, a significant association between NDM with all-cause or CVD mortality was not observed in this study, even though after adjustment for known risk factors, NDM was associated with a 30% increased risk (HR 1.3, 95% CI 0.9–2.0) for all-cause mortality, and an 80% increased risk (HR 1.8, 95% CI 0.9–3.6) for CVD mortality. As other longer-term studies have reported that people identified with NDM are also at significantly greater risk for both all-cause and CVD mortality [18, 28, 29], the non-significant finding reported in chapter 5 could have been due to the relatively small number of deaths among those in the NDM group (n=27) over the five-year follow-up period. Longer mortality follow-up of the AusDiab cohort will be required to further evaluate the potential all-cause mortality and CVD risks in people with NDM at baseline.

9.3.3 FPG is more strongly associated with CVD compared to 2hPG or HbA_{1c}

It was outlined in chapter 2 that FPG, 2hPG and HbA_{1c} reflect different glycaemic metabolic processes [22], which could potentially lead to different associations with mortality and CVD. In chapter 5, it was shown that compared to NGT, both IGT and IFG had a 50 to 60% greater all-cause mortality risk, but only IFG was significantly associated with an increased risk for CVD mortality after adjusting for age, sex, and other CVD covariates. Moreover, in chapter 6, the assessment of the continuous relationship between FPG, 2hPG and HbA_{1c} with all-cause and CVD mortality revealed that adding FPG to a model with 2hPG or HbA_{1c} significantly improved prediction of both all-cause and CVD mortality, whereas adding 2hPG to a model with FPG only improved prediction of all-cause mortality, but not CVD mortality. The addition of HbA_{1c} to a model with FPG did not improve either all-cause or CVD mortality prediction. This data therefore suggests that abnormal FPG is more strongly associated with CVD mortality than abnormalities in 2hPG or HbA_{1c} over a five year period.

Although some studies have reported a significant association between IFG and all-cause mortality [18] and CVD [17, 19, 21], others have shown that post-challenge blood glucose is a more important predictor of all-cause and CVD mortality than is fasting blood glucose [18, 20]. The differences between the findings outlined in this thesis and those of the DECODE and DECODA analyses could reflect different study methods. The DECODE and DECODA meta-analyses compared the IFG group to people without IFG, whereas the comparison group in the AusDiab study consisted of people who had both 'normal' FPG (< 6.1 mmol/l) and 2hPG (< 7.8 mmol/l) values, which allowed for better discrimination of mortality risk between those with IFG and NGT. Furthermore, these meta-analyses relied on different blood glucose samples (e.g. whole blood and plasma), and analysed the glucose measurements using different assays. This meant that glucose values obtained from whole blood had to be statistically manipulated prior to pooling these values with the plasma glucose results. Consequently, this could have influenced the precision of the results reported in these papers. The AusDiab study used a single assay to analyse all plasma glucose samples, and analyses were performed in a central laboratory

according to standardised criteria. These results suggest that compared to post-challenge blood glucose, abnormalities of fasting blood glucose may play a greater role in the risk of premature mortality and the development of CVD than previously recognised.

HbA_{1c} has been shown to be significantly associated with both all-cause mortality [36-39] and fatal and/or non-fatal CVD [31, 37, 39], however, none of these studies investigated whether these relationships were independent of other blood glucose measures. The study in chapter 6 showed that although HbA_{1c} was significantly associated with CVD mortality, and showed a borderline association with all-cause mortality, this relationship was attenuated with further adjustment for either FPG or 2hPG. This finding can be explained by the fact that HbA_{1c} reflects blood glucose concentration over the previous two to three months [22].

9.3.4 The nature of the relationship between blood glucose and CVD and all-cause mortality is linear for 2hPG and HbA_{1c}, but J-shaped for FPG

Although grouping individuals into either IFG and IGT categories might have practical value for classifying individuals with abnormalities in fasting glucose metabolism and post-challenge glucose metabolism, respectively [15], dividing a continuous variable into groups can mask the true relationship between a risk factor and outcome. This occurs because: (i) the risk relationship is often continuous according to the severity of the particular risk factor, (ii) people in the one group are considered to have the same risk for disease, even though their risk might vary according to their specific measurement of the risk factor, (iii) people on either side of a cut-point are artificially classified as having very different risks for the disease, when in fact their risks might be similar because they have very similar values of the risk factor, and (iv) categorising the risk factor conceals any potential non-linear relationship between the risk factor and disease [138]. Therefore, a recent joint report by the WHO and the International Diabetes Federation recommended future consideration should be given to replacing IFG and IGT with an overall risk assessment for diabetes and CVD which includes blood glucose as a continuous measure [16]. Thus, this thesis not only assessed the all-cause and CVD mortality risks associated with IFG and IGT (as undertaken in chapter 5), but expanded this evaluation to include an assessment of the continuous relationships between blood

glucose and all-cause and CVD mortality, as presented in the study outlined in chapter 6.

As discussed in chapter 2, few studies have assessed the continuous relationships between blood glucose and all-cause and CVD mortality, and findings are mixed with threshold [30, 31], continuous [21, 32] and J-shaped [33, 34] relationships being described. The study in chapter 6 showed that the risks of all-cause and CVD mortality progressively increased throughout the range of 2hPG and HbA_{1c}, whereas J-shaped relationships were observed for FPG. The linear relationship between 2hPG and all-cause mortality contrasts with the findings of other studies, which have reported a J-shaped relationship between 2hPG and all-cause mortality [33, 35]. However, the linear relationship between 2hPG and CVD mortality observed in the study outlined in chapter 6 does concur with most studies [17, 19, 35], but not all [30, 33], as J-shaped [33] and threshold relationships [30] have also been reported for post-challenge blood glucose. The observation of a linear relationship between HbA_{1c} and all-cause and CVD mortality concurs with most studies [36-39], except for one [31], which reported a threshold relationship. Finally, the J-shaped relationship between FPG and all-cause and CVD mortality as shown in chapter 6 has been reported by several other studies [33-35, 160, 161], and suggests that excessively low FPG levels are associated with elevated mortality and CVD risk. Therefore, the results presented in this thesis represent the largest single study to evaluate, in the same study population, the nature of the relationships between FPG, 2hPG and HbA_{1c} and both all-cause and CVD mortality, and confirm the findings from other studies that the relationship between blood glucose and all-cause and CVD mortality is continuous. These findings also highlight that although a reduction in CVD risk could be achieved by lowering either 2hPG or HbA_{1c}, excessive lowering of FPG below 5.1 mmol/l may increase the risk of death from all causes and CVD.

9.3.5 Blood glucose does not improve individual CVD risk identification over and above that provided by traditional CVD risk factors

CVD prevention involves targeting several modifiable CVD risk factors, including dyslipidaemia, elevated blood pressure and smoking. However, these traditional CVD risk factors together do not completely explain the risk for CVD [162], and

therefore, it is important to determine whether the addition of other risk factors to CVD prediction models, such as glucose, might improve individual CVD risk stratification. Although CVD risk scores such as Framingham include diabetes [162], it was demonstrated in chapter 2 that few studies have specifically evaluated whether glucose is able to significantly contribute to individual risk stratification over and above that provided by traditional CVD risk factors [45, 48, 52].

The study in chapter 6 presents novel findings on the role of FPG, 2hPG and HbA_{1c} in CVD risk prediction among people without diagnosed diabetes. This study showed that the ability of blood glucose to classify individual risk was only modest when traditional CVD risk factors were considered. This supports the findings of two other studies that showed neither 2hPG [45] nor HbA_{1c} [52] improved CVD risk discrimination after other CVD risk factors were considered. The phenomenon of a significant independent risk factor not substantially improving individual risk classification is, however, not uncommon, as such discrimination often requires very strong measures of association [49]. Nevertheless, among individuals without diagnosed diabetes, all three measures of blood glucose were each significantly associated with CVD mortality after adjusting for other CVD risk factors including age, sex, previous history of CVD, smoking, blood pressure, dyslipidaemia, lipid-lowering medication and abdominal obesity. Thus, these findings provide additional support for blood glucose playing an important role in the development of CVD and in characterising population CVD risk.

9.3.6 Abnormal glucose metabolism may be more strongly associated with myocardial infarction than with stroke or coronary artery revascularisation

Chapter 5 showed that compared to NGT, IFG and KDM were both significantly associated with CVD mortality, after adjusting for age, sex and CVD covariates. However, as noted in chapter 2, CVD can encompass several different outcomes, including myocardial infarction, stroke and procedures such as coronary revascularisation, and the association between hyperglycaemia and these outcomes may differ. Therefore, the aim of chapter 7 was to expand upon the analysis in chapter 5 and assess the association between glucose tolerance categories and myocardial infarction, stroke and coronary revascularisation. The findings of this

study showed that when CVD outcomes were considered separately, the risk of myocardial infarction was significantly increased for KDM, IFG, and IGT, but not NDM, whereas the risk of coronary revascularisation was only significantly increased for KDM, and no significant associations were observed for stroke.

These findings are important because very few epidemiological studies have specifically investigated the role of hyperglycaemia on the risk of different CVD outcomes. Of the studies that have, analyses are restricted to comparisons between CHD events and stroke, but findings are variable [18, 21, 56, 58, 59]. In the study outlined in chapter 7, KDM was associated with a two-fold risk of stroke of borderline significance, and neither NDM, IFG nor IGT were significantly associated with stroke. One of the reasons for the observed differences in risk for stroke and myocardial infarction in those with IFG and IGT could be explained by the possibility that hyperglycaemia needs to be more severe before it has a detrimental effect on cerebrovascular disease. However, the non-significant finding for stroke in this study could also be due to the heterogenous nature of stroke [53]. As there were too few stroke events to specifically investigate the relationship between abnormal glucose metabolism and different stroke sub-types, all stroke events were grouped together as the one outcome. This may have weakened the association between abnormal glucose metabolism and stroke because other studies have shown diabetes to be more strongly associated with certain stroke sub-types, particularly ischaemic stroke [163]. Further research is therefore required to: (i) assess the association between all levels of abnormal glucose metabolism and different stroke sub-types, and (ii) assess whether the risks associated with different stroke sub-types are different in comparison to the risks for myocardial infarction.

Little is known about the relationship between hyperglycaemia and revascularisation, as studies often combine revascularisation end-points with other CVD events, in particular myocardial infarction. Histological and physiological research shows that hyperglycaemia increases the vulnerability of atherosclerotic plaques which leads to a greater likelihood of rupture and thrombosis [3]. This suggests that people with abnormal glucose metabolism may be more likely to experience myocardial infarction than stable CHD with angina. The results in chapter 7 support this finding because IFG, IGT and KDM were found to be more strongly associated with

myocardial infarction than with coronary revascularisation. Indeed, KDM was associated with a HR of 3.9 for myocardial infarction and only a HR of 2.1 for revascularisation, and the risk differential widened once acute coronary revascularisation for the management of acute coronary syndrome was excluded in a sub-analysis. These findings imply that in order to prevent individuals with abnormal glucose metabolism experiencing a more serious or fatal CHD event, clinicians may need to intervene earlier, rather than waiting for symptoms of stable CHD with angina to develop. Alternatively, the stronger association observed between hyperglycaemia and myocardial infarction than for coronary revascularisation could also reflect differences in medical care. Clinicians may be less likely to intervene in those with diabetes, either because: (i) they consider people with diabetes to be less suitable for coronary revascularisation, or (ii) they do not adequately recognise the increased coronary risk associated with diabetes. However, further research on clinical management practices would be required to test this hypothesis.

9.3.7 Hyperglycaemia is a better predictor of CVD than insulin sensitivity as measured with HOMA-%S

Chapters 5 to 7 explored the association between abnormal glucose metabolism and all-cause mortality and CVD events. However, as discussed in chapter 2, insulin resistance may also be an important risk factor for CVD and premature mortality, yet, data from previous studies are inconsistent. The study in chapter 8, therefore, explored the relationship between HOMA-%S, a measure of insulin sensitivity (which is the reciprocal of insulin resistance), and all-cause mortality and CVD events. This study showed that HOMA-%S was not associated with all-cause mortality, and despite showing a stronger inverse relationship with CVD events, this was significantly attenuated after adjusting for HDL-C. These findings indicate that insulin resistance may have an indirect relationship with CVD through its association with dyslipidaemia, which supports the findings of several [88, 100-103], but not all studies [82-85, 87, 93, 97, 111]. The study outlined in chapter 8 extends the work of previous reports, because rather than adjusting for covariates simultaneously, it systematically evaluated the influence of each CVD risk factor on the association between HOMA-%S and CVD events. However, whether there is a direct relationship between insulin sensitivity and dyslipidaemia is uncertain, as both these

factors could simply arise independently as a result of their relationship with a common antecedent. This theory is supported by a previous study that has shown central obesity to be more strongly associated with and precede the development of metabolic abnormalities than insulin sensitivity measured by HOMA-%S [122].

Both insulin resistance and hyperglycaemia are markers of abnormal glucose homeostasis, and therefore, as noted in chapter 2, comparing the relative associations of these measures with mortality and CVD may provide some insights into the mechanisms of disease. However, as discussed in the literature review, few studies have simultaneously adjusted for both blood glucose and measures of insulin resistance, and of the studies that have evaluated this, findings are inconsistent. The study in chapter 8 showed FPG to be a better predictor of CVD than HOMA-%S. Furthermore, abnormal glucose metabolism was not a significant effect modifier of the association between HOMA-%S and CVD events. These findings, taken together with the results outlined in chapters 5 and 6, suggest that while hyperglycaemia and HOMA-%S are related to the development of CVD, FPG appears to show a stronger relationship with CVD that is independent of other traditional CVD covariates. This indicates that abnormal glucose metabolism, as measured with FPG, may play a more important role than insulin sensitivity in the development of CVD in people without diabetes

9.3.8 Age and baseline CVD risk do not strongly modify the association between abnormal glucose metabolism and all-cause mortality and CVD events

It is important to investigate whether the relationship between a risk factor and disease varies in different population groups, as this will help to identify individuals who may be at greater risk of developing the disease. In this thesis, the effects of age, sex and previous history of CVD (self-reported angina, myocardial infarction or stroke) on the associations between abnormal glucose metabolism and CVD events was examined in chapters 5 to 7.

Although the absolute burden of diabetes has been shown to increase with age [159], the relative risk of both all-cause mortality and CVD tends to diminish with age [156, 158, 159, 164-168]. The smaller excess risk attributable to diabetes that is observed

in older age groups may occur for two reasons: (i) people with diabetes who survive into older age possess some unmeasured protective factor that reduces their risk of dying, or (ii) older people without diabetes possess other risk factors that increases their likelihood of death. The studies in this thesis, however, showed that age only weakly modified the relationship between abnormal glucose metabolism and all-cause mortality and CVD. Chapter 5 showed that in those aged 45 to 65 years, the risk of all-cause mortality increased steadily across the glucose tolerance categories, but in people aged 65 years and over, the pattern was less consistent, as in comparison to the younger age group, the risk for all-cause mortality was slightly diminished in people with abnormal glucose metabolism, particularly those with NDM and KDM. Further analysis in chapter 7 showed that for the risk of CVD events, the interaction between abnormal glucose metabolism categories and age was of borderline significance (p=0.04). When the analysis was restricted to those without diagnosed diabetes (chapter 6), there were no significant interactions between age and any of FPG, 2hPG or HbA_{1c} for CVD or all-cause mortality.

This thesis found no strong evidence that having a previous history of CVD significantly modified the relationship between abnormal glucose tolerance and either all-cause mortality or CVD. Chapter 6 showed that in people without KDM, there was no significant interaction between previous history of CVD and FPG, 2hPG or HbA_{1c} for either all-cause or CVD mortality. Chapter 7 showed that the test for an interaction between abnormal glucose metabolism categories and previous history of CVD did not quite reach the conventional boundary for statistical significance. Together these findings suggest that having a previous history of CVD does not modify the relationship between hyperglycaemia and future CVD events, which is consistent with other studies that have shown that intermediate hyperglycaemia and/or diabetes are significant predictors of CVD in individuals both with [64, 174] and without [28, 45] a previous history of CVD.

9.3.9 Sex may modify the relationship between abnormal glucose metabolism and all-cause mortality but not CVD

It has been suggested that diabetes diminishes the protective effect afforded by female sex. As discussed in chapter 2, some studies in people with type 2 diabetes have found women to have a significantly shorter life expectancy compared to men

[9, 169]. Moreover, other studies [170-172], but not all [173], have also shown that diabetes confers a greater relative risk for mortality and CVD in women compared to men. Similar inconsistencies have also been reported for intermediate hyperglycaemia [19, 21]. In this thesis, sex-stratified analyses in chapter 5 showed that for women, only IGT and KDM, and for men, only KDM remained significant predictors of all-cause mortality after controlling for other covariates. For the risk of CVD events, chapter 7 showed that the test for interaction did not reach significance. As these analyses were based on categories of abnormal glucose metabolism, there may have been insufficient power to find an effect by sex. Therefore, it was important to also evaluate whether the relationship between the continuous blood glucose measures and mortality and CVD varied between men and women. When the continuous measures of glucose were considered, as outlined in chapter 6, a significant interaction between both FPG ($p=0.03$) and 2hPG ($p=0.02$) with all-cause mortality was observed. Sex-stratified analyses revealed that the relative risk of all-cause mortality per standard deviation (SD) (0.7 mmol/l) decrease in FPG < 5.1 mmol/l was greater in men, and the relative risk of all-cause mortality per SD (2.2 mmol/l) increase of 2hPG was greater in women. This finding indicates that among individuals without diagnosed diabetes, low levels of FPG in men, and high levels of 2hPG in women, signify an increased risk for all-cause mortality. However, there was no interaction between blood glucose and sex for CVD mortality.

9.4 Implications of thesis findings

The findings of the studies presented in this thesis have several implications for clinical practice, public health policy, and the conduct of future epidemiological studies.

9.4.1 Clinical implications

There are three major clinical implications from the results of this thesis. Firstly, chapters 5 and 7 showed that the risk of CVD events and premature mortality is not only significantly increased in individuals with established diabetes, but also in those with IFG and IGT. This suggests that CVD prevention strategies are warranted in

individuals with even moderate elevations in blood glucose. However, until further evidence becomes available to demonstrate that specifically lowering blood glucose is beneficial in reducing risk of death and macrovascular CVD, preventative measures should be targeted towards lowering other CVD risk factors such as smoking, obesity, elevated blood pressure and addressing dyslipidaemia, as there is strong evidence that the treatment of these risk factors is beneficial in preventing CVD in both the general population [175] and among those with diabetes [176]. Secondly, the findings from the study in chapter 6 showed that although FPG, 2hPG and HbA_{1c} were all significantly associated with an increased risk of CVD, in comparison to 2hPG and HbA_{1c}, FPG proved to be the strongest predictor. This suggests that an OGTT may only add a small and possibly clinically unimportant amount of information on individual mortality risk discrimination over and above FPG and HbA_{1c}. However, as the ability of blood glucose to classify individual CVD risk was only modest in comparison to other CVD risk factors such as smoking, previous history of CVD, hypertension, dyslipidaemia and obesity, the use of established CVD prediction scores in people without diagnosed diabetes is still necessary for risk stratification. Thirdly, the study outlined in chapter 7 showed a stronger relationship between abnormal glucose metabolism and fatal or non-fatal myocardial infarction than with coronary artery revascularisation, indicating that people with abnormal glucose metabolism may be at greater risk of more serious sequelae of CHD, as the findings indicated that the first presentation with CHD was often fatal. Therefore, intervention prior to the development of angina may be required in patients with abnormal glucose metabolism.

9.4.2 Public health policy implications

As outlined in chapter 1, both diabetes and CVD together have a considerable impact on the health of Australians, and for this reason, both conditions have been highlighted as *National Health Priority Areas* [177]. However, the findings from this thesis suggest that strategies to reduce the impact of CVD should also include the risks associated with intermediate hyperglycaemia as well as diabetes. The study in chapter 5 indicated that 65% of all CVD deaths occurred among individuals who had IFG, IGT or diabetes at baseline, which suggests that the public health benefits of addressing abnormal glucose metabolism could have a substantial impact on CVD

prevention in Australia. The continuous relationship between glucose and all-cause and CVD mortality noted in chapter 6 suggests that if the population blood glucose level was lowered (but not below a FPG of 5.1 mmol/l), if only by a small margin, this might substantially reduce the burden of CVD. Furthermore, as hypgerglycaemia is related to an increased risk of developing frank diabetes [16], lowering blood glucose may also reduce the likelihood of individuals converting to diabetes, in itself a strong risk factor for CVD.

9.4.3 Implications for future epidemiology studies

The findings from this thesis have two main implications for the design of future epidemiological studies. The first relates to the use of composite CVD end-points. The findings from the study in chapter 7 showed that categories of abnormal glucose metabolism may be more strongly associated with fatal or non-fatal myocardial infarction than with stroke or coronary artery revascularisation. Therefore, these results indicate that careful consideration may need to be given to the components included in a composite CVD end-point which might vary according to the particular question being addressed. Secondly, the study in chapter 4 demonstrated the importance of validating self-reported CVD data, as approximately 30% of all self-reported CVD outcomes were misclassified by participants. Misclassification was greatest for stroke and myocardial infarction, suggesting that the validation of these outcomes may be particularly important. Nevertheless, self-reported myocardial infarction and stroke may provide a reasonable measure of CHD and cerebrovascular disease, respectively, as misclassifications were primarily a result of myocardial infarction being confused with angina, and stroke with transient ischaemic attack. Moreover, very few actual CVD events (myocardial infarction, stroke, coronary artery revascularisation) were missed. Additionally, this study demonstrated the feasibility of CVD validation with both medical record adjudication and linkage to a statewide hospital morbidity database, showing a high level of concordance between the two methods. However, as linkage to a hospital morbidity database provides a far more efficient means of validating CVD events, the development of a national hospital morbidity database with individual level data would benefit the ascertainment of CVD outcomes in future epidemiological studies.

9.5 Areas for further research

9.5.1 Different populations

The Australian population comprises several different ethnicities, and although the AusDiab study did include people from a variety of ethnic backgrounds, 85% of the cohort were born in Australia, New Zealand or United Kingdom, and 95% spoke English, which indicates that the majority of the cohort population were Europid. Consequently, there were insufficient numbers of participants from other ethnicities, including Indigenous Australians, to permit further investigations on whether or not the findings reported in this thesis varied according to ethnicity. This is an important area for future research, as the burden of abnormal glucose metabolism and CVD may be different in these groups. Compared to the general population, both CVD, and in particular diabetes, occur at a markedly greater rate among Indigenous Australians [178]. Furthermore, some ethnic groups which represent large minorities in the Australian population, such as those from the Middle East and Asia, have also been shown to have a very high prevalence of diabetes and abnormal glucose metabolism [179]. However, the impact that this would have on CVD in migrants is uncertain, as compared to the general population, migrants to Australia have generally been shown to have better health – the so-called "healthy migrant effect" [180]. In order to investigate the impact of abnormal glucose metabolism on CVD and mortality in different population groups in Australia, future studies that specifically recruit participants from different ethnic backgrounds would be required. This could be accomplished by running several ethnic-specific studies, or by designing a single large cohort study that intentionally over-samples certain ethnic groups.

9.5.2 Longer follow-up

The studies in this thesis were based on a median five to six year follow-up period. As mentioned in section 9.1.2, this can be considered a strength, as the relationships observed between IFG and CVD and all-cause mortality, and between IGT and all-cause mortality and myocardial infarction (chapters 5 and 7) were less likely to have been explained by the conversion to diabetes. However, there was no way of testing

this as most of the individuals who died did not have a follow-up OGTT. A longitudinal study design which incorporates OGTTs at multiple time points would provide the data with which to examine whether individuals with IFG or IGT that convert to diabetes are at a greater risk for premature mortality and CVD events. This is important, as one study has shown that the risk of CVD was only significantly increased among those with IFG at baseline who converted to diabetes during the follow-up period [181], whereas the findings from another study suggested that this was not the case for IGT, which remained significantly associated with CVD and all-cause mortality irrespective of whether or not conversion to diabetes took place [182].

Longer follow-up would also provide greater statistical power to compare the relationships between abnormal glucose metabolism and CVD and all-cause mortality among different populations. Although this thesis did investigate whether there were any differences according to age, sex and previous history of CVD (chapters 5 to 8), some of the strata in these analyses had small numbers of outcome events, which may have reduced the power of the study to find an effect. Furthermore, more outcomes would also permit further exploration of the relationships between abnormal glucose metabolism and specific CVD outcomes, such as myocardial infarction, stroke and coronary revascularisation. This would then help to confirm the findings in chapter 7 that suggested that abnormal glucose metabolism may be more strongly associated with myocardial infarction than stroke and coronary revascularisation.

9.5.3 The contribution of abnormal glucose tolerance to cancer and other non-CVD causes of death

Cancer is a significant cause of death in Australia, with over one third of all deaths being attributable to malignancy. In addition to CVD and all-cause mortality, chapters 5 and 6 reported on the associations between abnormal glucose metabolism and non-CVD deaths. After adjusting for age and sex, the study in chapter 5 found that the risk of non-CVD mortality was increased two-fold for KDM and by 30% for IGT compared to NGT. In addition, the study in chapter 6 showed that among individuals without diagnosed diabetes, for every 2.2 mmol/l increase in 2hPG there was a 10% increased risk for non-CVD mortality. These associations held even after

further adjustment for smoking, waist-to-hip ratio and a previous history of CVD events. As two-thirds of all non-CVD deaths in these studies were due to malignancies, the increased risk of non-CVD mortality observed for abnormal glucose metabolism could have been driven by significant associations between abnormal glucose metabolism and cancer. These findings are consistent with other studies that have also observed a significant association between diabetes [183-185] and intermediate hyperglycaemia [186, 187] and cancer. However, as findings remain equivocal as to whether hyperglycaemia is specifically related to certain types of cancer, further research into this area should be undertaken. Unfortunately, with only five year follow-up data on cause-specific mortality, it was not possible to examine the relationship between abnormal glucose metabolism and specific cancer outcomes, as there were insufficient numbers of cancer deaths to provide meaningful results.

9.5.4 Does lowering of blood glucose improve CVD and mortality outcomes?

The studies in this thesis have shown a strong dose-response relationship between abnormal glucose metabolism and premature mortality and CVD. However, as this thesis is based on observational data, it could not be determined whether or not hyperglycaemia is a direct mediator of CVD and mortality, or just a marker of more severe disease. Specifically examining the effects of blood glucose lowering on CVD and mortality prevention in the general population would help to answer this question, as a more direct link between hyperglycaemia and CVD could be tested. Intervention studies in people with diabetes that have compared intensive glucose lowering with usual clinical care have consistently demonstrated that intensive blood glucose lowering leads to a greater reduction in microvascular events [188-190], than macrovascular disease [188-191]. Indeed, one study reported that excessive lowering of HbA_{1c} led to an increased risk of all-cause mortality [191]. Extended observational follow-up of both the UK Prospective Diabetes Study and the Diabetes Control and Complications Trial participants have suggested, however, that glucose-lowering treatment may have a role in CVD prevention [192, 193], but further evidence from trials with longer follow-up are required.

The beneficial effects of glucose-lowering treatment in people without diagnosed diabetes have not been well examined. A secondary analysis of the Stop-Non-Insulin-Dependent Diabetes Mellitus trial did show that in individuals with IGT and a BMI of between 25 and 40 kg/m^2, acarbose treatment (an α-glucosidase inhibitor) lead to a significant reduction in the development of major CVD events over three years. However, it was not clear from this study whether the reduction of CVD was related specifically to glucose lowering. Although acarbose was associated with a reduction in 2hPG over the three year period, a large and significant decline in blood pressure was also observed. Another study, currently underway, is the Outcome Reduction with an Initial Glargine Intervention (ORIGIN), which is planning to investigate the effects of insulin glargine compared to standard glycaemic treatment on subsequent CVD events and death in people with a previous history of CVD who have established diabetes, newly diagnosed diabetes, IFG or IGT. However, as people with IFG and IGT comprise only 12% of the trial population, it will not be possible to delineate the effects of the intervention in those with intermediate hyperglycaemia from the total trial population [194]. Therefore, further research on the impact of blood glucose lowering on CVD and mortality in people with all levels of abnormal glucose metabolism is warranted to identify any direct effects of glycaemia on CVD and mortality.

9.6 Conclusions

This thesis aimed to investigate the extent to which impaired glucose metabolism contributes to the burden of CVD morbidity, CVD mortality and all-cause mortality in the Australian population. Using data collected as part of the Australian Diabetes, Obesity and Lifestyle (AusDiab) study in conjunction with linkage of this cohort to the NDI and adjudication of self-reported CVD events, the series of studies presented in this thesis clearly indicate that impaired glucose metabolism is an important independent risk factor for CVD events and all-cause mortality in Australians.

With reference to the initial study hypotheses, the following conclusions can be made:

- the use of self-reported myocardial infarction, coronary artery revascularisation and stroke in epidemiological surveys is a valid measure of composite CVD outcomes, but when myocardial infarction and stroke outcomes are used in isolation, these should be validated against medical record information

- elevated plasma glucose is an important risk factor for CVD and all-cause mortality

- the association between elevated plasma glucose and CVD and all-cause mortality is independent of other traditional risk factors, such as hypertension and dyslipidemia, but the ability of blood glucose (FPG, 2hPG or HbA_{1c}) to classify individual risk is only modest when traditional CVD risk factors are considered

- IFG and IGT are associated with an increased risk of all-cause mortality, and IFG, not IGT is significantly associated with an increased risk of CVD mortality

- compared to HbA_{1c}, FPG and 2hPG are more important predictors of all-cause mortality, and compared to 2hPG and HbA_{1c}, FPG is a more important predictor for CVD mortality

- the nature of the relationships between 2hPG and HbA_{1c} and all-cause and CVD mortality is linear, but a J-shaped relationship characterises the association between FPG and all-cause and CVD mortality

- hyperglycaemia may be more strongly associated with myocardial infarction than stroke or coronary artery revascularisation procedures

- HOMA-%S is not associated with all-cause mortality; and is only modestly associated with CVD events, largely explained by its association with HDL-C. FPG is a better predictor of CVD than is HOMA-%S

- age and having a history of previous CVD may not strongly modify the association between abnormal glucose metabolism and all-cause mortality or CVD events; however, low levels of FPG in men, and high levels of 2hPG in women, appear to signify an increased risk for all-cause mortality. The risk for CVD events does not differ in women compared to men

The findings of this thesis have significant implications for public health, as they suggest that CVD prevention strategies should not only target diabetes, but all levels of hyperglycaemia.

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APPENDIX 1

A1. AusDiab – methods and response rates

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The Australian Diabetes, Obesity and Lifestyle Study (AusDiab)—methods and response rates

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Abstract

The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) addresses the urgent need for data on diabetes prevalence, risk factors and associated conditions in Australia. Here we describe the methods used and the response rates obtained. AusDiab was a population-based cross-sectional survey of national diabetes mellitus prevalence and associated risk factors in people aged $\geqslant 25$ years, conducted between May 1999 and December 2000 in the six states and the Northern Territory of Australia. The study involved an initial household interview, followed by a biomedical examination that included an oral glucose tolerance test (OGTT), standard anthropometric tests, blood pressure measurements and the administration of questionnaires. Of the 20 347 eligible people (aged $\geqslant 25$ years and resident at the address for $\geqslant 6$ months) who completed a household interview, 11 247 (55.3%) attended for the biomedical examination. Of those who completed the biomedical examination 55.1% were female. Comparisons with the 1998 Australian population estimates showed that younger age responders were under-represented at the biomedical examination, while the middle-aged and older age groups were over-represented. Weighting of the AusDiab data for age and gender have corrected for this bias. AusDiab, which is the largest national diabetes prevalence study undertaken in a developed nation to have used an OGTT, provides a valuable national resource for the study of the prevalence and possible causes of diabetes, as well as identifying possible risk factors that may lead to diabetes. Furthermore, it generates the baseline data for a prospective 5-year cohort study. The data will be important for national and regional public health and lifestyle education and health promotion programs.

Keywords: AusDiab; Response rates; Diabetes survey; Australia; Diabetes prevalence

1. Introduction

Globally, the prevalence of diabetes, particularly Type 2 diabetes is rapidly increasing [1].

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Indeed, it has been predicted that the global figure of people with diabetes will rise from current levels of about 150 million in 2000 to 300 million by 2025 [2]. However, with the exception of the USA [3], nationally representative, population based diabetes prevalence data among developed nations is scarce. In particular, few studies have involved an oral glucose tolerance test (OGTT).

In Australia, estimates of diabetes prevalence and other categories of glucose intolerance are confined to studies conducted 10–20 years ago on a small sample of residents from a rural town in Western Australia [4]. Most recent estimates of diabetes prevalence in Australia have relied on self-reported data, but since Type 2 diabetes can be asymptomatic for many years before it is diagnosed in a clinical situation, reliance on self-reported information invariably contributes to an underestimation of the true prevalence. Furthermore, such studies fail to provide information on the extent of other states of glucose intolerance, which are known to substantially increase risk of future diabetes.

To address the urgent need for more definitive data on the true current prevalence of diabetes and its associated risk factors in Australia, the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) was a cross-sectional study involving a standard OGTT conducted during 1999–2000 in all Australian States and the Northern Territory. The present paper provides a detailed description of the survey methods including the design, sampling techniques and survey protocols. Data on weighting of the sample, response rates and statistical techniques are also presented. The survey methods conform to those recommended by the World Health Organisation (WHO) [5], and the study was approved by the International Diabetes Institute ethics committee.

The AusDiab study aimed to determine the national prevalence of diabetes and other selected non-communicable diseases and their risk factors in a representative sample of adults aged 25 years and over from each of the states and the Northern Territory of Australia.

More specifically, the objectives of the study were to:

1. estimate the national and regional prevalence of diabetes and other forms of abnormal glucose tolerance;
2. estimate the prevalence of the cardiovascular risk factors within the Metabolic Syndrome, including obesity, hypertension, and lipid profile abnormalities;
3. assess the distribution and relationships of the cardiovascular risk factors indicated above;
4. assess temporal trends in risk factor prevalences with reference to previous Australian surveys;
5. describe health knowledge and attitudes and utilization of health services, and
6. provide baseline data for longitudinal cohort studies.

2. Methods

2.1. Target population/eligibility requirements

Non-institutionalised adults aged 25 years and over residing in private dwellings in each of the six states and the Northern Territory of Australia were included in the survey if they had resided permanently at the address for a minimum of 6 months prior to the survey. Persons with physical or intellectual disabilities that precluded participation in the study were not included.

2.2. Sampling frame

A stratified cluster sampling method was used, involving seven strata (six states and the Northern Territory) and clusters based on census collector districts (CDs—the smallest geographic unit defined by the Australian Bureau of Statistics at each census, with an average of 225 dwellings each). Within each state, 6 CDs were randomly selected with a selection probability proportional to the population size (population aged over 25 years). Due to the logistic and economic constraints of the survey, and to avoid the bias of including an unrepresentative number of high prevalence groups, the following exclusion criteria were adopted:

1. CDs containing fewer than 100 persons aged 25 years and over
2. CDs that formed part of a statistical local area that was classified as 100% rural according to 1996 census data [6]
3. CDs that contained more than 10% indigenous population.

Of the total pool of CDs available (34 410), 4141 CDs (12%) were excluded from selection on these grounds. From the excluded CD's, 762 (18.4%) had $>10\%$ indigenous population, 1464 (35.4%) were rural, 1100 (26.6%) had <100 persons aged $\geqslant 25$ years, while 815 (19.7%) had more than one factor of the exclusion criteria. The three exclusion categories meant that the total eligible population (adults aged $\geqslant 25$ years) was reduced by 6.44% from 11 341 070 to 10 610 855. This comprised 241 931 (33.1%) adults from CDs that had $>10\%$ indigenous population, 349 716 (47.9%) adults from CDs that were rural, 74 723 (10.2%) adults from CDs that had <100 people aged $\geqslant 25$ years and 63 845 (8.7%) adults from CDs that had more than one factor of the exclusion criteria.

2.3. Sample size determination

The sample size was selected based on precision of estimates to identify a national diabetes prevalence of 7.0% (an estimation based on results of previous surveys, and the expectation that the diabetes rate had increased over time). As a secondary objective of the study was to deliver useful state-specific prevalence estimates, the sampling frame was stratified at the state level. With very little loss of efficiency, an accurate national estimate can be obtained from weighted samples of equal size from the six states and the Northern Territory. Accounting for the clustering of the survey design, a sample size of 10 500 (1500 per state) was predicted to provide 95% confidence intervals of 6.2–7.8, around a diabetes estimate of 7.0%. This level of precision was regarded as acceptable, and the sample size was considered achievable and within the funding constraints of the survey. It should be noted however, that the sample size was calculated for total diabetes prevalence only and would be expected to have limited power to describe the prevalence of type 1 diabetes in this sample.

2.4. Sample selection

It was calculated that 6 CDs were required to provide the required sample size (1500 per state) within each state. Following an initial field visit, if the CD was considered inappropriate for sampling in that location, the selected CD was replaced with another randomly selected CD from the same state. Replacements occurred in seven instances during the course of the survey, for the following reasons:

1. The low population density of the CD made it economically and logistically impossible to conduct the survey activities within the allocated timeframe (3 CDs)
2. The area selected was predominantly an industrial/business zone (2 CDs)
3. No eligible 'neighbouring' CD was available (see below) (1 CD)
4. The area had been recently involved in a large-scale health survey, including diabetes testing (1 CD).

After the first three sites had been surveyed, it became clear that a single CD would not provide the required sample size at each location surveyed. Clusters were subsequently formed by combining the randomly selected index CD and its largest adjoining neighbours to achieve a minimum cluster size of 250 participants. The final sample comprised 3 single CDs, 22 pairs of CDs, 16 triplets and 1 quad.

2.5. Survey protocol and procedures

The AusDiab survey activities occurred over a 21-month period between May 1999 and December 2001. Approximately 2 months were allocated to the collection of data in each state and the Northern Territory. The AusDiab survey activities were divided into two phases—the household interview and the biomedical examination.

2.5.1. Household census and interview

Following a local media advertising campaign involving news items in local community newspa-

pers and local radio and/or television, all private dwellings within the sampled cluster received a hand-delivered (non-addressed) letter informing residents about the survey and advising that an AusDiab interviewer would visit to conduct the household interview. A brochure describing the study objectives, the interview and examination process, and study confidentiality was supplied in the initial contact letter. This brochure was provided only in English.

The first visit by the interviewer occurred approximately 3 days after the letter had been delivered. If the interviewer could not make contact with household members, a letter was left requesting the household to telephone a toll-free number to arrange a suitable interview time. The interviewers made a minimum of 2 visits and up to 5 visits before a household was classified as a non-contact.

Where possible, at each participating household a personal interview was conducted with every adult member aged 25 years and over who met the eligibility requirements. The interview ascertained marital status, level of education, date and country of birth, language spoken at home and history of diabetes or high blood sugar levels. In some instances, adult household members were unable to answer for themselves because of old age, illness, intellectual disability or difficulty with the English language. In these cases, a responsible 'proxy' was interviewed on their behalf. There were no provisions for interviews to be conducted in languages other than English. In order to obtain a personal interview with all eligible household members, interviewers made appointments to visit as often as was necessary to the household. In a small number of cases interviews were conducted over the telephone with the Household Survey Coordinator.

At the completion of the interview, all household members aged 25 years or older were invited to attend a local testing site for the biomedical examination. Participants were provided with a brochure explaining the biomedical examination procedures, together with the self-administered SF-36 General Health and Well-Being questionnaire, which they were asked to complete and bring to their biomedical examination appointment.

2.5.2. Biomedical examination

The biomedical examination was conducted at a local test site on weekdays (except Friday) and weekend days over an 8-day period in each sampled area. Local survey sites included community centres, scout headquarters, sporting venues, church halls and schools. Survey activities at the testing site commenced at 7:00 a.m. and typically finished at 2:00 p.m. On average, approximately 40 participants attended daily.

All responders gave written informed consent to participate in the survey upon arrival at the testing site. The AusDiab biomedical examination protocol followed closely the WHO recommended model for diabetes and other non-communicable disease field surveys [5,7]. The components of the biomedical examination are shown in Table 1. Following the initial collection of the fasting blood sample, an OGTT was performed on all participants, except those on insulin or oral hypoglycaemic drugs or those who were pregnant. The OGTT was performed according to WHO specifications. Participants moved through the biomedical examination procedures in a circuit-like manner that took approximately 2.5–3 h to complete. The SF-36 and dietary questionnaires were self-administered, while all other questionnaires were interviewer administered. All data from the participant record forms were entered both electronically and manually.

3. Results

3.1. Survey response

Response rates to the household interview and the biomedical examination are shown in Fig. 1. In total, the AusDiab interviewers approached 25 984 households in the 42 selected clusters. Of these, 6769 (26%) were classified as non-contacts. Reasons for non-contact (and hence non-participation) in the household interview included language difficulties (318 households), no access gained to the residence (e.g. because of dangerous dogs, security fences) (941), the householders not being contactable despite several attempts (5358), and other reasons such as drunkenness or disability of the householders (152).

Of the 19 215 residential properties where contact was achieved, 1095 were excluded because none of the occupants met the residency criteria of the survey, and a further 991 were excluded because all of the residents in the household were less than 25 years of age. Of the remaining 17 129 eligible households, 5178 refused to be interviewed and 472 were away for the duration of the study period, giving rise to a total of 11 479 households (70.2%) where an interview was achieved. Reasons for refusal included health concerns (486, 9.4%), being unable to attend because of work commitments (1159, 22.4%), feeling they were too old to participate (368, 7.1%), medical problems (1317, 25.4%), and 'other' reasons (1848, 35.7%).

Assuming that the proportion of ineligible households was similar between the contacted (2086/19 215 = 10.9% ineligible) and the non-contacted households, 49.6% (11 479/23 163) of eligible households participated in the household interview. The denominator here (23 163) is calculated as all private dwellings (25 984) minus all ineligible households (2821, which is comprised of

Table 1
Variables assessed within AusDiab

Category	Variable	Measurement instrument
Demographic characteristics	Age, sex, ethnicity	Household interview and interviewer-administered questionnaires at survey site
	Socio-economic status (education, occupation, income)	
	Diabetes status	
Medical and family history	Family history (diabetes)	Interviewer-administered questionnaires at survey site
	Chronic health conditions (cardiovascular disease, gout)	
	Women's health	
Life-style related factors	General health and well-being	SF-36 Questionnaire
	Alcohol/tobacco	Interviewer-administered questionnaires at survey site
	Physical activity	
	Diet	Anti-Cancer Council of Victoria Dietary Questionnaire (self-administered)
Health-behaviour related factors	Health knowledge, attitudes and practice data	Interviewer-administered questionnaires at survey site
	Health service utilisation patterns	
Physical measurements	Height	Stadiometer
	Weight	Beam balance scales
	Waist and hip circumference	Tape measure
	Body fat determination	Bioimpedance
	Blood pressure	Dinamap/mercury sphygmomanometer
	12-lead ECG	
Blood measurements (fasting)	Blood glucose	Glucose oxidase
	Blood lipids	Enzymatically—Olympus AU600 analyser
	HbA_{1c}	Boronate affinity high performance liquid chromatography
Urine measurements (spot morning sample)	Albumin	Immunoturbidimetric method—Olympus AU600 analyser
	Creatinine	Olympus AU600 analyser

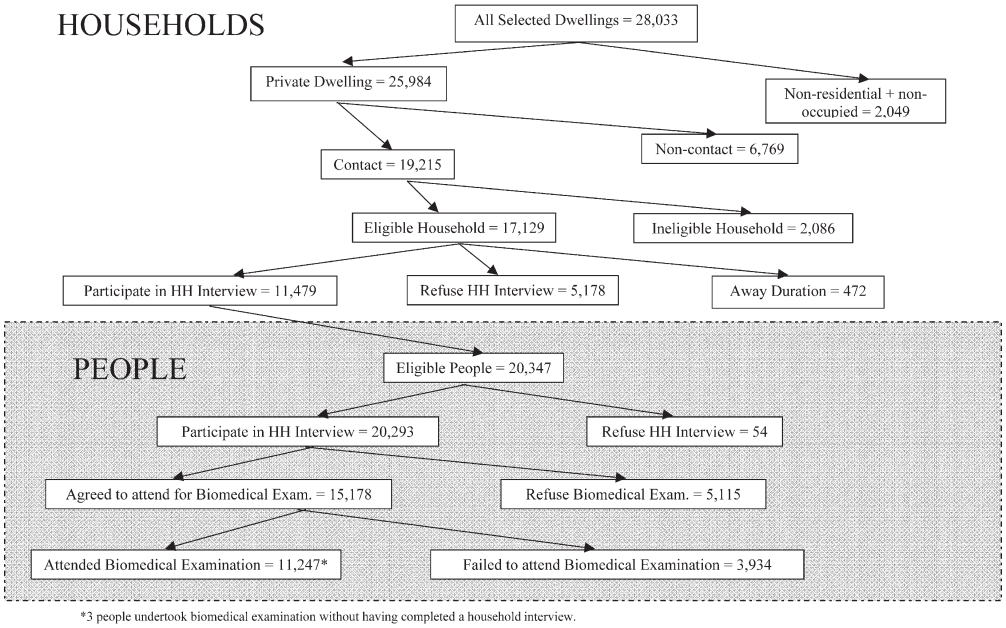

Fig. 1. Flowchart of households and persons selected to participate in AusDiab.

2086 contacted ineligible households, plus 735, which is an equivalent percentage of non-contacted ineligible households). This response rate is a conservative estimate, as more of the non-contacted households are likely to be unoccupied or have fewer occupants than contacted households.

In the 17 129 households that were confirmed as containing at least one eligible participant, 20 347 eligible adults were interviewed. Of those who participated in the household interview, 11 247 (55.3%) took part in the biomedical examination. This response rate for the biomedical examination ranged from 49.5% in Queensland and 49.6% in South Australia to 61.8% in Western Australia (Table 2). Assuming that the numbers of eligible adults residing in the 5178 households that refused the household interview was the same as in those which participated, and combining the household response rate (11 479/17 129 = 67%) with the biomedical examination response rate (11 247/20 347 = 55.3%) the overall response rate can be estimated to be 37%.

3.2. Profile of responders and weighting of the survey sample

To account for the clustering and stratification of the survey design, and to adjust for non-response, the data have been weighted to match the age and gender distribution of the 1998 estimated residential population of Australia aged over 25 years [8]. The weighting factor is based on the probability of selection in each cluster. The number of males and females in each cluster aged 25 years and over identified in the 1996 census was used to calculate the probability of selection in each cluster. The weight was then calculated based on the probability of selection, adjusted to reflect the age and sex structure of the 1998 estimated residential population over the age of 25. Groups based on age deciles and gender defined the weighting variable. As there are two

distinct populations in our sample—one who participated in the household interview and a subset of this population who attended the biomedical examination, two weighting factors have been applied, one to all responders to the biomedical examination and another to all responders to the household interview.

Among the responders to the biomedical examination ($n = 11\,247$), 44.9% were male, with the mean age being 51.5 years (Table 3). This compares to 49.0% male in the 1998 Australian population, and a mean age in the 1996 census [6] over 25 years of 48.1 years. Among the non-responders ($n = 9049$), 51.2% were male and the mean age was 47.7 years. Weighting of the sample to the estimated 1998 residential Australian population corrected the gender and age bias, with 49.0% (95% CI, 47.9–50.1) of the weighted responders to the biomedical examination being male, and the mean age being 48.2 years (95% CI, 46.6–49.9).

Table 4 provides a comparison between responders and non-responders to the biomedical examination for both unweighted and gender and age-adjusted estimates, with respect to various demographic characteristics. For the crude, unadjusted estimates, significant differences were observed for percent married, the percentage of English speaking participants, the percent born in the UK, and the percentage who suspected they had diabetes, but no differences were noted for the percentage born in Australia, the percentage born outside the UK or Australia, the percentage who had completed the highest year of school, and the percentage who had ever been told they had diabetes. Adjustment for age and gender rectified the difference between responders and non-responders for the percentage married, but differences were still observed in the percentage who suspected that they had diabetes, the percent born in the UK and the percentage of English speakers. Additionally, after adjustment for age and gender, the percentage who had completed the final year of high school, technical education or University was higher for responders.

4. Discussion

AusDiab is the largest cross-sectional study of the prevalence of diabetes and its precursors ever performed in a developed nation. Through its capacity to provide the first definitive data on the true magnitude of the diabetes epidemic in Australia, AusDiab will not only be a valuable resource for health care planners in Australia, but will also serve as an important research tool for the study of diabetes and associated diseases on a longitudinal basis.

The AusDiab experience provides a valuable insight into the execution of population-based,

Table 2
Response rates of eligible residents to the biomedical examination by state/territory

State/Territory[a]	Eligible residents (n)	Respondents to household interview (n)	Respondents to biomedical examination (n)	Biomedical examination response rate (%)[b]
VIC	2396	2391	1434	59.8
WA	2527	2485	1561	61.8
NSW	2719	2717	1515	55.7
TAS	3339	3339	1848	55.3
SA	3618	3618	1796	49.6
NT	2446	2446	1459	59.6
QLD	3302	3297	1634	49.5
Total	20 347	20 293	11 247	55.3

[a] VIC = Victoria, WA = Western Australia, NSW = New South Wales, TAS = Tasmania, SA = South Australia, NT = Northern Territory, QLD = Queensland.
[b] Calculated as biomedical examination responders as a percentage of eligible residents.

Table 3
Response rates of eligible residents to the biomedical examination by age and gender

Gender/age group	Eligible residents (n)[a]	Respondents to household interview (n)[a]	Respondents to biomedical examination (n)[a]	Biomedical examination response rate (%)[b]
Male 25–34	1757	1747	590	33.6
Male 35–44	2342	2331	1093	46.7
Male 45–54	2290	2281	1345	58.7
Male 55–64	1516	1515	928	61.2
Male 65–74	1125	1122	731	65.0
Male 75+	677	677	362	53.5
Female 25–34	1894	1890	803	42.4
Female 35–44	2510	2503	1465	58.4
Female 45–54	2355	2352	1546	65.6
Female 55–64	1649	1647	1096	66.5
Female 65–74	1286	1285	837	65.1
Female 75+	927	927	451	48.7

[a] Nineteen eligible people refused to give their age and are thus missing from this table, of whom 16 were respondents to the household interview, and none were respondents to the biomedical examination.
[b] Calculated as biomedical examination responders as a percentage of eligible residents.

cross-sectional surveys involving the use of an OGTT. Since AusDiab required careful consideration of the logistics required to achieve a national sample within the funding and timeframe constraints imposed, particular emphasis was given to the establishment of a study design that reflected the 'best available' model. This extensive 12-month planning process was crucial to the successful implementation of the study.

Several aspects of the methods used in sample selection and the study design of AusDiab warrant further discussion. First, the inclusion criteria contained only those CDs that contained less than 10% indigenous population. Existing data provide clear evidence of a very high prevalence of diabetes among the indigenous population in Australia [9]. To overcome the chance selection of one or more CDs with a large proportion of indigenous people, and thus minimize the potential bias introduced to the national and state diabetes estimates, we considered it more practical to restrict the inclusion criteria to those CDs likely to contain smaller proportions of indigenous people rather than account for any potential bias at the analysis stage. Furthermore, this approach was considered important for the operations of the study, since aspects such as questionnaire design would have required extensive modifications to reflect the cultural differences. It is unlikely that this restriction would have impacted greatly on the generation of national estimates since the indigenous population is numerically a very small minority group in Australia ($\approx 2\%$ of the total Australian population), and indeed, represented only 0.8% of the total AusDiab sample. Preparations are presently underway to address these issues through a survey that will employ similar survey methods used within AusDiab in urban indigenous Australians living in Darwin, Northern Territory.

The decision to sample equal numbers from each stratum reflects a compromise between the primary and secondary objectives of the survey. It is probable that a study design that sampled from the states proportional to their size would have been more efficient in terms of providing a more accurate national diabetes prevalence estimate, however accurate estimates for all states (in particular the smaller states) would have been compromised. Since weighting of the data prior to the analysis stage enables us to allow for over-representation of the smaller states and under-representation of the larger states, it is unlikely that our primary objective was compromised unduly by this decision.

Table 4
Comparison between biomedical examination responders and non-responders, both unweighted, and age and gender-adjusted to the 1998 estimated residential population aged over 25 years

Characteristic	Responders to biomedical exam. (unweighted)	Non-responders to biomedical exam. (unweighted)	Responders (age and gender adjusted to 1998 population[a])	Non-responders (age and gender adjusted to 1998 population[a])
Married	71.5 (68.8–74.2)	67.0 (64.4–69.5)	68.5 (65.5–71.5)	67.6 (65.0–70.2)
Country of birth: Australia	76.0 (72.9–79.1)	77.1 (73.8–80.5)	77.6 (74.6–80.6)	76.7 (73.3–80.1)
Country of birth: UK	11.3 (9.7–12.8)	8.7 (7.2–10.2)	10.3 (8.9–11.7)	8.8 (7.3–10.4)
Country of birth: Other	12.7 (10.2–15.2)	14.1 (10.9–17.3)	12.1 (9.7–14.5)	14.4 (11.1–17.7)
Language spoken: English	96.0 (94.6–97.4)	93.7 (91.2–96.3)	96.1 (94.9–97.4)	93.6 (91.0–96.2)
Education: completed high school/university/technical education	55.8 (51.4–60.1)	51.7 (46.4–57.1)	58.2 (54.0–62.4)	51.3 (46.0–56.6)
Ever told have DM?	6.4 (5.7–7.1)	6.2 (5.2–7.1)	5.9 (5.3–6.5)	6.4 (5.4–7.3)
Suspect have DM?	1.5 (1.3–1.7)	0.5 (0.4–0.7)	1.5 (1.3–1.7)	0.5 (0.4–0.7)

Estimates are percentages (95% CI).
[a] June 30, 1998 Australian population [8].

It is also noted that, due to the exclusion criteria of the study, the results may not be generalisable to either the indigenous population or the rural population of Australia. The primary aim of the study, however, was to provide estimates which were accurate for the Australian population over 25 years as a whole and these exclusion criteria should not significantly affect that aim.

The response rates to AusDiab can be interpreted in several ways. In many studies where a defined population is used as the sample pool, an absolute response rate can be accurately calculated. For example, when using an electoral role in the sample selection, the number of residents in each household is accurately known, allowing the demographic profile of both the non-responders and the responders to be calculated. In AusDiab, the sample pool was comprised of households in CDs based on the 1996 Australian census, conducted 2 years prior to commencement of the AusDiab survey. An accurate estimate of the number of residents in households where contact was not achieved, as well as the age and gender profile of these households cannot be accurately obtained. This is due to the possibility that in those households where contact could not be achieved, many may have been unoccupied, or the resident population in each household may be lower (assuming that the more people residing in a household, the more likely it is that someone will be home when an interviewer calls).

Our estimates suggest, however, that reasonably good response rates were obtained from those households where contact could be achieved. Furthermore, considering the duration and nature of the testing procedures involved in the biomedical examination for each individual, the response to the biomedical examination is acceptable. Nevertheless, additional in-depth analyses will be necessary to explore whether specific non-response biases exist at both the national and state level.

Regarding the analysis of non-response bias presented, there are several points worth noting. Firstly, the difference in the percentage of English speakers between responders and non-responders shown in Table 4, while being significant, was fairly small (96.1 vs. 93.6%) and is unlikely to have had a significant impact on diabetes or other prevalence estimates. Similarly, the percentage of responders born in the UK was only slightly greater than the percentage of non-responders born in the UK (10.3 vs. 8.8%), although again, this difference was significant. Most of the difference in country of birth between responders and non-responders was removed by age and gender-standardisation. It is unlikely that the percentage of people born in the UK would have an important effect on diabetes prevalence estimates, since many cultural similarities exist for those born in the UK and those born in Australia.

The greatest differences between responders and non-responders were observed in suspicion of diabetes and level of education. Firstly, the percentage of those who suspected they had diabetes (but have never been told they do) was significantly higher in the responders (1.5%) compared to the non-responders (0.5%). Only one in 12 of those who suspected they had diabetes were actually found to have the disease, compared to one in 25 of those who did not suspect they had diabetes. Taking into account the very low prevalence of those who suspected they had diabetes, and the low prevalence of those found to actually have diabetes when they suspected they had diabetes, the difference between responders and non-responders with respect to suspicion of diabetes would have increased the total number of newly diagnosed cases of diabetes by 6 or 7 persons at most. This would be expected to have only a negligible effect on the total prevalence estimates for diabetes.

Participants who attended the biomedical examination were more likely to have completed the final year of high school, University or other higher education (58.2 vs. 51.3%) than non-responders. This would indicate that the higher socio-economic groups were over-represented in AusDiab. This difference could potentially bias estimates of diabetes, as well as other studied variables. However, for glucose intolerance, as well as other cardiovascular disease risk factors such as dyslipidaemia, physical activity, alcohol consumption and smoking, there is a negative association with socio-economic status [10]. Therefore, our estimates of these disease states, if a socio-economic bias does indeed exist, are likely

to underestimate the true prevalence. Of course, education level is only one indicator of socio-economic status, and other variables such as income level, occupation and type of residence will need to be considered in further analyses of response bias. Detailed comparisons between responders and the Australian population aged over 25 (using both census data and other previous surveys), particularly in the areas of socio-economic status, language spoken and suspicion of diabetes, will be valuable in assessing more precisely the impact of any response bias in the AusDiab survey.

AusDiab is a major achievement in the study of diabetes in Australia. The study not only provides much needed data on the current magnitude of the diabetes epidemic that exists in Australia but also fills a 10-year void in knowledge on current levels of many of the associated cardiovascular disease risk factors that can only be determined through blood collection. Furthermore, an important extension to this initiative will be the follow-up of the AusDiab cohort, that will provide the first opportunity ever in Australia to examine the natural history of diabetes and its complications, as well as the incidence of cardiovascular disease among this representative sample of Australians with diabetes or impaired glucose metabolism.

Acknowledgements

We are most grateful to the following for their support of the study: The Commonwealth Department of Health and Aged Care, Eli Lilly (Aust) Pty Ltd, Janssen–Cilag (Aust) Pty Ltd, Knoll Australia Pty Ltd, Merck Lipha s.a., Alphapharm Pty Ltd, Merck Sharp & Dohme (Aust), Roche Diagnostics, Servier Laboratories (Aust) Pty Ltd, SmithKline Beecham International, Pharmacia and Upjohn Pty Ltd, BioRad Laboratories Pty Ltd, HITECH Pathology Pty Ltd, the Australian Kidney Foundation, Diabetes Australia (Northern Territory), Queensland Health, South Australian Department of Human Services, Tasmanian Department of Health and Human Services, Territory Health Services, Victorian Department of Human Services and Health Department of Western Australia.

For their invaluable contribution to the field activities of AusDiab, we are enormously grateful to Annie Allman, Marita Dalton, Adam Meehan, Claire Reid, Alison Stewart, Robyn Tapp and Fay Wilson.

And our special thanks goes to the local collaborating centers, including Sir Charles Gairdner Hospital (Western Australia), the Prince of Wales Hospital (New South Wales), the Menzies Centre for Population Health Research (Tasmania), the Queen Elizabeth Hospital (South Australia), the Menzies School of Health Research (Northern Territory), Queensland Health, the Monash Medical Centre, Department of Nephrology (Victoria) and the Centre for Eye Research Australia (Victoria).

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APPENDIX 2

A2. Ethics approvals

International Diabetes Institute

ETHICS COMMITTEE

CERTIFICATE OF APPROVAL

This is to certify that Project No. 3/99

THE AUSTRALIAN DIABETES,OBESITY AND LIFESTYLE STUDY

Chief Researchers: Professor Paul Zimmet
Dr Daniel J. McCarty
Dr David Dunstan
Dr Maximilian deCourten

has been approved in accordance with your application and subsequent amendments on the understanding that you observe the National Health and Medical Research Council Statement on Human Experimentation.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any change to the application which is likely to have a significant impact on the ethical aspects of this project will require approval from the Ethics Committee.

A progress report is due in June 1999 and a summary of your findings is requested on completion of the project.An audit may be conducted by the Ethics Committee at any time.

Extension of your project beyond this date will require approval from the Ethics Committee. Request for Amendment, Request for Extension, and Progress Report forms are available from The Secretary, Ethics Committee, International Diabetes Institute, 260 Kooyong Road, Caulfield 3162.

Approval date: 02/3/1999
Expiry date 02/3/2001

[redacted]

Chairperson, Ethics Committee

p\sfournel\lp\crtapdol

International Diabetes Institute

ETHICS COMMITTEE

CERTIFICATE OF APPROVAL

This is to certify that Project No. 3/2002

THE AUSTRALIAN PROSPECTIVE DIABETES STUDY (APDS)

Chief Researchers: Professor Paul Zimmet
Dr Jonathan Shaw

has been approved in accordance with your application and subsequent amendments on the understanding that you observe the National Health and Medical Research Council Statement on Human Experimentation.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any change to the application which is likely to have a significant impact on the ethical aspects of this project will require approval from the Ethics Committee.

A progress report is due in June 2004 and a summary of your findings is requested on completion of the project. An audit may be conducted by the Ethics Committee at any time.

Extension of your project beyond this date will require approval from the Ethics Committee. Request for Amendment, Request for Extension, and Progress Report forms are available from The Secretary, Ethics Committee, International Diabetes Institute, 250 Kooyong Road, Caulfield 3162.

Approval date: 13/6/2003
Expiry date: 13/ 6/2005

[redacted]

Chairperson, Ethics Committee

p\stoumel\libby\crtapapds

DIABETES INSTITUTE
ABN 16 007 342 412

28 April 2004

Dr Jonathan Shaw
Director Research.IDI
250 Kooyong Rd
CAULFIELD3162

Dear Jonathan,

RE:Project 3/2002: THE AUSTRALIAN PROSPECTIVE DIABETES STUDY (APDS)
Consent Form to Access Medical Records version 1: dated 19/4/04.
Patient Explanatory Brochure/Booklet versions dated March 2004.
Additional Questionnaires (birth weight and health care use)
Study Extension to 13/6/2006

Following recent consideration of your study amendment application, I am pleased to advise that the Committee has recommended that the above be approved.

Thank you for submitting your research proposal to the Ethics Committee

Yours sincerely

[redacted]

Professor Paul Komesaroff
Chair IDI Ethics Committee

p/lp/apdsapproval

250 Kooyong Rd, CAULFIELD VICTORIA, AUSTRALIA 3162 • Tel (+613) 9258 5050 • Fax (+613) 9258 5090 • www.diabetes.com.au
INCORPORATING • WHO Collaborating Centre for Diabetes and Health Promotion • MOW Diabetes Education Centre
IN AFFILIATION WITH • Deakin University • IN ASSOCIATION WITH • Caulfield General Medical Centre and Monash University

International Diabetes Institute

Ethics Committee

Certificate of Approval

This is to certify that Project 3/2002

THE AUSTRALIAN PROSPECTIVE DIABETES STUDY (APDS)
EXTENSION

Chief Researchers: Professor Paul Zimmet
A/P Jonathan Shaw

has been approved as per your application and subsequent amendments on the understanding that you observe the National Health and Medical Research Council Statement on Human Experimentation.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Period of Approval: 14/6/2005 to 30/6/2010

Extension of your project beyond this date will require approval from the Ethics Committee. An annual progress report is required and is due on 30 June 2006. Request for Amendment, Request for Extension, and Progress Report forms are available from The Secretary, Ethics Committee, International Diabetes Institute, 250 Kooyong Road, Caulfield 3162.

Chairperson, Ethics Committee

Professor Paul Komesaroff [redacted]

Date 11/11/05

CARE • EDUCATION • RESEARCH

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Celebrating 20 years 1985 – 2005

ABN 16 007 342 412

11/05/2007

A/Prof Jonathan Shaw
Director Research
IDI, 250 Kooyong Rd
CAULFIELD 3162

Dear Jonathan,

RE; Project No 3/2002 The Australian Prospective Diabetes Study. (APDS)

PROTOCOL AMENDMENT Dated 8th May 2007

.

Following consideration of your request for approval of an amendment to the above study by the Ethics Committee, I am pleased to advise that the amendment has been approved.

Thank you for bringing these changes to the Committee's attention

Kind regards

[redacted]

Professor Paul Komesaroff
Chair
IDI Ethics Committee

P/lp/amendapprove

PO BOX 227, CAULFIELD SOUTH, VICTORIA 3162, AUSTRALIA
250 Kooyong Rd, CAULFIELD, VICTORIA 3162, AUSTRALIA • Tel (+613) 9258 5050 • Fax (+613) 9258 5090 • www.diabetes.com.au
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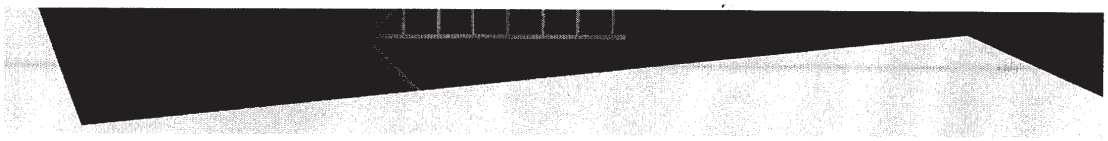
IN AFFILIATION WITH • Deakin University • IN ASSOCIATION WITH • Caulfield General Medical Centre and Monash University

IDI 007



Australian Government
Australian Institute of Health and Welfare

26 Thynne Street
Fern Hill Park
Bruce ACT
GPO Box 570
Canberra ACT 2601
Ph 02 6244 1000
Fax 02 6244 1299

Dr Jonathan Shaw
International Diabetes Institute (IDI)
250 Kooyong Road
CAUFIELD VIC 3162

Dear Dr Shaw

Re: EC358 The Australian Prospective Diabetes Study (APDS) - A five year follow up study of the Australian Diabetes, Obesity and Lifestyle study (AusDiab) population cohort

The Australian Institute of Health and Welfare's Ethics Committee has found your submission to access the National Death Index (NDI) to be acceptable on ethical grounds. Enclosed is a copy of the certificate issued by Ethics Committee, decision number EC358 of 25 May 2004. Please quote this decision number (EC358) in all future correspondence.

The preferred format for data for matching projects is described in the NDI data provision package, which can be downloaded from the AIHW web-site: http://www.aihw.gov.au/cancer/ndi/index.html.

Please organize the data for your project in the preferred format and send it to the Institute on a disk or by emailing an encrypted and/or password protected EXCEL or text file. If you chose to send the data via email, please send an advance email containing any passwords and decryption instructions.

The postal address is:

Health Registers and Cancer Monitoring Unit
Australian Institute of Health and Welfare
GPO Box 570
Canberra ACT 2601.
Attention: Ian McDermid (02 6244 1230)

The courier address is

26 Thynne Street (6A Traeger Court)
Fern Hill Park, Bruce ACT

Email addresses are:

cancer@aihw.gov.au (for general queries)
Ian.McDermid@aihw.gov.au (for transfer of data)

MONASH University

Standing Committee on Ethics in Research Involving Humans (SCERH)
Research Office

Assoc Prof Jonathan Shaw
School of Nursing and Midwifery
Faculty of Medicine, Nursing and Health Sciences
Alfred Hospital

18 December 2006

2006/986MC - High blood sugar is linked to heart disease, stroke and mortality

Dear Researchers,

The above research project has been considered by the Standing Committee on Ethics in Research Involving Humans and approval has been given. This approval will be ratified at meeting A1/2007 on 6 February 2007. It is possible that issues may be raised by the Committee at that meeting. If you do not hear anything further you may assume that approval for the project is confirmed.

Terms of approval

1. This project is approved from 18 December 2006 to 30 June 2010 and this approval is only valid whilst you hold a position at Monash University.
2. It is the responsibility of the Chief Investigator to ensure that, if relevant, all information that is pending is forwarded to SCERH. You will then receive a letter from SCERH confirming that we have received the information.
3. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval and to ensure the project is conducted as approved by SCERH.
4. You should notify SCERH immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.
5. **Amendments to the approved project:** Changes to any aspect of the project require the submission of a Request for Amendment form to SCERH and must not begin without written approval from SCERH. Substantial variations may require a new application.
6. **Future correspondence:** Please quote the project number and project title above in any further correspondence.
7. **Annual reports:** Continued approval of this project is dependent on the submission of an Annual Report. Please provide the Committee with an Annual Report determined by the date of your letter of approval.
8. **Final report:** A Final Report should be provided at the conclusion of the project. SCERH should be notified if the project is discontinued before the expected date of completion.
9. **Monitoring:** Projects may be subject to an audit or any other form of monitoring by SCERH at any time.
10. **Retention and storage of data:** The Chief Investigator is responsible for the storage and retention of original data pertaining to a project for a minimum period of five years.

All forms can be accessed at our website www.monash.edu.au/resgrant/human-ethics

We wish you well with your research.

[redacted]

pp Mrs Lyn Johannessen
Acting Human Ethics Officer (on behalf of SCERH)

Cc: Ms Elizabeth Barr

Postal - Monash University, VIC 3800, Australia
Building 3E, Room 111, Clayton Campus, Wellington Road, Clayton
Telephone +61 3 9905 5490 Facsimile +61 3 9905 1420
Email scerh@adm.monash.edu.au www.monash.edu.au/research/ethics/human/index.html
CRICOS Provider No. 00008C ABN 12 377 614 012

MONASH University

Standing Committee on Ethics in Research Involving Humans (SCERH)
Research Office

Assoc Prof Jonathan Shaw
International Diabetes Institute
250 Kooyong Rd
Caulfield 3162

30 May 2007

2006/986MC: High blood sugar is linked to heart disease, stroke and mortality

Dear Researchers,

Thank you for submitting your Request for Amendment form with respect to the above named project.

This is to advise that the requested amendments dated 15 May 2007, received in our office on 21 May 2007, have been approved and the project can proceed according to your approval given on 18 December 2006.

Thank you for keeping the Committee informed.

[redacted]
Mrs Lyn Johannessen
Acting Human Ethics Officer (on behalf of SCERH)

Cc: Prof Andrew Tonkin, Ms Elizabeth Barr

Postal – Monash University, Vic 3800, Australia
Building 3E, Room 111, Clayton Campus, Wellington Road, Clayton
Telephone +61 3 9905 5490 Facsimile +61 3 9905 1420
Email scerh@adm.monash.edu.au www.monash.edu/research/ethics/human/index/html
ABN 12 377 614 012 CRICOS Provider #00008C

CONFIDENTIALITY OF HEALTH INFORMATION COMMITTEE (CHIC)
An Independent Committee appointed by the Minister for Health in Western Australia

Please address all correspondence to:-
Executive Officer - CHIC
Information Collection and Management
1st Floor 'C' Block
189 Royal Street
EAST PERTH WA 6004

ph: (08) 9222 4194
fax: (08) 9222 4236

Dr Timothy Welborn
Dept of Medicine, UWA
55 Hamden Road
NEDLANDS WA 6009

Dear Dr Welborn

#200722 **Verification of cardiovascular disease events: The Australian Diabetes, Obesity and Lifestyle Study**

Date of commencement	01/09/2007		
Date of completion	28/02/2009		
Researchers accessing identifiable data	Dr Timothy Welborn Prof Andrew Tonkin	Prof Paul Zimmet Elizabeth Barr	A/Prof Jonathan Shaw
Data linkage required	Yes (linkage to Ausdiab data)		
Datasets to be accessed	Hospital Morbidity Data System		
Ethics approval	International Diabetes Institute valid from 14/06/2005 expires 30/06/2010 Monash University dated 18/12/2006 (amended 30/05/2007) expires 30/06/2010		

Thank you for your application, which was received on 28/05/2007. The Confidentiality of Health Information Committee (CHIC) reviewed the protocol at the meeting held on 13/06/2007.

Approval is granted for you to proceed with your project subject to and conditional upon compliance with the following points:

- It is the responsibility of the researcher(s) to advise CHIC of any change to the above information or to the design protocol. Major changes to protocol that affect access to and use of data from the Department of Health datasets must be approved by CHIC and your Ethics Committee.
- CHIC has a mandate to monitor the use of any data released for access. Monitoring includes the submission of an annual progress report, and a final report required at the completion of the project. Failure to submit reports may result in termination of access to data.
- CHIC reserves the right to monitor the progress of a project more intensively, as it sees fit. This monitoring may include site visits, interviews or documentation checks.

This approval is contingent on your successful liaison with the relevant Data Collections Managers.

Please note that data must be in an encrypted format when being transported from the WA Department of Health. Further, for additional security, CHIC recommends that the identifiable demographic data be transported separately from the other personal health information.

We wish you well with your project.

Yours sincerely

[redacted] [redacted]

Dr David Blackledge
CHAIRPERSON
CONFIDENTIALITY OF HEALTH INFORMATION COMMITTEE

Dr Tim Threlfall
DIRECTOR GENERAL'S DEPUTY REPRESENTATIVE

18 June 2007

cc. Ms Elizabeth Barr

SD/fm/2007-042
Ext 2999

13th March 2007

Emeritus Prof Tim Welborn
C/ Liz Barr
International Diabetes Institttue
250 Kooyong Road
CAULFIELD VIC 3162

Dear Prof Tim Welborn

HUMAN RESEARCH ETHICS 2007-042 Australian diabetes, obesity and lifestyle study (AusDiab)

Please be advised that the Human Research Ethics Committee has granted expedited ethical approval of the project. I note that Emeritus Prof Tim Welborn is the principal investigator on the Sir Charles Gairdner Hospital site and as such will take responsibility for the study on this site. The approval is for the above named protocol and participant documents. Approval is granted on the understanding that the project will commence within twelve months of the date of this letter or a new application may have to be submitted. Equally if the project is discontinued before the expected date of completion the committee must be informed and the reasons provided for the cessation.

Please be advised that this Committee complies with the National Statement on Ethical Conduct in Research involving Humans by the National Health and Medical Research Council (NH&MRC) and as such has responsibility to monitor the progress of all approved projects until completion to ensure that they continue to conform to approved ethical standards.

It is the responsibility and obligation of the researcher under the Good Clinical Practice (GCP) guidelines to advise the Committee of any departure from the original protocol that could impact on the ethical approval of the study. Please note that the attachment entitled "Reporting Guidelines for Adverse Events and Deviations from Protocol" forms part of this approval letter. Under these reporting guidelines you are required to submit formal notice of any changes to documentation, relevant information arising out of ongoing safety monitoring and annual reports on the human rights aspects of your study. An annual report form for your study will be posted to you several weeks in advance of the anniversary of the project's approval date.

As the responsibility for the conduct of the trial lies with you as the investigator, you should sign all communications to the committee.

Please quote Ethics number: 2007-042 on all correspondence associated with this study.

Yours sincerely

MS SUE DAVIS
CHAIR
HUMAN RESEARCH ETHICS COMMITTEE

APPENDIX 3

A3. Consent forms

Australian Diabetes, Obesity and Lifestyle Study

CONSENT TO PARTICIPATE & FORWARD RESULTS

I, _____ _____, of
(First Name) *(Last name)*

_____ _____ _____
(Address) *(Suburb)* *(Postcode)*

Date of Birth_____

have been asked to participate in the Australian Diabetes, Obesity and Lifestyle Study. I give my consent on the understanding that:

i. the survey has been approved by the International Diabetes Institute Human Ethics Committee.
ii. the purpose of the survey, the expected length of time the examination will take, and an indication of any discomfort which may be expected, has been explained to me.
iii. the information, urine and blood specimens collected in the course of the study will be used for research purposes only, the results of which will be published in scientific journals or reports in such a way that individual participants cannot be identified.
iv. the results of my assessment will be given to me and/or my doctor if I wish.
v. my own answers in any of the questionnaires undertaken and the results of my tests will not be released to anyone, even to my own doctor, without my specific permission.
vi. I can withdraw from the project at any time.
vii. I have had all my questions concerning involvement in the survey answered to my satisfaction.

Under the provision that my name will be used only by researchers involved in this project,
I **do** □ **/ do not** □ (please tick one box) agree to have my name added to the register of participants available to be followed up for future studies on diabetes and diabetes-related conditions.

Signed_____ Date_____/_____/_____

Witness_____ Date_____/_____/_____

To whom would you like your results sent? *(Please tick the appropriate box)*

To myself ☐ To my doctor* ☐

* If you want your results sent to your doctor, please write the name and address below.

Dr_____

_____ _____ _____
(Address) (Suburb) (Postcode)

International Diabetes Institute
250 Kooyong Road, Caulfield Victoria 3162

CONSENT FORM FOR RESEARCH PROJECT FOR COMPETENT SUBJECTS

TITLE: The AusDiab Study - A five year follow up study of the AusDiab population based cohort *(NHMRC application ID 233200)*

CONSENT TO ACCESS MEDICAL RECORDS

I,_____ *(First Name) (Last name)*

of

_____ _____ _____
(Address) *(Suburb)* *(Postcode)*

Date of Birth_____

have been asked to participate in the AusDiab Study. As part of this study, I hereby give my consent for copies of my medical records to be provided to the AusDiab investigators at the International Diabetes Institute. This is on the understanding that:

i. the survey has been approved by the International Diabetes Institute Human Ethics Committee.
ii. my own answers in any of the questionnaires undertaken and the results of my tests will not be released to anyone, even to my own doctor, without my specific permission.
iii. I can withdraw from the project at any time.
iv. I have had all my questions concerning involvement in the survey answered to my satisfaction.
v. My consent does not extend beyond 5 years from the date of signing.

Signed_____
Date_____/_____/_____

Witness_____
Date_____/_____/_____

Version 1. 19.04.2004

APPENDIX 4

A4. Existing health conditions questionnaire

AusDiab Existing Health Conditions Questionnaire

ID number: *[insert mail merge field]*
Name: *[insert mail merge field]*
Date of birth: *[insert mail merge field]* **(confirm)**
Sex: *[insert mail merge field]*

Results from 1999-2000

Diabetes: *[insert mail merge field]*
Angina: *[insert mail merge field]*
Stroke: *[insert mail merge field]*
Heart Attack: *[insert mail merge field]*

1a) Have you ever been told by a doctor or nurse that you have **diabetes**?

☐ Yes **(women go to Q1b, men go to Q2a)**
☐ No (if yes last time, investigate and record reason for discrepancy) **(otherwise go to Q3)**

1b) **(For women only)** was your diabetes first diagnosed when you were pregnant?

☐ Yes **(go to Q1c)**
☐ No **(go to Q2a)**

1c) Has a doctor ever told you that diabetes continued immediately after the end of any pregnancy?

☐ Yes **(go to Q2b)**
☐ No **(go to Q1d)**

1d) Has a doctor ever told you that diabetes developed again at a time that you were not pregnant?

☐ Yes **(go to Q2c)**
☐ No **(go to Q2d)**

2a) How old were you when you were first told you had diabetes?

_____ years **(go to Q2e)** ☐ Don't know **(go to Q2e)**

2b) How old were you at the end of that pregnancy?

_____ years **(go to Q2e)** ☐ Don't know **(go to Q2e)**

2c) How old were you when you were told that diabetes had come back?

_____ years **(go to Q2e)** ☐ Don't know **(go to Q2e)**

2d) Is it correct that you either no longer have diabetes or you are currently pregnant?

☐ Yes **(if either is true) (go to Q3)** ☐ No **(review Q1a-1d)**

2e) What treatment are you currently receiving for your diabetes?

☐ Diet only **(go to Q3)**
☐ Tablets **(go to Q3)**
☐ Insulin **(go to Q2f)**
☐ Insulin and tablets **(go to Q2f)**
☐ Other **(go to Q3)**

2f) At what age did you start having daily insulin injections?

_____ years **(go to Q3)**

3) Have you ever suffered from **gout**?

☐ Yes ☐ No

3a) Has a doctor ever told you that you have broken or fractured any bones since you were 25 years of age?

☐ Yes **(complete table)**
☐ No **(move on to question 3b)**

Please complete the following table if you have had **any breaks/fractures since the age of 25**:

Fracture/Break Site	Age	Cause	Comments/Other

Codes:

FRACTURE/BREAK SITE:
1 = hip
2 = rib
3 = forearm
4 = hand
5 = ankle
6 = spine
7 = humerus
8 = wrist
9 = leg
10 = other (please specify)

CAUSE:
1 = As a result of a fall from standing height or less
2 = Because of a harder fall
3 = From a car accident or other severe trauma
4 = Don't know
5 = Other (please specify)

3b) Do you currently have any kidney problems?

☐ Yes **(go to Q3c)**
☐ No **(go to Q4)**
☐ Don't know **(go to Q4)**

3c) Are your kidney problems a result of:

☐ Infection ☐ Stones ☐ Other

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4) Have you ever suffered from or experienced a stroke or any heart problems. Heart problems include angina, heart attack, heart bypass operation, an angioplasty or stent? **(Check results from 1999-2000 on page 1 of this questionnaire)**

☐ Yes **(Ask Q4a, then go to q5 on part 2 of this questionnaire)**
☐ No **(ask consent question 4a then END of questionnaire, <u>BUT</u> if YES last time, investigate and <u>record</u> reason for discrepancy)**
☐ Don't Know **(Ask Q4a, then go to q5 on part 2 of this questionnaire)**

4a) For us to confirm your medical history we need to check your medical records. Would you mind signing a consent form allowing us to check your details through a medical records search?

(Place barcode sticker (ID number) at top of consent form and ensure participant reads and signs the form and has any questions explained to them)

5) Have you ever been told by a doctor or nurse that you have **angina**?

☐ Yes
☐ No **(if YES last time, investigate and <u>record</u> reason for discrepancy)**

6a) Have you ever been told by a doctor or nurse that you have had a **heart attack** (includes a 'coronary', coronary occlusion, coronary thrombosis, myocardial infarction)?

☐ Yes **(go to Q6b)**
☐ No **(go to Q7a) (if YES last time, investigate and <u>record</u> reason for discrepancy)**

6b) Please estimate the date of the heart attack(s): (/) (mm/yy)
(/) (mm/yy)
(/) (mm/yy)

6c) To which hospital(s) were you admitted when you had the heart attack(s)?

(name, location) .....

(name, location) .....

(name, location) .....

6d) If you were transferred to another hospital(s), could you please tell us the name and location of that hospital(s)?

(name, location) .....

(name, location) .....

(name, location) .....

7a) Have you ever been told by a doctor or nurse that you have had a **stroke**?

☐ Yes **(go to Q7b)**
☐ No **(go to Q8a) (if YES last time, investigate and <u>record</u> reason for discrepancy)**

7b) Please estimate the date of the stroke(s): (/) (mm/yy)
(/) (mm/yy)
(/) (mm/yy)

7c) To which hospital(s) were you admitted when you had the stroke(s)?

(name, location) .....

(name, location) .....

(name, location) .....

7d) If you were transferred to another hospital(s), could you please tell us the name and location of that hospital(s)?

(name, location) .....

(name, location) .....

(name, location) .....

8a) Have you ever had a heart bypass operation (includes coronary bypass)?

☐ Yes **(go to Q8b)**
☐ No **(go Q9a)**

8b) Please estimate the date of the heart bypass operation(s): (/) (mm/yy)
(/) (mm/yy)
(/) (mm/yy)

8c) To which hospital(s) were you admitted when you had the heart bypass operation(s)?

(name, location) .....

(name, location) .....

(name, location) .....

8d) If you were transferred to another hospital(s), could you please tell us the name and location of that hospital(s)?

(name, location) .....

(name, location) .....

(name, location) .....

9a) Have you ever had an angioplasty or stent for your heart (includes 'coronary angioplasty', 'coronary stent', 'balloon')?

☐ Yes **(go to Q9b)**
☐ No **(end of questionnaire)**

9b) Please estimate the date of the angioplasty or stent(s): (/) (mm/yy)
(/) (mm/yy)
(/) (mm/yy)

9c) To which hospital(s) were you admitted when you had the angioplasty or stent(s)?

(name, location) .....

(name, location) .....

(name, location) .....

9d) If you were transferred to another hospital(s), could you please tell us the name and location of that hospital(s)?

(name, location) .....

(name, location) .....

(name, location) .....

APPENDIX 5

A5. Media coverage

Journal Report
06/18/2007

Pre-diabetes more than doubles risk of heart disease death

American Heart Association rapid access journal report

DALLAS, June 19 — The risk of dying from heart disease increases with the earliest sign that the body is having trouble metabolizing glucose, according to research reported in *Circulation: Journal of the American Heart Association.*

In the Australian Diabetes, Obesity and Lifestyle Study (AusDiab), participants with impaired fasting glucose, a condition considered pre-diabetes, "after five years were more than twice as likely to die of cardiovascular disease," said Elizabeth L.M. Barr, M.P.H., lead author of the study at the International
Diabetes Institute in Melbourne, Australia.

"Moreover, diabetes and pre-diabetes (impaired glucose tolerance and impaired fasting glucose) accounted for 65 percent of all heart disease deaths in the study of 10,429 Australians."

Researchers classified participants as having known diabetes if they reported that their physician diagnosed them with the condition and were taking drugs to lower blood sugar or had blood tests that confirmed diabetes. People who had not previously been diagnosed with diabetes but had blood tests that indicated diabetes were classified as newly diagnosed diabetes.

After adjusting for other risk factors for heart disease mortality such as age, sex, history of heart disease, smoking, blood pressure, total cholesterol to high-density lipoprotein (HDL, "good" cholesterol) ratio, and waist circumference, the increased risk associated with impaired fasting glucose was about the same as the risk associated with known diabetes.

"The five-year risk of cardiac mortality was 2.6 times higher among people who had diabetes and was 2.5 times higher in those with impaired fasting glucose," Barr said.

Impaired glucose tolerance, by contrast, was associated with a 20 percent increase in risk of cardiac mortality, which was not statistically significant, unlike impaired fasting glucose and known diabetes, which were both independent predictors of heart disease death. Impaired glucose tolerance was, however, predictive of all-cause mortality.

Compared with people who metabolized glucose normally, the five-year total mortality risk was 50 percent higher for people with impaired glucose tolerance and 60 percent higher for people with impaired fasting glucose.

Study participants age 25 or older were enrolled in 1999 and 2000 and followed for a median of 5.2 years. At baseline, all participants underwent an oral glucose tolerance test, fasting serum total cholesterol, triglycerides and HDL measurements.

Impaired fasting glucose was defined as a glucose concentration of 6.1 mmol/l or higher, but less than 7.0 mmol/l, and two-hour plasma glucose of less than 7.8 mmol/l. Impaired glucose tolerance was defined as two-hour plasma glucose of at least 7.8 mmol/l, but less than 11.1 mmol/l and fasting plasma glucose of less than 7.0 mmol/l.

Researchers found:

- 298 deaths occurred during the median 5.2 years of follow-up — an all-cause mortality rate of 5.5 per 1,000 person-years.
- 88 of those deaths were due to heart disease.
- Almost 12 percent of the people who had known diabetes when

they entered the study died during follow-up, versus 6.2 percent of those newly diagnosed with diabetes, 5.2 percent of participants with impaired glucose tolerance and 3.9 percent of those who had impaired fasting glucose.
- The five-year death rate for those who had normal glucose metabolism at baseline was 1.7 percent.

“This study confirms the clinical importance of pre-diabetes, and suggests the need to target glucose abnormalities with lifestyle interventions (diet and exercise) to prevent these from progressing to pre-diabetes and diabetes,” Barr said.

Five million adults in the United States have undiagnosed diabetes and 56.5 million have pre-diabetes, according to the American Heart Association.

Co-authors are Paul Z. Zimmet, Ph.D.; Timothy A. Welborn, Ph.D.; Damien Jolley, M.Sc.; Dianna J. Magliano, Ph.D.; David W. Dunstan, Ph.D.; Adrian J. Cameron, M.P.H.; Terry Dwyer, M.D.; Hugh R. Taylor, M.D.; Andrew M. Tonkin, M.D.; Tien Y. Wong, Ph.D.; John McNeil, Ph.D.; and Jonathan E. Shaw, M.D.

Editor’s note: The American Heart Association program The Heart Of Diabetes[SM] is a national education and action program to help reduce the risk for cardiovascular disease — the leading cause of death for people with diabetes. People with type 2 diabetes who wish to join the free program can call the American Heart Association toll-free at **1-800-AHA-USA1 (1-800-242-8721)** or visit: **americanheart.org/diabetes**.

Statements and conclusions of study authors that are published in the American Heart Association scientific journals are solely those of the study authors and do not necessarily reflect association policy or position. The American Heart Association makes no representation or warranty as to their accuracy or reliability.

NR07 – 1161 (Circ/Barr)

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REUTERS UK

Diabetes poses health risks early on, study finds

Mon Jun 18, 2007 9:01pm BST

CHICAGO (Reuters) - Diabetes is dangerous even before the disease becomes full-blown, boosting the risk of death from heart disease in its earliest form, Australian researchers said on Monday.

Before most people develop type 2 diabetes, they have trouble metabolizing sugar, a problem known as pre-diabetes that affects 56 million people in the United States.

Elizabeth Barr of the International Diabetes Institute in Melbourne, Australia, said that a large study found people with pre-diabetes had more than double the risk of death from heart disease after five years.

Type 2 diabetes is linked with obesity, poor diet and lack of exercise and is becoming a growing problem in many parts of the world. It can lead to blindness, limb loss, heart disease and early death.

Barr and colleagues studied 10,429 Australians age 25 or older for about five years. Patients were considered pre-diabetic if they had abnormal blood glucose levels after fasting.

These patients have a 2.5 times higher risk of death from heart problems than those who metabolized glucose normally, said the researchers, whose work was published in the journal Circulation.

"This study confirms the clinical importance of pre-diabetes, and suggests the need to target glucose abnormalities with lifestyle interventions," Barr said in a statement.

Studies have shown that people with pre-diabetes can prevent type-2 diabetes through dietary changes and increased physical activity.

ABC News

Study flags diabetes heart risk

Posted Tue Jun 19, 2007 11:39am AEST

A new Australian study has found that people with early signs of diabetes face a high risk of heart disease even before the condition is full blown.

Elizabeth Barr of the International Diabetes Insitute in Melbourne studied 10,000 people aged over 25.

She found those who had pre-diabetes had a 2.5 times higher risk of death from heart problems than those who were healthy.

More than 2 million Australians have pre-diabetes.

- ***Map:** Melbourne 3000*

Tags: health, diseases-and-disorders, diabetes, heart-disease, science-and-technology, research, melbourne-3000

Abnormal Glucose Metabolism and Mortality

In a population-based, Australian study, even mild forms of hyperglycemia were associated with increased risk.

Diabetes increases the risks for all-cause and cardiovascular-disease mortality. To explore whether hyperglycemia at nondiabetic levels increases such risks as well, investigators from the Australian Diabetes, Obesity, and Lifestyle Study compared outcomes data from participants with normal glucose tolerance (NGT) with those from participants with known diabetes mellitus (KDM), newly diagnosed diabetes (NDM), impaired fasting glucose (IFG), or impaired glucose tolerance (IGT).

Between May 1999 and December 2000, glucose tolerance status was determined for 10,428 participants, who were subsequently followed for a median of 5.2 years. The all-cause mortality rate for the entire cohort was 5.5 per 1000 person-years (298 deaths in all). Overall, 11.8% of participants with KDM died, compared with 6.2% of participants with NDM, 5.2% of those with IGT, 3.9% of those with IFG, and 1.7% of those with NGT. Mortality decreased with decreasing severity of glucose intolerance in both men and women. Risks for all-cause and CVD mortality were elevated for all categories of abnormal glucose metabolism, although the increase was not statistically significant for all-cause and CVD mortality in participants with NDM, or for CVD mortality in participants with IGT. Of the 260 deaths with cause-specific mortality data, 172 (66.2%) were classified as non-CVD, and 102 non-CVD deaths (59.3%) were attributed to malignant neoplasms. KDM and IFG, but not IGT, were independent predictors of CVD mortality after adjustment for age, sex, and traditional CVD risk factors.

Comment: These results reinforce the strong link between abnormal glucose metabolism and increased mortality. Clinicians must place greater emphasis on screening for and primary prevention of hyperglycemia, especially given the current epidemics of obesity and metabolic syndrome.

— ***Joel M. Gore, MD***

Published in Journal Watch Cardiology *June 27, 2007*

Citation(s):

Barr ELM et al. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance. The Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation* 2007 Jun 18; [e-pub ahead of print].
(http://dx.doi.org/10.1161/CIRCULATIONAHA.106.685628)