Relationships between Different Measures of Glycaemia and Their Relevance to Diabetic Complications

Thesis submitted for the degree of Doctor of Philosophy by research at the University of Oxford

by

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Trinity Term, 2006

Abstract

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Diabetes is a chronic disease characterised by high blood glucose levels. Monitoring of blood glucose plays an important role in the management of diabetes. Although, several different glycaemic measures are used in clinical practice, glycated haemoglobin (HbA1c) is increasingly accepted as the standard on which many clinical decisions are based. In this thesis, the relationship between HbA1c and different blood glucose measures will be examined and their relevance to diabetic complications will be investigated. This work is for people at increased risk of type 2 diabetes and for those with newly diagnosed type 2 diabetes.

Using the blood glucose profiles available from continuous glucose monitoring, the relationships between HbA1c and derived blood glucose measures were studied. Mean blood glucose and blood glucose peaks equally predicted HbA1c. The correlation of FPG to HbA1c was weaker than that of mean blood glucose.

The haemoglobin glycation index (HGI) is defined as the difference between a subject's HbA1c and that expected from regression of HbA1c on FPG and captures the variation in HbA1c not attributable to FPG. I used Early Diabetes Intervention Trial (EDIT) and UK prospective diabetes study (UKPDS) data to determine whether HGI is reproducible and whether differences relate to potential confounders. In both studies, HGI was reproducible and not explained by the potential confounders examined.

I examined whether there were additive effects of FPG, HbA1c or HGI on diabetic complications using UKPDS data since it was found previously that either HbA1c or FPG was related to diabetic complications in UKPDS. For microvascular complications, both FPG and HbA1c were independent risk factors. When HGI was related to microvascular complications, HGI made an independent contribution adjusting for either FPG or HbA1c. For macrovascular complications, HbA1c but not FPG was an independent risk factor. HGI did not make an independent contribution to macrovascular complications adjusting for HbA1c.

My work showed that the same HbA1c level does not necessarily have the same clinical meaning in two different subjects. There can be marked differences in individual levels of HbA1c relative to FPG values in people with type 2 diabetes. Thus, HbA1c levels should be interpreted with caution and treatment decisions need to be made considering individual glycaemic control. HGI may be useful, informing healthcare professionals of the likely magnitude and direction of difference between observed HbA1c levels and those expected in the light of prevailing blood glucose levels.

Acknowledgements

This thesis is the result of three and half years of work throughout which I have been accompanied and supported by many people. I am grateful to have this opportunity to express my gratitude to all of them.

The first person I would like to thank is my supervisor Dr. Richard Stevens. He has provided me not only with supervision and scientific advice but also with continual encouragement. I would not have had this doctoral experience if it had not been for his support. I am also very grateful to another supervisor, Professor Rury Holman, for inspiring me with his enthusiasm, integral view on research and for helping me to understand the importance of my research by giving me a clinician's perspective.

I would like to thank Dr. Anne Clark for providing me with motherly support and tips which helped me to stay on the right track. I would also like to thank my colleagues within the statistical modelling group, Ruth Coleman and Genevieve Heins, for being such good friends throughout. I am grateful to all the rest of the academic and support staff within the Diabetes Trials Unit, particularly Irene Stratton and Carole Cull for their suggestions and advice regarding my statistical analysis. I would also like to thank all the OCDEM graduate students, particularly Minal, Mary, Katherine, Diva and Janine who have shared joy and difficulties during the PhD course.

Finally, I would like to say 'thank-you' to all my friends and family, wherever they are, particularly to my parents and most importantly of all to my husband, Hyeongjin, for being so patient and supportive during the last year.

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Abbreviations Used in This Thesis

2HPG	2h post-challenge glucose
ADA	American Diabetes Association
BG	blood glucose
CGM	continuous glucose monitoring
CI	confidence interval
CVD	cardiovascular disease
DCCT	Diabetes Control and Complications Trial
DM	diabetes mellitus
DR	discriminant ratio
EDIT	Early Diabetes Intervention Trial
FBG	fasting blood glucose
FPG	fasting plasma glucose
GG	glycosylation gap
HbA1c	glycated haemoglobin
HGI	haemoglobin glycation index
HR	hazard ratio
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
MBG	mean blood glucose
NGT	normal glucose tolerance
OGTT	oral glucose tolerance test
PPG	postprandial glucose
UKPDS	UK prospective diabetes study
WHO	World Health Organisation

1 Literature review and outline of thesis

1.1 Scope of this thesis

Diabetes mellitus is a chronic disease characterised by high blood glucose (sugar) levels, i.e. hyperglycaemia, which is strongly associated with diabetic complications. Therefore, monitoring of blood glucose levels plays an important role in management of diabetes and different measures are in use to determine glycaemic exposure. In this thesis, relationships between different blood glucose measures over time will be investigated first and then their relevance to diabetic complications will be examined. This work is focused on people at increased risk of type 2 diabetes and those with newly diagnosed type 2 diabetes (the most common type) but not type 1 diabetes.

1.2 Definition and diagnosis of diabetes

Diabetes mellitus is a metabolic disease characterised by high blood glucose levels, i.e. hyperglycaemia, caused by an absolute or relative failure of insulin secretion and varying degrees of insulin insensitivity. Insulin is a hormone which is a major regulator of the level of glucose in the blood. Chronic high blood glucose levels are associated with diabetic complications including dysfunction of various organs such as eyes, kidneys, nerves, heart and blood-vessels.

Type 1 and Type 2 diabetes

There are two major types of diabetes – type 1 and type 2. Type 1 diabetes, which accounts for 5-10% of those with diabetes, was known previously as insulin-dependent diabetes mellitus (IDDM) since insulin is needed to treat it. It is an auto-immune disease usually diagnosed in children and young adults. People with type 1

diabetes cannot produce any insulin and need to be on permanent insulin replacement therapy. Type 2 diabetes, which accounts for 80-90% of diabetes, was known as non-insulin dependent diabetes (NIDDM) since insulin treatment is not essential at least initially although the majority will eventually require insulin to help maintain optimal glycaemic control. Type 2 diabetes, which usually occurs later in life, is caused by a declining insulin secretary capacity in people who often have reduced insulin sensitivity.

Other forms of diabetes

There are also rarer forms of diabetes such as gestational diabetes and maturity onset diabetes of the young (MODY). Gestational diabetes is defined as onset of diabetes or any degree of glucose intolerance first recognised during pregnancy (American Diabetes Association 2006). MODY is associated with monogenetic defects in the β-cells in the pancreas which produce and release insulin. MODY is characterised by onset of hyperglycaemia at an early age (generally before age 25 years old) (American Diabetes Association 2006). People with MODY have impaired insulin secretion but usually have normal insulin sensitivity.

Pre-diabetes

Subjects whose glucose levels are lower than the criteria for diabetes mellitus (DM) but above those for normal glucose tolerance (NGT) have been recognised as an intermediate group by the World Health Organisation (WHO) (World Health Organisation 1999) and the American Diabetes Association (ADA) (American Diabetes Association 2005). People in this intermediate group are now referred to as

having "pre-diabetes" by the ADA indicating their relatively high risk for development of diabetes (American Diabetes Association 2006).

Diagnostic criteria for pre-diabetes and diabetes

The classification of subjects is based on fasting plasma glucose (FPG) and 2h post-challenge glucose (2HPG) levels from an oral glucose tolerance test (OGTT) using criteria selected by the WHO (World Health Organisation 1999) and the ADA (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). FPG is measured in a blood sample taken after fasting for at least 8 hours. 2HPG is measured in a blood sample taken 2 hours after oral administration of 75g anhydrous glucose. The 1999 WHO criteria for the diagnosis of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes are shown in Table 1.1 (World Health Organisation 1999). Pre-diabetes includes people with IFG and/or IGT (American Diabetes Association 2006).

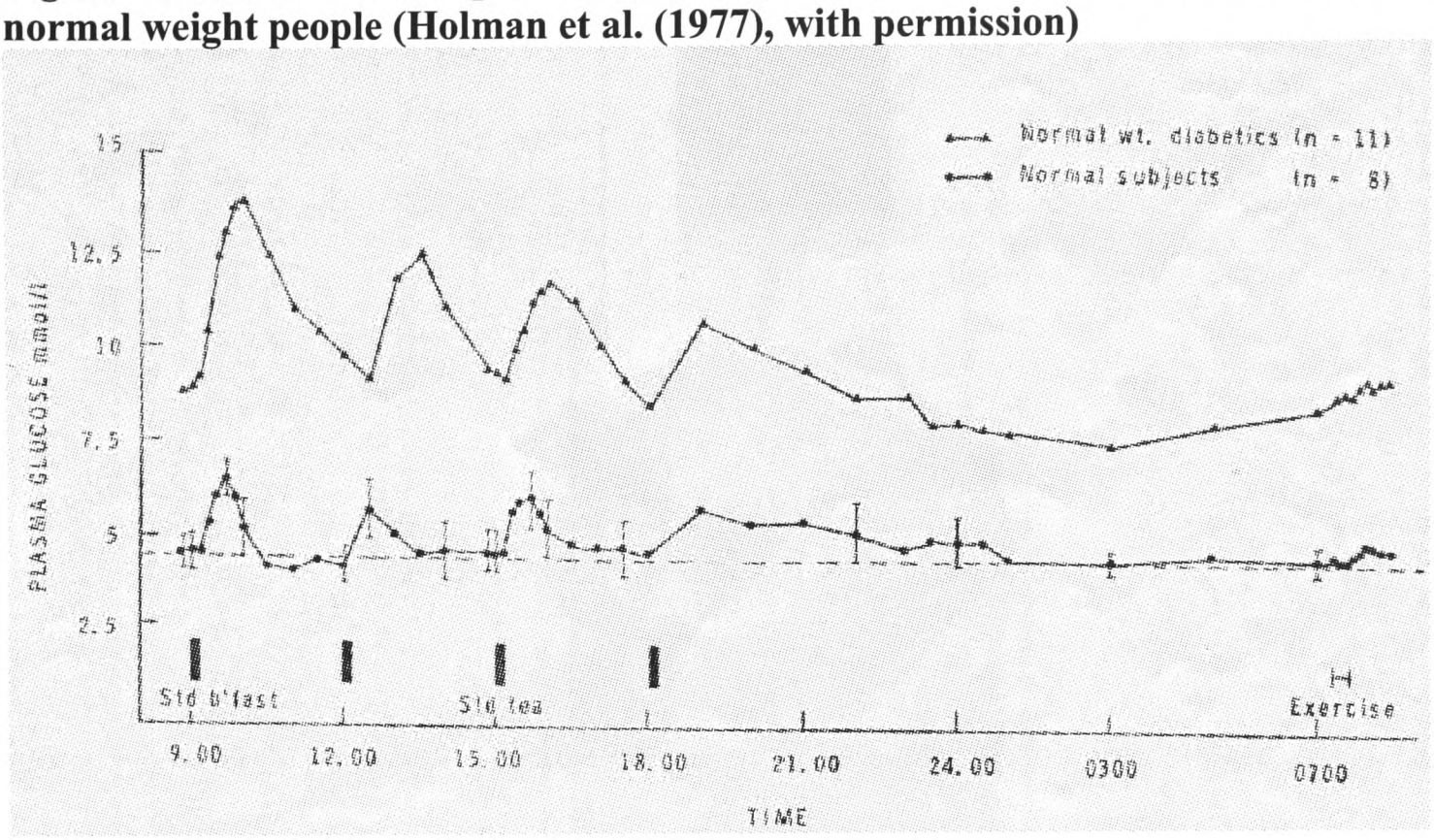
Table 1.1 Plasma glucose criteria for diagnosing IFG, IGT and Diabetes

Glucose levels	Normal (NGT)	Pre-diabetes	Diabetes Mellitus
Fasting (mmol/l)	FPG < 6.1	6.1 ≤ FPG <7.0 (IFG)	FPG ≥ 7.0
Post-challenge (mmol/l)	2HPG < 7.8	7.8 ≤ 2HPG <11.1 (IGT)	2HPG ≥ 11.1

1.3 Blood glucose profiles in people with diabetes

In the absence of diabetes, blood glucose levels stay within narrow limits throughout the day due to insulin which controls tissue uptake of glucose. Blood glucose levels rise after meals and are usually lowest in the morning, before the first meal of the day. In people with type 2 diabetes, the excursion of blood glucose after meals is higher than in non-diabetic people since their peak insulin level is delayed and insufficient and fasting blood glucose levels are elevated (Holman and Turner 1977).

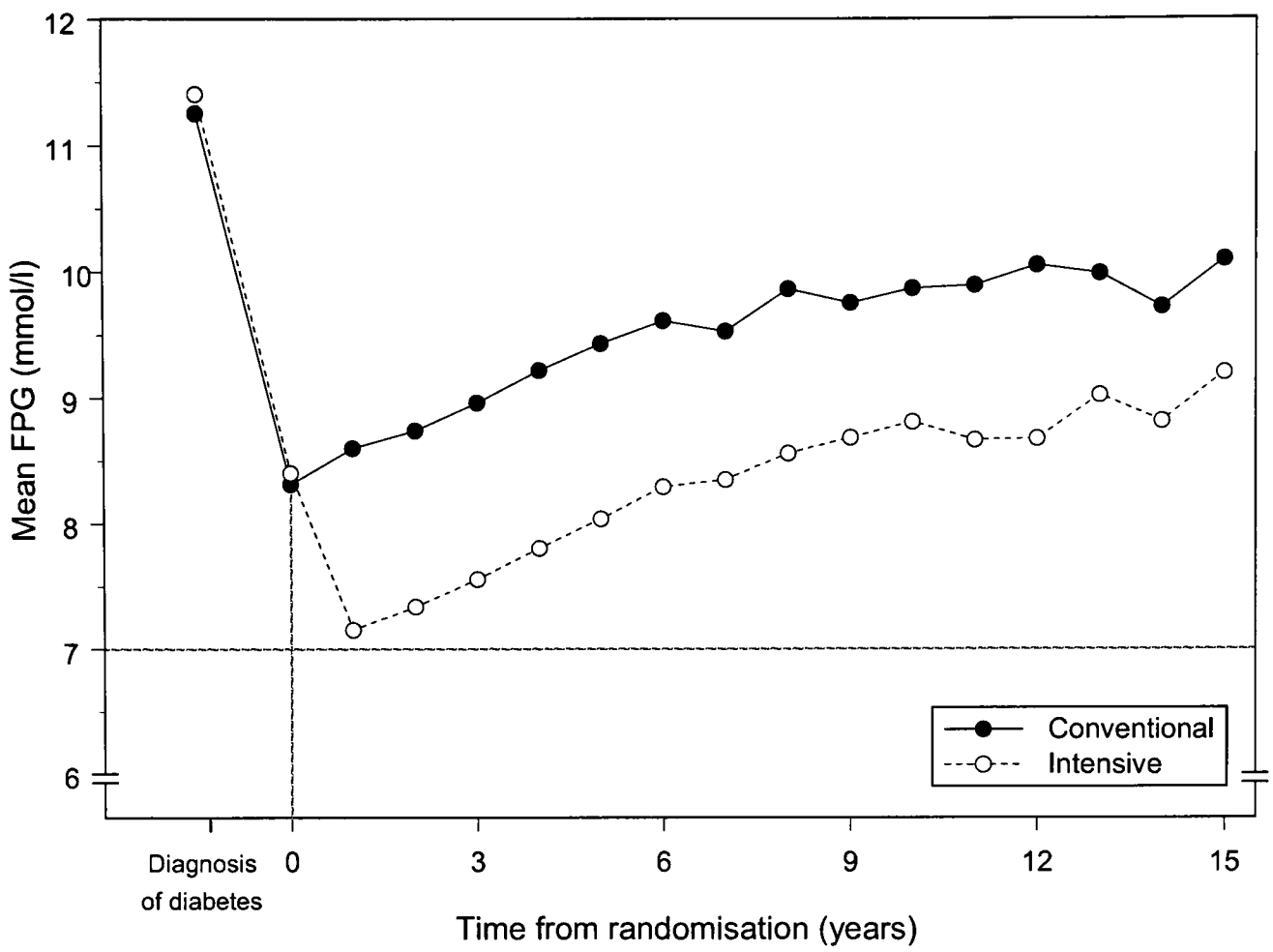
Figure 1.1 Mean 24 hour plasma glucose profiles for non-diabetic and diabetic normal weight people (Holman et al. (1977), with permission)



In Figure 1.1, Holman et al. (Holman and Turner 1977) showed mean 24 hour plasma glucose profiles in diabetic and non-diabetic people, depicting the elevated basal glucose level on which the exaggerated glucose excursions after meals are superimposed.

1.4 Progressive hyperglycaemia in type 2 diabetes

Figure 1.2 Fasting plasma glucose levels in conventional and intensive glucose control policy groups in the UKPDS



Diabetes is a complex and progressive disease. The UK prospective diabetes study (UKPDS) (UKPDS Group 1998) was a large, multicentre, randomised trial of patients with newly diagnosed type 2 diabetes. It was designed to study whether more intensive blood glucose control could reduce the risk of diabetic complications. After the diagnosis of diabetes, patients entered a dietary run-in lasting three or four months, followed by randomisation. Figure 1.2 shows FPG levels over time in the UKPDS. In both the conventional and intensive glucose control policy groups, FPG decreased markedly during the dietary run-in period and then increased steadily over time despite the initial further decrease in FPG during the first year in the intensive group. This indicates that hyperglycaemia in type 2 diabetes increases over time regardless of the therapies used in the UKPDS.

1.5 Health care burden of diabetes

The number of people who suffer from diabetes is increasing all over the world and it has become a huge public health burden (McKinlay and Marceau 2000; Wagner, Sandhu et al. 2001; Bagust, Hopkinson et al. 2002; World Health Organisation 2003). This is due to the chronic nature of diabetes and the serious complications associated with long disease duration. Data from the United States (American Diabetes Association 1998) and Europe (Jönsson B 2002) indicate that the health care expenditure for patients with diabetes mellitus is significantly higher than for comparable patients without diabetes. It is also suggested that the majority of such 'excess' costs can be attributed to diabetes-related complications (Selby, Ray et al. 1997; Redekop, Koopmanschap et al. 2002; Brandle, Zhou et al. 2003).

1.6 Blood glucose monitoring

Although people with type 2 diabetes are often hypertensive (Vijan and Hayward 2003) and frequently have dyslipidaemia (UKPDS Group 1997), most monitoring methods have focused on glucose since hyperglycaemia is the defining characteristic of the diabetic state (Goldstein, Little et al. 2004). Accordingly, assessing glucose status plays an important role in diabetes care. Results of monitoring are used to assess therapy efficacy and to make adjustments in diet, exercise and medications in order to achieve optimal blood glucose levels.

Fasting glucose

Fasting glucose, measured after fasting at least 8 hours overnight, is widely used by health care providers to assess glycaemic control (American Diabetes Association

2001). The ADA also recommends a fasting glucose test for the diagnosis of diabetes (American Diabetes Association 2004).

Preprandial glucose

Preprandial glucose is the glucose level measured prior to any meal by definition. Thus, preprandial glucose may be only a few hours after the previous food intake in contrast to fasting glucose.

Postprandial glucose

In people without diabetes, the postprandial glucose (PPG) peaks about 1 hour after a meal and returns to normal preprandial levels within 2-3 hours (American Diabetes Association 2002). In type 2 diabetic patients, PPG excursions are not controlled adequately since peak insulin levels are delayed and insufficient. The ADA has concluded that measuring glucose 2 hours after the start of a meal provides a reasonable assessment of postprandial peak hyperglycaemia in type 2 diabetes (American Diabetes Association 2001). PPG testing is not well established since PPG reproducibility is affected by various factors such as timing, meal size, nutrient composition, physical activity, insulin secretion and insulin sensitivity (Buse 2003). It has received more attention recently however as several studies have shown an association between PPG and diabetic complications, especially cardiovascular disease (Balkau, Shipley et al. 1998; Balkau, Bertrais et al. 1999; de Vegt, Dekker et al. 1999; Decode Study Group on behalf of the European Diabetes Epidemiology Group 2001). The development of medications which specifically target PPG has also resulted in an increased interest in PPG levels (Schrot 2004); These medications include rapid-acting and long-acting insulin analogs, rapid acting insulin

secretagogues, alpha-glucosidase inhibitors, injectable amylin analogs, glucagon like peptide 1 (GLP-1) mimetics and dipeptidyl peptidase-IV (DPP-IV) inhibitors.

2-hour post-challenge glucose

PPG is sometimes measured after a standardised meal but an oral glucose tolerance test (OGTT) has been mostly used in epidemiological studies and clinical practice due to its simplicity. OGTT can be performed at clinic. A person after an overnight fast is given a drink containing 75g anhydrous glucose. After drinking it, the glucose level is measured 2 hours later and called the 2-hour post-challenge glucose (2HPG) level. A post-challenge glucose level can also be measured at different times such as 30 minutes, 60 minutes and 90 minutes after the glucose load for research purposes.

Postprandial glucose vs. 2-hour post-challenge glucose

Although post-challenge glucose is used as a surrogate for PPG, how well post-challenge glucose reflects PPG is not well understood since few studies have examined this issue (Bonora and Muggeo 2001). Of course, there is difference between PPG and post-challenge glucose since the ingestion of a mixed meal which contains different nutrients such as protein, lipids and carbohydrates is different from that of a glucose drink which contains carbohydrate only. Nevertheless, a strong correlation between 2h glucose levels after a mixed meal and an oral glucose tolerance test (r=0.97) has been reported (Wolever, Chiasson et al. 1998).

Whole blood glucose vs. plasma glucose

Glucose levels in plasma (one of the components of blood) are generally 10-15% higher than glucose measurements in whole blood. Most laboratory tests measure the

glucose in plasma while some home blood glucose meters measure the glucose in whole blood. This is a potential source of confusion with plasma glucose levels being the preferred option (Saudek, Derr et al. 2006).

1.7 Blood glucose profile

Blood glucose testing mentioned above is a 'spot' measurement that represents a single point in a 24 hour blood glucose profile. Intermittent blood glucose measurements throughout the day have been used to better define blood glucose profiles. Some studies have used seven-point blood glucose profile, measuring blood glucose before and after each meal and before bed time (The DCCT Research Group 1986). Some studies have used 4-6 point blood glucose profiles (Avignon, Radauceanu et al. 1997; Bonora, Calcaterra et al. 2001; Monnier, Lapinski et al. 2003; Cavalot, Petrelli et al. 2006). The recent introduction of continuous glucose monitoring (CGM) technology has enabled more complete glucose profile monitoring with blood glucose measurements, for example, every 5 minutes for up to 3 days. Further details of CGM will be discussed in chapter 2.

1.8 Glycated protein monitoring

Although blood glucose monitoring is important for day-to-day management of diabetes, it does not necessarily provide patients and health care professionals with an adequate long-term measure of glycaemic control. Measurements of glycation such as glycated haemoglobin (HbA1c) and glycated serum proteins (fructosamine) can quantify average glycaemia over weeks or months.

Glycated serum proteins

Glucose can bind to proteins through a non-enzymatic chemical reaction called "glycation". Fructosamine is used to measure the degree of glycation of blood serum proteins – predominantly albumin. It reflects an average of blood glucose levels over the preceding 2 to 3 weeks. However, the use of glycated serum proteins in clinical practice has been questioned since the reliability of the fructosamine assay is variable (Saudek, Derr et al. 2006).

Glycated haemoglobin (HbA1c)

Glucose can bind to haemoglobin in red blood cells through glycation. Glycated haemoglobin, measured as the HbA1c, is proportional to an average of blood preceding 2-3 months (Goldstein, Little et al. 2004). The typical normal range (that found in healthy persons) is 3.9% to 6.1% (Kilpatrick 2000). The ADA and the UK Department of Health have recommended a target HbA1c of less than 7.0% for people with diabetes (Department of Health 2001; American Diabetes Association 2002) although more recently a target of 6.5% has been suggested. (European Association for the Study of Diabetes (IDF Clinical Guidelines Task Force 2005), American Association of Clinical Endocrinologists (AACE) (The AACE and the ACE 2001))

HbA1c provides an average measure of blood glucose levels over preceding 2 to 3 months as red blood cells have a life span of about 120 days. (Nathan, Singer et al. 1984; Meigs, Nathan et al. 1996; Saudek, Derr et al. 2006). Nevertheless, it has been shown that most recent blood glucose levels (i.e., 3-4 weeks earlier) contribute about 50% of the HbA1c level and older blood glucose levels (i.e., 90-120 days) contribute

only up to 10% of the HbA1c level (Tahara and Shima 1995). Thus, HbA1c can be regarded as a "weighted" average of blood glucose levels during the preceding 120 days. The HbA1c test assumes a normal red blood cell aging process. Hence people with altered red blood cell turnover e.g. recent blood loss or anaemia, are not suitable for this test. In these circumstances, the alternative fructosamine test may be used.

Kilpatrick (Kilpatrick 2000) mentioned that although HbA1c has an advantage which enables us to monitor a long-term glycaemic control, it also has a disadvantage since it cannot measure the stability of glucose control. For example, a patient with widely fluctuating blood glucose levels can have the same HbA1c level as a patient whose blood glucose levels change little throughout the day.

HbA1c in management of diabetes

HbA1c is increasingly described as the gold standard measure for assessing both blood glucose control and future risk of diabetic complications (Jeffcoate 2004). It is the basis on which clinical decisions are made and efficacy of treatment is assessed, reflecting the evidence for the strong association between HbA1c and diabetic complications (The DCCT Research Group 1986; Klein 1995; UKPDS Group 1998). Much clinical research has provided this evidence, notably two major long-term multicentre studies, the Diabetes Control and Complications Trial (DCCT) in type 1 diabetes (The DCCT Research Group 1986) and the UKPDS (UKPDS Group 1998) in type 2 diabetes. Accordingly, a target HbA1c value is recommended by several health care professional bodies. Moreover, in the U.K. a new financial reward system has been introduced recently in which general practitioners are encouraged to lower their patients' HbA1c below a target level (Department of Health 2001).

1.9 1,5-Anhydroglucitol monitoring

1,5-Anhydroglucitol (1,5 AG) has been proposed as a new marker of short-term glycaemia over 10-14 days (Buse, Freeman et al. 2003). It was found that people with diabetes have decreased levels of 1,5 AG in serum compared to those with normal glucose tolerance (Yoshioka, Saitoh et al. 1983). However, 1,5 AG as well as fructosamine are not widely used as a long-term glycaemic control like HbA1c since they have not been investigated in as much detail as HbA1c. The relationship between these measures and other measures of glycaemia and its potential use in clinical practice still remain uncertain.

1.10 Relationship between HbA1c and diurnal measures of blood glucose

1.10.1 Relative contributions of pre- and postprandial glucose to HbA1c

Although HbA1c is widely recognised as an average of blood glucose over 2-3 months which reflects both preprandial and postprandial glucose levels, the relative contribution of these two measures to the HbA1c level is still under debate. Several studies have investigated the relative contribution of preprandial and postprandial glucose level to HbA1c values and found contradictory results (Trovati, Burzacca et al. 1992; Avignon, Radauceanu et al. 1997; Bouma, Dekker et al. 1999; Bonora, Calcaterra et al. 2001; Hillman, Herranz et al. 2004). For example, Bonora et al. found preprandial glucose but not postprandial glucose was significantly associated with HbA1c in a multivariate analysis (Bonora, Calcaterra et al. 2001) whereas Avignon et al. found postprandial glucose but not preprandial glucose was

significantly associated with HbA1c (Avignon, Radauceanu et al. 1997). This discrepancy might be explained by the study of Monnier et al. (Monnier, Lapinski et al. 2003) which found the relative contribution of fasting glucose increases whereas that of postprandial glucose decreases when patients progress from moderate to more severe hyperglycaemia.

In 2001, the ADA (American Diabetes Association 2001) concluded that "there are insufficient data to determine accurately the relative contribution of the FPG and PPG to HbA1c." This question has become more crucial since the evidence for the association between postprandial glycaemia and diabetic complications has been emerging (Lowe, Liu et al. 1997; Barrett-Connor and Ferrara 1998; Balkau, Bertrais et al. 1999; de Vegt, Dekker et al. 1999; Rodriguez, Lau et al. 1999; Shaw, Hodge et al. 1999).

1.10.2 Discordance between HbA1c and blood glucose levels

The DCCT showed a strong correlation between HbA1c and mean blood glucose (MBG) (r=0.80) in their type 1 diabetes population but it also indicated that a patient with MBG of 10 mmol/l could have any HbA1c level between 7% and 11% (The DCCT Research Group 1987). In fact, the variation in HbA1c not attributable to glycaemia has been recognised and investigated by several studies (Yudkin, Forrest et al. 1990; Hudson, Child et al. 1999; Hempe, Gomez et al. 2002; Cohen, Holmes et al. 2003; McCarter, Hempe et al. 2004).

High and low glycators

Yudkin et al. (Yudkin, Forrest et al. 1990) used the term "high and low glycators" to describe people who had relatively high or low HbA1c levels compared to that expected given their 2h blood glucose levels. This classification was persistent after 4.4 years in 42 subjects (mostly non-diabetic).

Haemoglobin glycation index (HGI)

The haemoglobin glycation index (HGI) has been proposed as a numerical measure of whether a subject's HbA1c is higher or lower than that expected for their blood glucose levels (Hempe, Gomez et al. 2002). HGI is the vertical offset from the linear regression line when HbA1c values are fitted on MBG or fasting blood glucose levels. HGI provides a measure of the HbA1c component not attributable to an individual's blood glucose levels.

Figure 1.3 Illustration of HGI

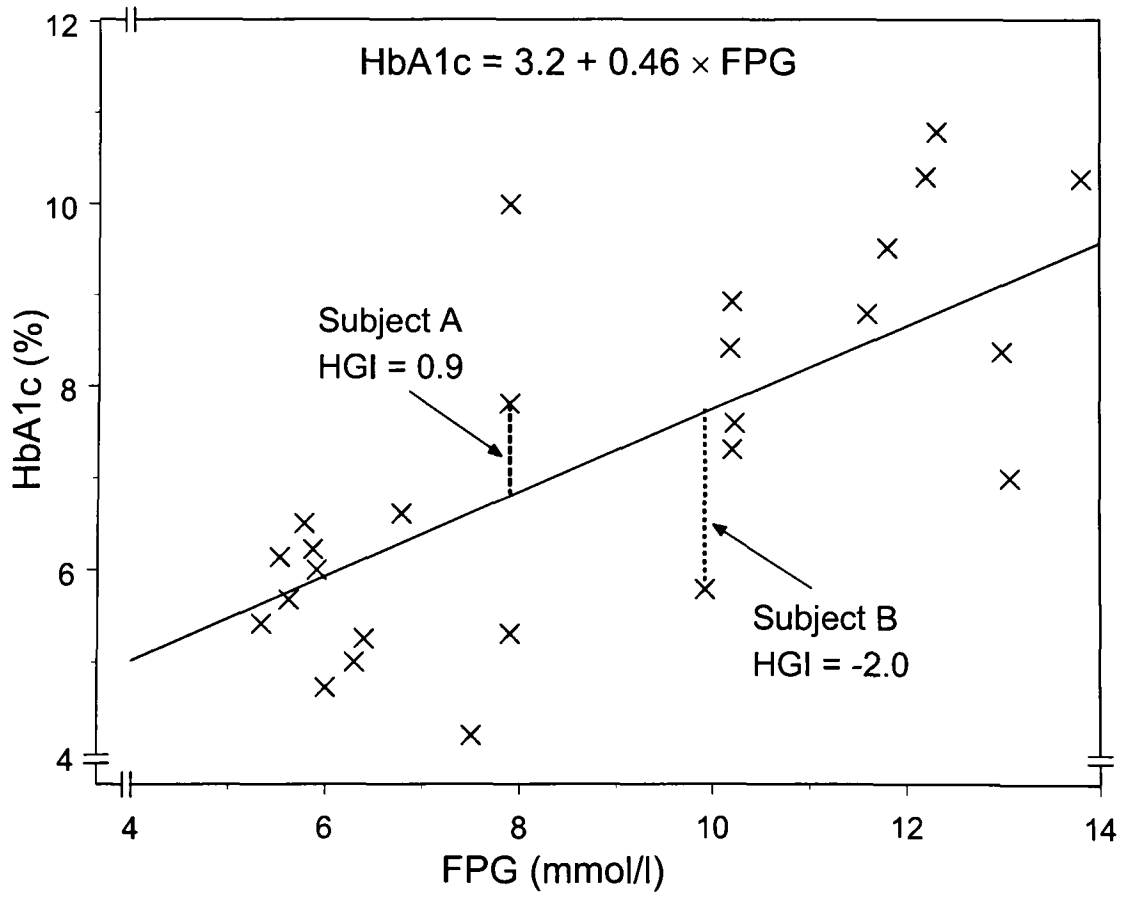


Figure 1.3 shows how HGI is calculated. For example, if a linear regression equation from the population is HbA1c = 3.2 + 0.46 × FPG and subject A has HbA1c of 7.8% and FPG of 8.0mmol/l, then the predicted HbA1c is 6.9% (= 3.2 + 0.46 × 8.0) and this person's HGI is 0.9 (= 7.8 – 6.9). If subject B has HbA1c of 5.8% and FPG of 10mmol/l, this person's HGI is -2.0 (= 5.8 – 7.8).

In 37 of 128 patients with type 1 diabetes (29%) their mean HGI was significantly different from zero, indicating their observed HbA1c was higher or lower than their HbA1c predicted from their MBG (Hempe, Gomez et al. 2002). Their HGI values were reproducible over 2.3 years.

Glycosylation gap (GG)

The glycosylation gap (GG) (Cohen, Holmes et al. 2003), has been proposed as another approach to capturing the variation in HbA1c not attributable to glycaemia. GG is the vertical offset from the linear regression line when HbA1c values are fitted on fructosamine. A study of 65 patients with type 1 diabetes demonstrated a good correlation between two consecutive GG values determined 23 weeks apart (r=0.81) (Cohen, Holmes et al. 2003).

Haemoglobin glycation index vs. glycosylation gap

HGI and GG are comparable since both are 'residuals' of HbA1c in statistical terminology and represent fractions of HbA1c not related to other blood glucose measures. Because HbA1c measures the percentage of haemoglobin bound to glucose in erythrocytes, HGI and GG capture the difference between intracellular glycaemic exposure measured by HbA1c and extracellular glycaemic exposure measured by

MBG in one case (HGI) and fructosamine in the other case (GG). HGI and GG appear to be equivalent methods for assessing the variation in HbA1c not attributable to glycaemia.

1.11 Glycaemia and microvascular complications

Diabetes can cause microvascular complications such as eye disease (retinopathy), renal disease (nephropathy) and nerve damage (neuropathy). A number of epidemiological studies and clinical trials showed that hyperglycaemia is independently associated with microvascular complications after adjustment for other risk factors such as blood pressure, lipids and weight (Knuiman, Welborn et al. 1986; Ballard, Humphrey et al. 1988; The DCCT Research Group 1993; Klein 1995; Klein, Klein et al. 1996; UKPDS Group 1998; Shichiri, Kishikawa et al. 2000; Tapp, Shaw et al. 2003).

Epidemiological data

The Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) was a prospective study which followed 996 type 1 diabetic patients and 1370 type 2 diabetic patients for 10 years. They found a significant relationship between HbA1c and the incidence of microvascular complications (diabetic retinopathy, loss of vision and nephropathy) (Klein 1995). More recently, the Australian Diabetes, Obesity and Lifestyle study (AusDiab), a population based study, also found a significant association between HbA1c and diabetic retinopathy in a multivariate analysis, using data from 333 patients with type 2 diabetes (Tapp, Shaw et al. 2003).

Clinical trials

Two major trials, the DCCT in type 1 diabetes and the UKPDS in type 2 diabetes provide the strongest evidence for the association between tight glycaemic control and reduction in risk of microvascular complications. In the DCCT (The DCCT Research Group 1993), 1441 patients with type 1 diabetes were randomly assigned to conventional therapy (1 or 2 daily insulin injections) and intensive therapy (an external insulin pump or by 3 or more daily insulin injections). The intensive treatment group achieved a median HbA1c of 7%, whereas the conventional treatment group achieved 9% throughout the study period. After a mean follow up of 6.5 years, the risk of developing retinopathy in the intensive treatment group was reduced by 76%, the risk of developing albuminuria by 54%, and the risk of developing neuropathy was reduced by 60% compared to the conventional treatment group. In the UKPDS (UKPDS Group 1998), 3867 newly diagnosed type 2 diabetes were randomised to intensive glucose control policy with sulphonylurea (oral hypoglycaemic agent) or with insulin, or a conventional glucose control policy with diet after a 3 months dietary run-in period. The difference in median HbA1c between the intensive policy group (7.0%) and the conventional policy group (7.9%) after the median 10 years follow-up reduced the risk of microvascular endpoints in the intensive group by 25% compared with the conventional group.

Additive effects of different glycaemic measures

Although many studies have found a significant effect of hyperglycaemia on microvascular complications, additive effects of different glycaemic measures have not been investigated in detail. There are few studies which have tested different measures of glycaemia simultaneously in a multivariate analysis (Knuiman, Welborn

et al. 1986; Liu, Pettitt et al. 1993; Cohen, Holmes et al. 2003; McCarter, Hempe et al. 2004). The glycoslyation gap (GG) was significantly associated with nephropathy in 40 patients with type 1 diabetes. The relationship was still significant after adjustment for either glycated haemoglobin (HbA1c) or fructosamine (Cohen, Holmes et al. 2003). The haemoglobin glycation index (HGI) was also found to be significantly associated with retinopathy and nephropathy after adjustment for MBG, using data from the DCCT (McCarter, Hempe et al. 2004). For retinopathy, the high-HGI group had 3 times greater risk (30%) compared with those in the low-HGI group (10%). For nephropathy, the high-HGI group had 6 times greater risk (6%) compared with those in the low-HGI group (1%). However, these results were criticised by Genuth et al. (Genuth, Lachin et al. 2005). They argued that HGI is a residual of HbA1c which is highly correlated with HbA1c and thus the results just showed that diabetic complications are associated with HbA1c. Since these studies suggest additive effects of different measures of glycaemia on microvascular complications in type 1 diabetes, it seems necessary to determine whether this finding can be extended to microvascular complications in type 2 diabetes.

1.12 Glycaemia and macrovascular complications

People with diabetes have more than a 2-4 fold increased risk for cardiovascular death compared with non-diabetic people (Moss, Klein et al. 1991; Stamler, Vaccaro et al. 1993; Haffner, Lehto et al. 1998; Saydah, Miret et al. 2001). Indeed, cardiovascular disease (CVD) contributes about 80% of all deaths among people with type 2 diabetes (de Marco, Locatelli et al. 1999). Although diabetes is associated with hypertension and dyslipidaemia, these traditional CVD risk factors do not fully explain the

excessive CVD risk in people with diabetes. In fact, several studies found an association between hyperglycaemia and CVD risk (Bonora and Muggeo 2001).

Epidemiological data

Several epidemiological studies have examined the relationship of CVD risk in people with diabetes to hyperglycaemia measured by the fasting glucose, 2HPG or the HbA1c level (Moss, Klein et al. 1991; Singer, Nathan et al. 1992; Welin, Eriksson et al. 1992; Fu, Chang et al. 1993; Uusitupa, Niskanen et al. 1993; Kuusisto, Mykkanen et al. 1994; Andersson and Svardsudd 1995; Gall, Borch-Johnsen et al. 1995; Turner, Millns et al. 1998; de Vegt, Dekker et al. 1999). With few exceptions, researchers in these studies found an association between worsening glycaemic control and an increased risk of CVD. In fact, hyperglycaemia has been linked to risk for CVD not only in diabetic people but also in non-diabetic people (Fuller, Shipley et al. 1983; Balkau, Shipley et al. 1998; Coutinho, Gerstein et al. 1999; Khaw, Wareham et al. 2001). A meta analysis which combines data from 95783 non-diabetic people found a significant relation of CVD events to either fasting or 2h blood glucose levels in separate models (Coutinho, Gerstein et al. 1999).

Clinical trials

Clinical trials have not demonstrated a definitive association between tight glycaemic control and reduction in the risk macrovascular complications (The DCCT Research Group 1995; Colwell 1996; UKPDS Group 1998). In the DCCT, the CVD event rate was low because the patients were young. Still there were more CVD events in the conventional group compared with the intensive group (40 vs. 23) but it was not statistically significant (p=0.08) (The DCCT Research Group 1995). In the UKPDS,

the conventional group had higher absolute risk than the intensive group (17.4 vs. 14.7 events per 1000 patient-years) but the difference reached a borderline significance (p=0.052 for myocardial infarction) (UKPDS Group 1998). Recently, the DCCT/ Epidemiology of Diabetes Interventions and Complications (EDIC) study has found that intensive diabetes therapy reduced the risk of any CVD events by 42% and the risk of non-fatal myocardial infarction, stroke or death from CVD by 57% (The DCCT/ EDIC Study Research Group 2005). A recent meta analysis of published trials suggests that improved glycaemic control might reduce CVD risk by 63% in type 1 diabetes and 19% in type 2 diabetes (Stettler, Allemann et al. 2006).

Additive effects of different glycaemic measures

Epidemiological studies, which have tested different measures of glycaemia together in multivariate analyses to determine whether their effects are additive on macrovascular complications, have been inconclusive (Hanefeld, Fischer et al. 1996; Lehto, Ronnemaa et al. 1997; Florkowski, Scott et al. 1998; Decode Study Group on behalf of the European Diabetes Epidemiology Group 2001; Roselli della Rovere, Lapolla et al. 2003; Cavalot, Petrelli et al. 2006). When two or more measures of glycaemia were tested simultaneously, only one measure was independently associated with macrovascular complications. For example, the DECODE study group found that 2HPG but not FPG was independently associated with all-cause mortality (HR: 1.73, 95% CI [1.45 to 2.06], $p<0.001$) and CVD mortality (HR: 1.40, 95% CI [1.02 to 1.90], $p<0.005$) in people with diabetes (Decode Study Group on behalf of the European Diabetes Epidemiology Group 2001). In a study of 447 subjects with type 2 diabetes, it was reported that HbA1c but not FPG was independently associated with CVD mortality (HR for 1.8% HbA1c change: 1.9, 95%

CI [1.04 to 3.47]) (Florkowski, Scott et al. 1998). A study of 529 patients with type 2 diabetes demonstrated that only blood glucose after lunch but not FPG and HbA1c was independently associated with CVD events for both women (HR: 5.54, 95% CI [1.45 to 21.20]) and men (HR: 2.12, 95% CI [1.04 to 4.32]) (Cavalot, Petrelli et al. 2006).

1.13 Hypoglycaemia

To prevent or delay the development of diabetic complications, intensive glycaemic control is necessary. However, intensive glycaemic control with insulin or some oral medications can increase risk of hypoglycaemia, i.e. a lower blood glucose level than normal amount of glucose in the blood. Hypoglycaemia causes several problems such as feeling weak, difficulty in concentration, and confusion since glucose, the fuel of the brain, is not supplied to the brain properly (Cryer, Davis et al. 2003). If hypoglycaemia is severe, brain damage or even death can happen. Hypoglycaemia also affects people psychologically (Meece 2006); the fear of hypoglycaemia makes people with diabetes reluctant to attempt more intensified treatment. These effects of hypoglycaemia make glycaemic control in people with diabetes complex.

1.14 Outline of the thesis

As well as this introduction and literature review chapter, the thesis comprises four chapters presenting my original work and analyses, and a final discussion chapter. The thesis is arranged as follows. In chapter 2, the relationship between HbA1c and blood glucose measures derived from the complete blood glucose profile is examined using data provided by continuous glucose monitoring. In chapter 3 and 4, the relationships between HbA1c and FPG over time in people with pre-diabetes and type

2 diabetes are examined. In chapter 5, the relationships of HbA1c and FPG to diabetic complications are investigated, testing whether HbA1c and FPG make independent contributions to macrovascular and/or microvascular complications in type 2 diabetes. In chapter 6, the findings are summarised and discussed.

2 Relationship between HbA1c and blood glucose profiles: continuous glucose monitoring in type 2 diabetes

2.1 Background and aim

Glycated haemoglobin (HbA1c) has been widely used to monitor long-term blood glucose control in diabetic subjects (Kilpatrick 2000; Jeffcoate 2004; Saudek, Derr et al. 2006). It has become recognised as a laboratory marker of increased risk for diabetic complications (The DCCT Research Group 1986; Klein 1995; UKPDS Group 1998; Selvin, Marinopoulos et al. 2004). HbA1c has been linked to various measures of glycaemia but it is most widely accepted as indicator of average blood glucose over the preceding 2-3 months (Nathan, Singer et al. 1984; Meigs, Nathan et al. 1996; Kilpatrick 2000; Jeffcoate 2004; Saudek, Derr et al. 2006) However, the relative contribution of preprandial and postprandial glucose to HbA1c level is still under debate (Trovati, Burzacca et al. 1992; Avignon, Radauceanu et al. 1997; Bonora, Calcaterra et al. 2001; Hillman, Herranz et al. 2004)

Previous studies used blood glucose profiles from infrequent sampling (The DCCT Research Group 1986; Trovati, Burzacca et al. 1992; Avignon, Radauceanu et al. 1997; Bonora, Calcaterra et al. 2001; Hillman, Herranz et al. 2004) but the recent technology of continuous glucose monitoring provides us with a more complete blood glucose profile, measuring blood glucose levels every 1-10 minutes (Klonoff 2005). Using continuous glucose monitoring data, it has been shown that average 24 hour glucose profiles correlate strongly with HbA1c in type 1 diabetes (Salardi, Zucchini et

al. 2002; Sharp and Rainbow 2002). It has been found that MBG and peak blood glucose levels have similar correlations with HbA1c in type 1 diabetes (Deiss, Hartmann et al. 2004). A study of 11 people with type 2 diabetes also found similar correlation of MBG and the blood glucose peaks to HbA1c (Praet, Manders et al. 2006). Thus, it is of interest to evaluate the relationship between HbA1c and blood glucose measures derived from continuous glucose monitoring data in 50 people with type 2 diabetes. The aims of this phase of my work were to: 1) examine relationships between HbA1c and blood glucose measures derived from continuous glucose monitoring data; 2) compare the reproducibility of the derived blood glucose measures; 3) find which derived glucose measure best predicted HbA1c.

This project was started when Dr. John Burke (Barnet and Chase Farm Hospitals NHS, London) who had been collecting continuous glucose monitoring data asked me to plan and execute the statistical analysis for his publication. In an interim analysis (n=23), he had calculated Pearson's correlation coefficients and found that the percentage of blood glucose readings greater than 10 mmol/l had the strongest correlation to HbA1c. This, however, was not significantly higher than the correlation between MBG and HbA1c. Considering the small sample size, I decided to perform a power calculation and a list of different statistical methods.

A power calculation was performed, using a bootstrap method and a pilot data of 30 patients. A univariate linear regression of HbA1c on MBG and that on the percentage of blood glucose readings above 10 mmol/l were compared. To obtain 80% power to achieve the difference in the likelihoods > 2 (based on Akaike Information Criteria (AIC) (Akaike H 1969)), a sample size of 70 new patients would be required.

The list of different statistical methods I decided to use was as follows. 1) Spearman's correlation coefficients were calculated instead of Pearson's correlation coefficients so that results would not be sensitive to a few observations with high blood glucose and HbA1c. 2) The discriminant ratios (Levy, Morris et al. 1999) (explained in method section) were calculated to compare the reproducibility of different measures. 3) Multiple linear regression analyses were performed to find the best predictor of HbA1c. 4) Area under the blood glucose curve above a cut-off value (explained in method section) was also used, representing a patient's actual offset from the cut-off value. 5) Mean amplitude of glucose excursion (MAGE) (Service, Molmar et al. 1970) was dropped from the analyses since the definition of MAGE was not clear and M value (Schlichtkrull, Munch et al. 1965) (explained in method section) was tested instead. 6) A squared term of blood glucose levels (explained in method section) was tested together with a linear term of blood glucose levels (MBG) to determine whether the peaks of blood glucose levels are independently associated with HbA1c adjusting for MBG.

2.2 Technology of continuous glucose monitoring system

Five continuous glucose monitoring meters have been approved by the U.S. Food and Drug Administration (Klonoff 2005). Of these, the Medtronic Minimed® (Medtronic®, Sylmar, CA, USA) continuous glucose monitoring system (CGMS) (Bode, Sabbah et al. 2000; Gross, Bode et al. 2000) was used for this study. The CGMS sensor is placed under the skin, usually in the abdominal area. It measures the glucose concentration of the interstitial fluid - extracellular fluid providing the cells of the body with nutrients and a means of waste removal - every 10 seconds. The sensor

is connected to a pager-sized monitoring device by a cable. The device acquires a sensor signal every 10 seconds and calculates an average glucose value every 5 minutes. These values are stored in the monitor's memory. Patients need to measure their capillary blood glucose concentrations at least 4 times in each 24 hour period for calibration. Individual sensors can be worn for up to 72 hours. After the study, the data are downloaded using the CGMS software.

2.3 Patients and procedures

Using this newer CGMS technology, this study was performed to determine the relationship between various parameters of blood glucose control over a three-day monitoring period of CGMS and a contemporaneous HbA1c. 50 type 2 diabetic subjects all of whom gave informed consent to take part in the study which was approved by our local Ethics Committee. Patients agreed to wear a glucose monitor for up to 3 days and were instructed how to use the CGMS and encouraged to maintain their usual activities and eating habits while wearing the CGMS. They were also instructed to measure and enter capillary blood glucose sample results into the monitors 4 times a day. For all subjects, the CGMS sensors were inserted around 10 am and were removed three days later around 12 pm. No patient had a problem at the insertion sites. The records were downloaded and blood glucose levels were calculated using the Minimed 1.7A program. A venous blood sample was taken for measurement of HbA1c soon after the CGMS was inserted. All 50 patients completed the study and their blood glucose readings were downloaded to a PC after 3 days. Table 2.1 shows patients characteristics at baseline. There were 10 females and 40 males with mean age of 62 (± SD 9 years) and a duration of diabetes of 8.7 (± SD 5.6 years).

Table 2.1 Patients characteristics at baseline (n=50)

	Mean (SD), Median (1st, 3rd quartile) or %
Age (years)	62 (9)
Male	80%
Duration of diabetes (years)	8.7 (5.6)
Fasting blood glucose (mmol/l)	7.8 (6.5, 8.8)
HbA1c (%)	8.1 (7.3, 9.2)
Number of CGMS readings made	867 (826, 886)

2.4 Statistical methods

2.4.1 Blood glucose profiles included in the analyses

For each patient, blood glucose readings (the CGMS data) were truncated to a multiple of 24 hours and each 24-hour glucose profile was checked for any missing value. If more than 5 consecutive blood glucose readings were missing, the 24-hour glucose profile was not included in the analysis. Otherwise, missing values were linearly interpolated.

Table 2.2 Summary of missing data

	N
Patients who had missing values	34
Missing values per patient for 3 days as median (IQR) (n=34)	16 (6, 47)
Total number of linearly interpolated missing values	46
	No. of person-days
24-hour profile deleted due to more than 5 consecutive missing values	34
24-hour profiles containing interpolated missing values	15

Table 2.2 shows a summary of missing data. For 3-day blood glucose profiles, 16 patients had no missing readings and 34 patients had some missing readings. 34 person-days from 25 patients were deleted due to more than 5 consecutive missing values and 46 missing values from 15 patients were linearly interpolated. Reasons for the missing data were not documented but a study which used CGMS data reported inadequate sensor response or other technical failures as reasons for missing data in their study (Deiss, Hartmann et al. 2004). 3-day blood glucose profiles from 25

patients, 2 day blood glucose profiles from 16 patients, and 1 day blood glucose profiles from 9 patients were included in the analyses.

Figure 2.1 Complete and incomplete 24-hour blood glucose profiles from CGMS

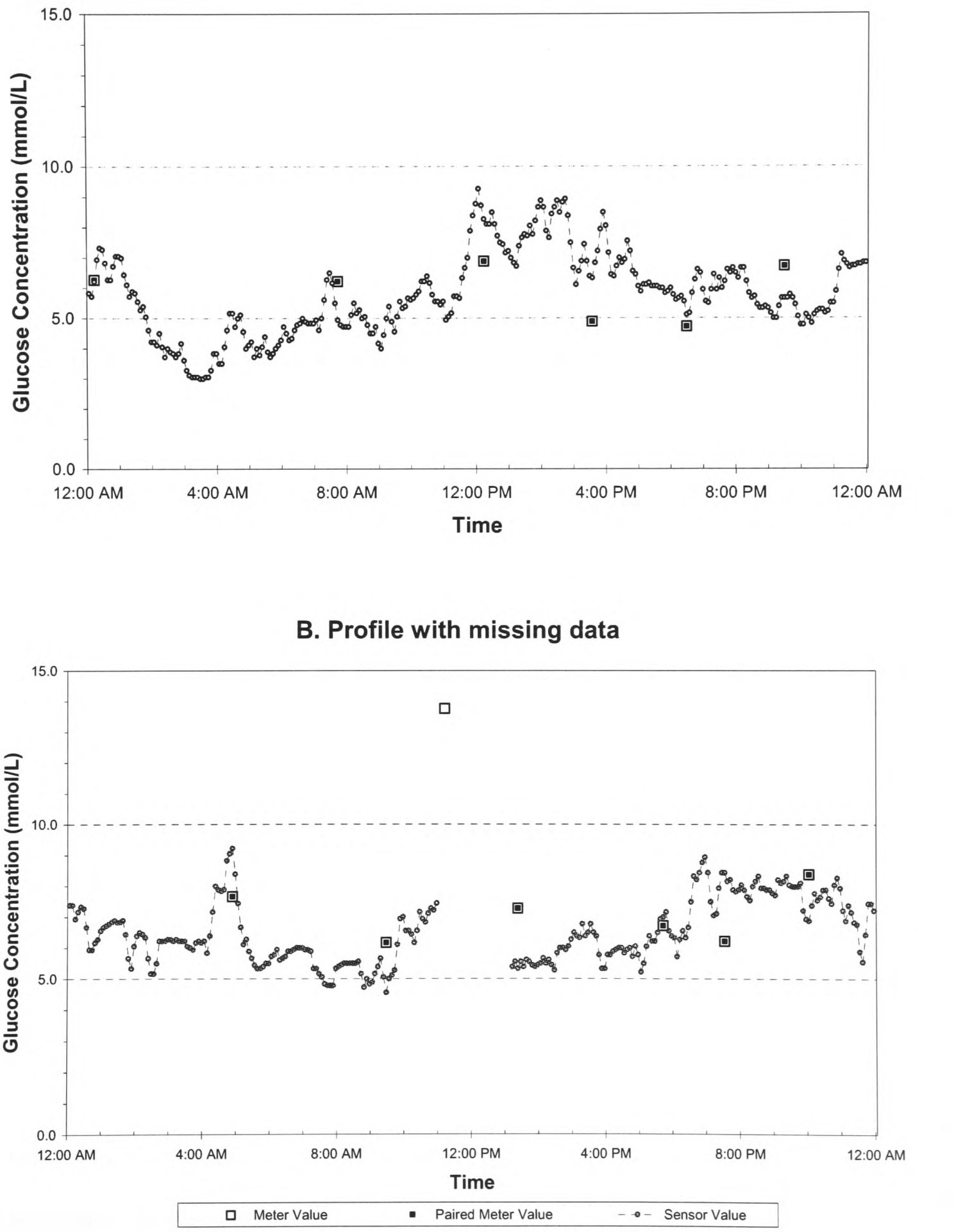


Figure 2.1 shows typical 24-hour blood glucose profiles from CGMS. The first plot shows a complete profile and the second plot shows a profile with missing data.

2.4.2 Derived blood glucose measures from the CGMS data

Several blood glucose measures derived from the CGMS data were considered for possible predictors of HbA1c. The list of the blood glucose measures is as follows:

- Fasting blood glucose (FBG) on day 1
- MBG
- Standard deviation of blood glucose (SD)
- Area under the blood glucose curve above 8 mmol/l (Area>8)
- Area under the blood glucose curve above 10 mmol/l (Area>10)
- Area under the blood glucose curve above 12 mmol/l (Area >12)
- M value $= \frac{1}{N}\sum\left|10\log\frac{\mathrm{BG}}{4.44}\right|^3 + (\max BG - \min BG)/20$
- Mean of blood glucose squared $= \frac{1}{N}\sum \mathrm{BG}^2$
- percentage of blood glucose readings greater than 8 mmol/l (%BG>8)
- percentage of blood glucose readings greater than 10 mmol/l (%BG>10)
- percentage of blood glucose readings greater than 12 mmol/l (%BG>12)

In each case, 'blood glucose (BG)' refers to blood glucose reading estimated by the CGMS as described above.

Area under the curve (AUC) above a cut-off value

Area under the blood glucose curve above 8 mmol/l was calculated, dividing each patient's blood glucose profile into two areas by the line of y = 8. The area above the

line of y = 8 was calculated and divided by the total number of days which contributed to the analyses. Area under the blood glucose curve above 10 mmol/l and 12 mmol/l were calculated in a similar way. The value of 10 mmol/l was chosen as a cut-off value since it is usually accepted as the renal threshold in clinical practice. This means that if a patient's blood glucose level is higher than 10 mmol/l, his or her kidney cannot reabsorb all the glucose filtered by the glomerulus (a part of kidney) and consequently some glucose is excreted in urine (glycosuria). This glycosuria causes diabetic symptoms (excessive urination and thirst) and may affect the relationship between blood glucose levels and HbA1c. The values of 8 mmol/l was chosen since it is similar to 7.8 mmol/l which used to be the FBG cut-off value for the diagnosis of diabetes recommended by the ADA and WHO (National Diabetes Data Group 1979; World Health Organization 1980). The value of 12 mmol/l was chosen by the clinician who initiated this project.

M value

The M value (Schlichtkrull, Munch et al. 1965) provides a single number which reflects both mean glycaemia and the fluctuations of blood glucose. The formula is a logarithmic transformation of the deviation of blood glucose level from an arbitrarily chosen value, 6.67mmol/l. In the present study the value of 4.44 mmol/l was chosen, following Service et al.'s recommendation (Service, O'Brien et al. 1987). They viewed 4.44 mmol/l as mean of diurnal glycaemia of non-diabetic people. For comparison, the M value was also calculated using 6.67 mmol/l.

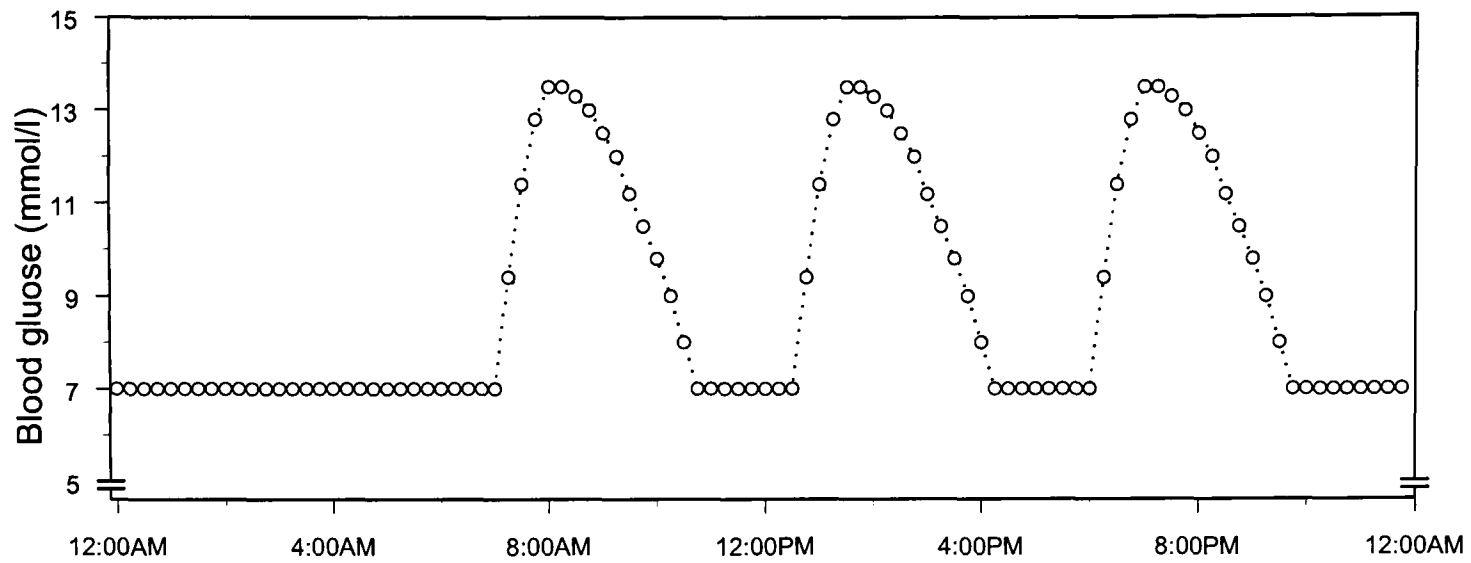
Quadratic term of blood glucose levels

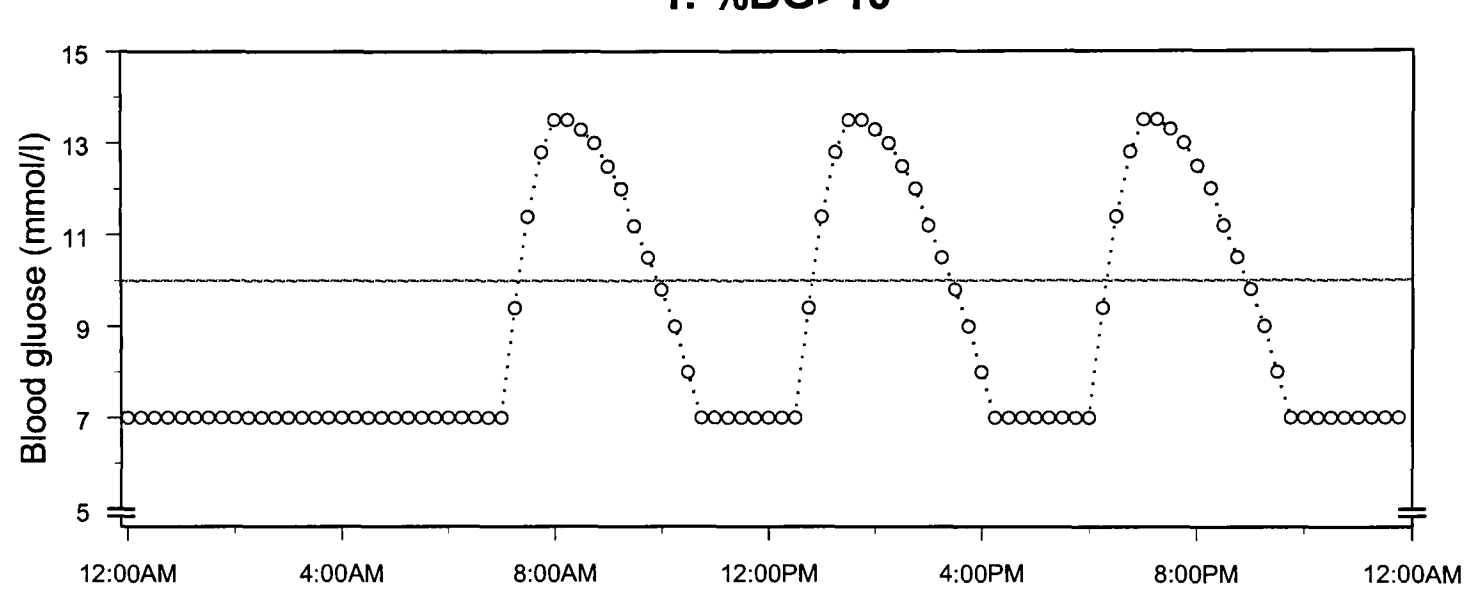
Mean of blood glucose squared was proposed as a quadratic term of blood glucose levels to test whether the peaks of blood glucose levels have independent contribution to HbA1c after adjustment for MBG. It will only be tested together with a linear term (MBG) in a liner regression model.

Frequency of blood glucose readings above a cut-off value

For comparison, percentage of blood glucose readings greater than 8 mmol/l (%BG>8), 10 mmol/l (%BG>10) and 12 mmol/l (%BG>12) were also used in analyses. Percentage of blood glucose readings greater than 8 mmol/l was calculated as the number of data points from the CGMS that were greater than 8 mmol/l, divided by the total number of data points from the CGMS. Percentage greater than 10 mmol/l and 12 mmol/l were calculated similarly. These measures represent how long blood glucose levels were above a cut-off value (time spent above a cut-off value).

Figure 2.2 Illustration of idealised 24-hour continuous blood glucose profile and graphical representation of derived measures (Area>10 and %BG>10)





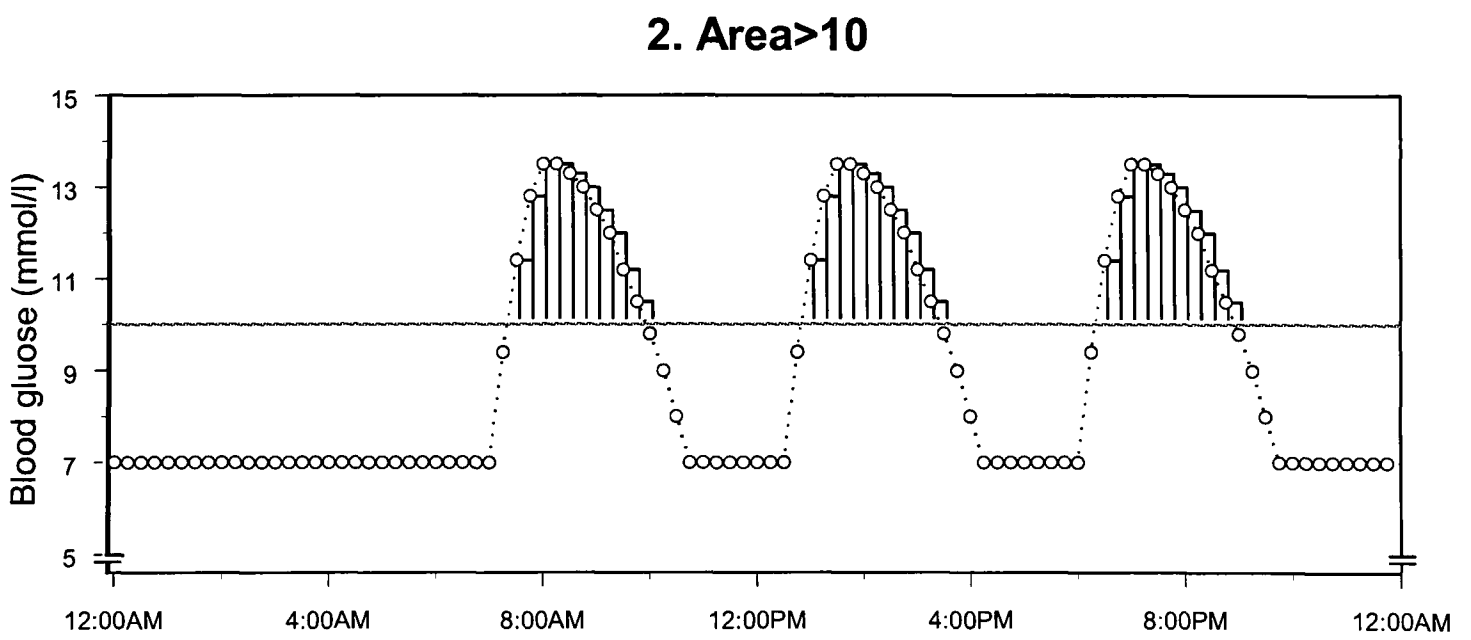


Figure 2.2 shows idealised 24-hour continuous blood glucose profile and graphical representations of %BG>10 and Area>10. Suppose blood glucose is measured every 15 minutes as depicted in Figure 2.2. There are total 96 blood glucose readings for 24

hours. In the middle pane, %BG>10 is 31% (=30/96) since there are 30 blood glucose readings greater than 10mmol/l. In the bottom pane, Area>10 is represented by bars. Those glucose readings which constitute each peak above 10mmol/l are 11.4, 12.8, 13.5, 13.5, 13.3, 13.0, 12.5, 12.0, 11.2 and 10.5mmol/l. To calculate an area, difference between each blood glucose reading and 10mmol/l is multiplied by the time interval (15 minutes in this example). Thus, Area>10 is 355.5 in this example.

2.4.3 Statistical Analysis

To examine the association between derived blood measures from the CGMS data and HbA1c levels, Spearman's rank correlation coefficients were calculated. To compare the reproducibility of the derived blood glucose measures, discriminant ratios (DR) (Levy, Morris et al. 1999) were calculated. DR is the ratio of the underlying between-subject standard deviation to within-subject standard deviation using duplicate measurements (please refer to Appendix 1 for the details). Two blood glucose profiles of 24 hours each in 41 patients were used to calculate the discriminant ratios. To find the best determinants of HbA1c, a stepwise selection procedure was used. To verify the chosen model, each blood glucose measure was tested for an association with HbA1c after adjustment for MBG or percentage of blood glucose readings greater than 10 mmol/l. All p-values for linear regression analyses were calculated by likelihood ratio tests. Linearity assumptions were checked graphically (data not shown).

2.5 Results

Table 2.3 Spearman's correlation coefficients between HbA1c and the blood glucose measures derived from CGMS data

	rho	95% CI		P-value
HbA1c vs M value with 4.44 mmol/l	0.74	0.58	0.84	<0.0001
HbA1c vs MBG	0.73	0.56	0.84	<0.0001
HbA1c vs Area>8	0.72	0.55	0.83	<0.0001
HbA1c vs %BG>10*	0.71	0.55	0.83	<0.0001
HbA1c vs Area>10*	0.71	0.54	0.83	<0.0001
HbA1c vs %BG>8	0.70	0.53	0.82	<0.0001
HbA1c vs %BG>12**	0.70	0.52	0.82	<0.0001
HbA1c vs Area>12**	0.68	0.49	0.80	<0.0001
HbA1c vs M value with 6.67 mmol/l	0.53	0.30	0.71	<0.0001
HbA1c vs FBG	0.53	0.30	0.71	<0.0001
HbA1c vs SD	0.47	0.22	0.66	0.0006

* 7 patients had Area>10 = 0, %BG>10 = 0

** 12 patients had Area>12 = 0, %BG>12 = 0

Table 2.3 shows Spearman's correlation coefficients between HbA1c and the blood glucose measures derived from CGMS data (nonparametric analysis). All associations were significant at the 5% level. Measures reflecting the peaks of glucose profiles (MBG, area under the curve above a cut-off values or time spent above a cut-off value) had similar correlation coefficients (rho = 0.68 to 0.74). A measure not reflecting the peaks of glucose levels such as fasting blood glucose (FBG) had weaker correlation (rho = 0.53) compared to MBG. The standard deviation which captures the fluctuation of blood glucose levels had the weakest correlation to HbA1c (rho=0.47). The M value calculated with 4.44 mmol/l was strongly correlated to HbA1c (rho = 0.74) but the M value calculated with 6.67 mmol/l had a weaker correlation to HbA1c (rho = 0.53).

Table 2.4 Spearman's correlation coefficients between the blood glucose measures derived from CGMS data

	MBG	M value (4.44 mmol/L)	Area>8	%BG>10*	%BG>8	Area>10*	%BG>12**	Area>12**	FBG	SD
MBG	1.00									
Area>8	0.97	1.00								
M value (4.44 mmol/l)	0.96	0.99	1.00							
%BG>10*	0.96	0.98	0.99	1.00						
%BG>8	0.96	0.95	0.94	0.95	1.00					
Area>10*	0.94	0.98	0.99	0.98	0.90	1.00				
%BG>12**	0.91	0.95	0.95	0.95	0.87	0.97	1.00			
Area>12**	0.90	0.95	0.95	0.93	0.85	0.97	0.99	1.00		
FBG	0.67	0.64	0.65	0.65	0.74	0.60	0.60	0.58	1.00	
SD	0.57	0.72	0.71	0.69	0.51	0.77	0.74	0.78	0.24	1.00
M value (6.67 mmol/l)	0.51	0.63	0.64	0.64	0.51	0.68	0.70	0.70	0.24	0.66

* 7 patients had Area>10 = 0, %BG>10 = 0

** 12 patients had Area>12 = 0, %BG>12 = 0

Table 2.4 shows Spearman's correlation coefficients between the blood glucose measures derived from the CGMS data. All measures were significantly associated with other measures at the 5% level except for FBG vs. standard deviation of blood glucose readings (p=0.092) and FBG vs. the M values calculated with 6.67 mmol/l (p=0.087). Measures reflecting the peak blood glucose levels all were highly correlated with MBG (Spearman's rho = 0.91 to 0.98).

Table 2.5 Discriminant ratios of the blood glucose measures derived from CGMS data (n=41)

	Corrrected Between-Subject SD	Within-Subject SD	Discriminant Ratio	DR 95% CI	
Quadratic term of BG	34.96	15.42	2.27	1.78	2.99
MBG	1.84	0.82	2.24	1.75	2.95
M value with 4.44 mmol/l	306.09	138.10	2.22	1.73	2.93
Area>8	1772.81	822.40	2.15	1.68	2.85
Area>10	1152.15	543.03	2.12	1.65	2.81
Area>12	661.35	315.70	2.09	1.63	2.78
%BG>10	0.22	0.11	2.02	1.56	2.68
%BG>12	0.17	0.09	1.83	1.39	2.45
%BG>8	0.26	0.16	1.57	1.17	2.14
M value with 6.67 mmol/l	80.21	59.49	1.35	0.96	1.87
SD	0.67	0.60	1.10	0.73	1.59
FBG	N/A	N/A	N/A	N/A	N/A

Table 2.5 shows the discriminant ratios of the derived blood glucose measures from the CGMS data. 9 patients with 1 day blood glucose profile were not included in the analysis. A higher discriminant ratio indicates more reproducibility. MBG (DR=2.24) had similar discriminant ratios to other measures (DR 1.83 to 2.27) except for time spent above 8 mmol/l (%BG>8) (DR=1.57), M value calculated with 6.67 mmol/l (DR=1.35) and SD (DR=1.10). These results suggest that MBG is more reproducible than %BG>8, M value with 6.67 mmol/l and SD whereas MBG and other measures have similar reproducibility. Quadratic term of blood glucose level had the highest discriminant ratio (DR=2.27) whereas standard deviation of blood glucose levels had the lowest DR (DR=1.10). M value calculated with 4.44 mmol/l had higher

discriminant ratio (DR=2.22) compared to M value calculated with 6.67 mmol/l (DR=1.35), indicating the reproducibility of M value can be affected by an arbitrarily chosen number.

Table 2.6 P-values from linear regression models of HbA1c on derived blood glucose measures from the CGMS data

	Unadjusted	Adjusted for MBG	Adjusted for %BG>10*
MBG	<0.0001	***N/A***	0.45
%BG>12**	<0.0001	0.078	0.37
%BG>10*	<0.0001	0.13	***N/A***
M value with 6.67 mmol/l	<0.0001	0.18	0.91
Area>8	<0.0001	0.21	0.85
M value with 4.44 mmol/l	<0.0001	0.24	0.70
Area>10*	<0.0001	0.33	0.97
Quadratic term of BG	—	0.33	—
FBG	<0.0001	0.42	0.14
Area>12**	<0.0001	0.82	0.72
%BG>8	<0.0001	0.94	0.69
SD	0.0019	0.95	0.49

* 7 patients had Area>10 = 0, %BG>10 = 0

** 12 patients had Area>12 = 0, %BG>12 = 0

Table 2.6 shows the results of univariate regression analyses (unadjusted). All derived measures from the CGMS data were significantly associated with HbA1c level at the 5% level. Area under the blood glucose curve above a cut-off value was recommended instead of percentage of blood glucose above a cut-off value, since it reflects actual offsets of blood glucose levels from the cut-off value rather than a proportion of the time a patient's blood glucose level was above the cut-off value. However, these two measures were highly correlated (Spearman's rho=0.98) and only percentage of blood glucose above 10 mmol/l ($p<0.0001$) was selected by the stepwise selection procedure. The model explained 54% of total variation in HbA1c level.

Table 2.6 above shows the results of multivariate regression analysis. After adjustment for percentage of blood glucose above 10 mmol/l, all other measures were not significant (all $p > 0.1$). After adjustment for MBG, percentage of blood glucose above 10 mmol/l was not significantly associated with HbA1c ($p=0.13$). All other variables were not significant at the 5% level after controlling for MBG (all $p > 0.1$) except that percentage of blood glucose above 12 mmol/l reached a borderline significance ($p=0.078$). When a quadratic term of blood glucose level (mean of blood glucose squared) was tested together with a linear term (MBG) in a regression model, the quadratic term was not significantly associated with HbA1c ($p=0.33$).

2.6 Discussion

Correlations between HbA1c and derived glucose measures from the CGMS data

This chapter found that MBG and time spent above 10 mmol/l, both of which reflect peaks of glucose profiles, had similar correlations to HbA1c. This is consistent with the findings in type 1 diabetes (Deiss, Hartmann et al. 2004) and type 2 diabetes (Praet, Manders et al. 2006). This suggests that the previous finding that HbA1c reflects average blood glucose remains valid when more complete glucose profiles are employed. A measure not reflecting the peaks of glucose profiles such as FBG had a weaker correlation to HbA1c. Using more complete blood glucose profiles provided by the CGMS, this chapter could not conclude whether MBG or blood glucose peaks is the better predictor of HbA1c.

Measures of variability in blood glucose profile

The results presented in this chapter confirm recent research which showed that HbA1c is not related to glycaemic fluctuations around mean measured by standard

deviation (SD) of blood glucose profiles (Derr, Garrett et al. 2003). In this chapter, the M value (Schlichtkrull, Munch et al. 1965), a measure reflecting glycaemic fluctuations as well as MBG was also tested. The M value was significantly associated with HbA1c but it can vary depending on an arbitrarily selected number used to calculate glucose deviation. The reproducibility of the M value was also dependent on the selected number for the calculation of the M values. Thus, it appears that M value cannot be recommended for other analyses.

Potential limitations

There are several limitations in this study. One is that the CGMS can only capture the blood glucose levels during 3 days whereas HbA1c is thought to be associated with blood glucose levels during the preceding 2-3 months. The result of this chapter is dependent on the assumption that each patient' blood glucose profile during 3 days is similar to that of past 2-3 months. Another limitation is that the result of this study is dependent on the accuracy of the blood glucose estimates by the CGMS. Suppose the CGMS is relatively insensitive to blood glucose levels below 10 mmol/l and more sensitive to blood glucose levels above 10 mmol/l then the percentage of blood glucose above 10 mmol/l would be most correlated with HbA1c. However, recent studies reported that the CGMS provides reliable estimates of blood glucose levels across different ranges of blood glucose levels (Gross, Bode et al. 2000; Sachedina and Pickup 2003). One study (The Diabetes Research in Children Network (DIRECTNET) Study Group 2003) reported that accuracy was worse during hypoglycaemia (BG<3.9 mmol/l) but it does not affect my result since most blood glucose readings (97%) were higher than 3.9 mmol/l in the present study. This study is also limited by the number of patients as discussed above. 70 new patients were

needed for the study but only data from the 50 patients including 30 patients in the pilot study were available. Therefore, this study should be regarded as a hypothesis generating study and further studies need to investigate whether the peak blood glucose has contribution to HbA1c.

Summary

Using blood glucose profiles provided by continuous glucose monitoring, this chapter showed that the MBG and blood glucose peaks equally predict HbA1c. In chapter 3, the relationship between HbA1c and blood glucose measures (fasting and post-challenge glucose levels) will be examined in 474 people with pre-diabetes.

3 Relationship between HbA1c and FPG values in people at increased risk of type 2 diabetes over a 6 year period

3.1 Background and aim

Although HbA1c reflects average blood glucose over the preceding 2 to 3 months, people can have much higher or lower HbA1c levels than expected for a given average blood glucose level. To capture this variation in HbA1c not attributable to blood glucose level, the haemoglobin glycation index (HGI) has been proposed (Hempe, Gomez et al. 2002). HGI captures whether a subject's HbA1c is higher or lower than expected for their blood glucose levels. In type 1 diabetes, HGI was reproducible over 2.3 years (Hempe, Gomez et al. 2002). The same authors also found a similar result, using the data of 1441 type 1 diabetic subjects from the DCCT (McCarter, Hempe et al. 2004).

The aim of this chapter is to determine whether the variation in HbA1c not attributable to FPG, captured by HGI, is reproducible in people at increased risk of type 2 diabetes, i.e. thought to have "pre-diabetes" over 6 year period. It will be also examined whether the reproducibility of HGI could be explained by other subject characteristics including 2h post-challenge plasma glucose (2HPG). To answer these clinical questions, four different statistical analyses will be employed and an additional aim of this chapter is to compare these statistical methods.

3.2 Study population

Data from the Early Diabetes Intervention Trial (EDIT) was used for the analysis. Briefly, 631 subjects with two consecutive elevated FPG levels in the range of 5.5 to 7.7 mmol/l were included in this 6 year study (Holman, Blackwell et al. 2003). There were 9 clinical centres. People were randomised in a 2 by 2 factorial design to therapy with acarbose vs. placebo and, simultaneously to metformin vs. placebo. Two oral glucose tolerance tests (OGTT) were performed one week apart at year 0, year 3 and year 6. HbA1c was measured by high performance liquid chromatography (HPLC) method.

Data from 474 subjects with the requisite data were included in the analysis after 157 subjects were excluded due to missing data at baseline or at three-year follow-up. Additional data was available at six-year follow-up in 297 subjects. FPG and 30 min, 60 min, 90 min and 2h post-challenge plasma glucose during OGTT were used as a mean of two measurements which were taken a week apart. Table 3.1 shows subject characteristics at baseline, year 3 and year 6 by subjects' glycaemic status at year 0. The glycaemic status was classified by 1999 WHO criteria (World Health Organisation 1999): normal glucose tolerance (NGT), impaired FPG (IFG), impaired glucose tolerance (IGT), and diabetes mellitus (DM). Isolated IFG was defined as IFG without IGT. 68 subjects were classified as DM due to the use of 1999 WHO criteria instead of 1985 WHO criteria used in the EDIT protocol.

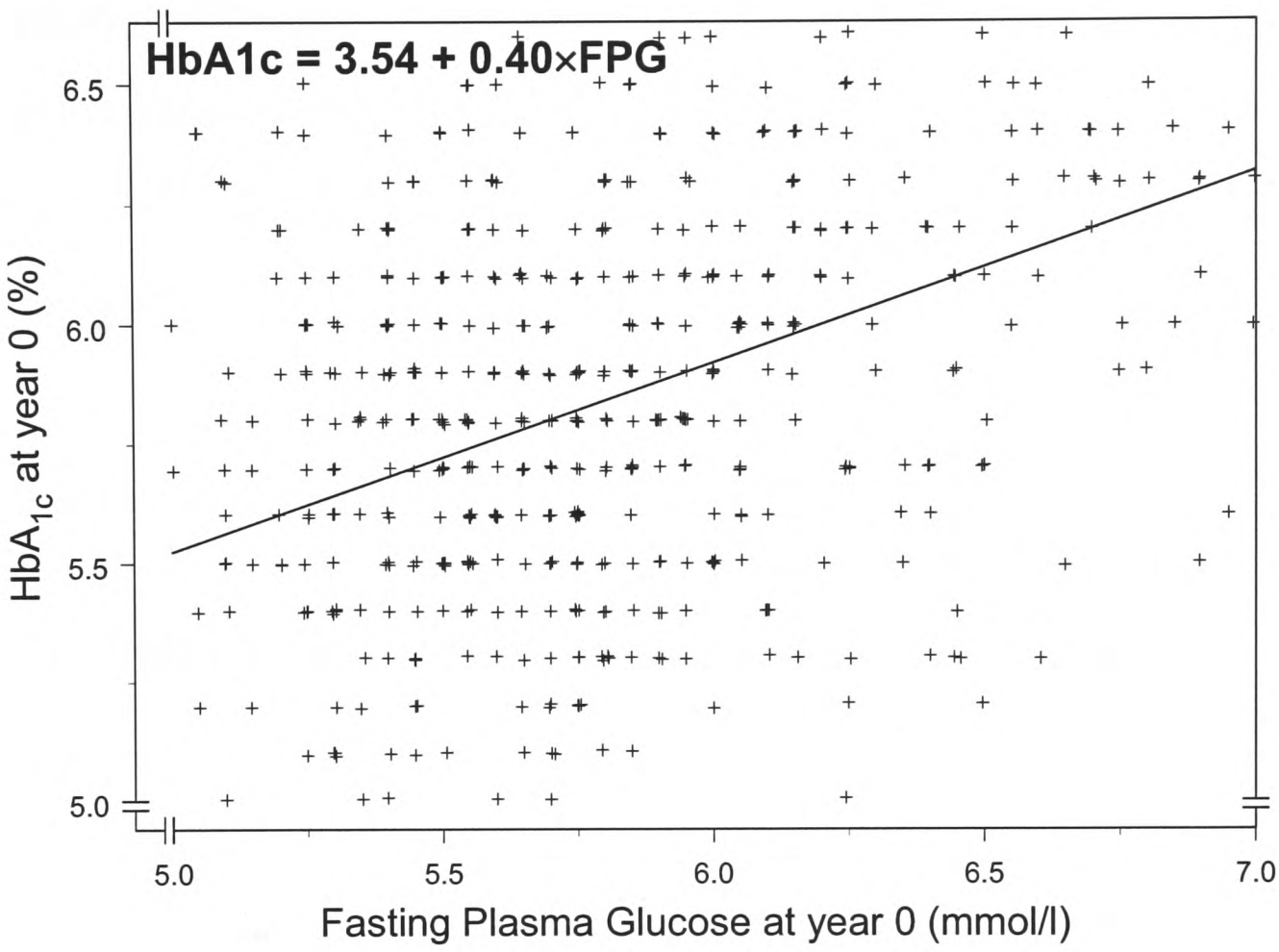
Table 3.1 Subject characteristics at year 0, year 3 and year 6 by glycaemic status at year 0 as mean (SD) or median (1st, 3rd quartile) or %

Year 0	NGT (n=198)	Isolated IFG (n=32)	IGT (n=176)	DM (n=68)
Age (years)	51 (9)	53 (9)	54 (10)	55 (10)
Male (%)	51%	72%	45%	38%
Randomization (% Control/Acarbose/Metformin/Both)	25/ 22/ 29/ 24%	34/ 25/ 16/ 25%	24/ 28/ 22/ 26%	25/ 19/ 24/ 32%
Smoker (% current/ex/never)	16/ 37/ 46%	25/ 41/ 34%	12/ 44/ 44%	7/ 43/ 50%
Body mass index (kg/m^2)	27.2 (4.2)	29.3 (4.3)	28.8 (4.6)	30.4 (4.3)
Systolic blood pressure (mmHg)	125 (14)	133 (15)	131 (18)	137 (17)
HDL cholesterol (mmol/l)	1.27 (0.39)	1.15 (0.31)	1.20 (0.34)	1.14 (0.33)
LDL cholesterol (mmol/l)	3.4 (0.9)	3.4 (1.0)	3.5 (0.9)	3.5 (0.9)
Triglyceride (mmol/l)	1.1 (0.9 to 1.6)	1.3 (1.0 to 1.7)	1.3 (1.0 to 1.8)	1.4 (1.1 to 2.3)
Fasting plasma glucose (mmol/l)	5.6 (5.4 to 5.8)	6.3 (6.2 to 6.5)	5.8 (5.5 to 6.0)	6.7 (6.2 to 7.2)
2h post-challenge plasma glucose (mmol/l)	6.6 (5.9 to 7.2)	7.0 (6.3 to 7.5)	8.8 (8.2 to 9.6)	12.3 (11.6 to 13.3)
HbA1c (%)	5.7 (5.4 to 6.0)	6.0 (5.7 to 6.3)	5.8 (5.6 to 6.1)	6.3 (6.0 to 6.6)
Year 3	NGT (n=198)	Isolated IFG (n=32)	IGT (n=176)	DM (n=68)
Smoker at year 3 (% current/ex/never)	14/ 40/ 46%	28/ 38/ 34%	12/ 45/ 43%	9/ 40/ 51%
Systolic blood pressure at year 3 (mmHg)	124 (14)	128 (14)	128 (17)	129 (15)
HDL cholesterol at year 3 (mmol/l)	1.31 (0.36)	1.20 (0.25)	1.27 (0.35)	1.20 (0.32)
LDL cholesterol at year 3 (mmol/l)	3.3 (0.8)	3.2 (0.9)	3.3 (0.8)	3.3 (0.8)
Triglyceride at year 3 (mmol/l)	1.1 (0.8 to 1.5)	1.2 (0.9 to 1.8)	1.2 (0.9 to 1.8)	1.4 (1.0 to 2.1)
Fasting plasma glucose at year 3 (mmol/l)	5.6 (5.4 to 5.9)	6.2 (5.9 to 6.7)	5.7 (5.5 to 6.1)	6.6 (6.0 to 7.1)
2h post-challenge plasma glucose at year 3 (mmol/l)	6.9 (5.6 to 7.8)	7.6 (6.5 to 9.0)	8.5 (7.4 to 10.1)	12.2 (10.6 to 13.5)
HbA1c at year 3 (%)	5.7 (5.5 to 6.0)	6.0 (5.8 to 6.3)	5.9 (5.6 to 6.2)	6.3 (6.0 to 6.8)
Year 6	NGT (n=154)	Isolated IFG (n=19)	IGT (n=115)	DM (n=10)
Fasting plasma glucose at year 6 (mmol/l)	5.7 (5.4 to 5.9)	6.2 (5.9 to 6.4)	5.8 (5.5 to 6.1)	6.4 (5.8 to 6.6)
2h post-challenge plasma glucose at year 6 (mmol/l)	6.9 (5.8 to 7.7)	6.6 (5.9 to 7.9)	8.6 (7.3 to 10.0)	10.0 (9.1 to 11.3)
HbA1c at year 6 (%)	5.7 (5.5 to 6.0)	6.1 (5.7 to 6.2)	5.9 (5.6 to 6.2)	6.2 (5.9 to 6.5)

NGT: normal glucose tolerance, Isolated IFG: isolated impaired FPG, IGT: impaired glucose tolerance, DM: diabetes mellitus

3.3 Haemoglobin Glycation Index (HGI)

Figure 3.1 HbA1c vs. FPG at year 0 in EDIT (n=622)



To calculate HGI, a linear regression of HbA1c on FPG was fitted using all the available data at year 0 (n=622). Figure 3.1 shows the plot of HbA1c against FPG at year 0 in the EDIT. The R-square statistic was 0.25, indicating 25% of total variation in HbA1c is explained by FPG. The fitted linear equation was

$$\text{HbA1c} = 3.54 + 0.40 \times \text{FPG} \qquad \text{(Equation 3.1)}$$

After that, predicted HbA1c was calculated, entering patients' FPG at any given time point into the linear equation (Equation 3.1). Then HGI was calculated subtracting predicted HbA1c from patients' actual HbA1c at any given time point.

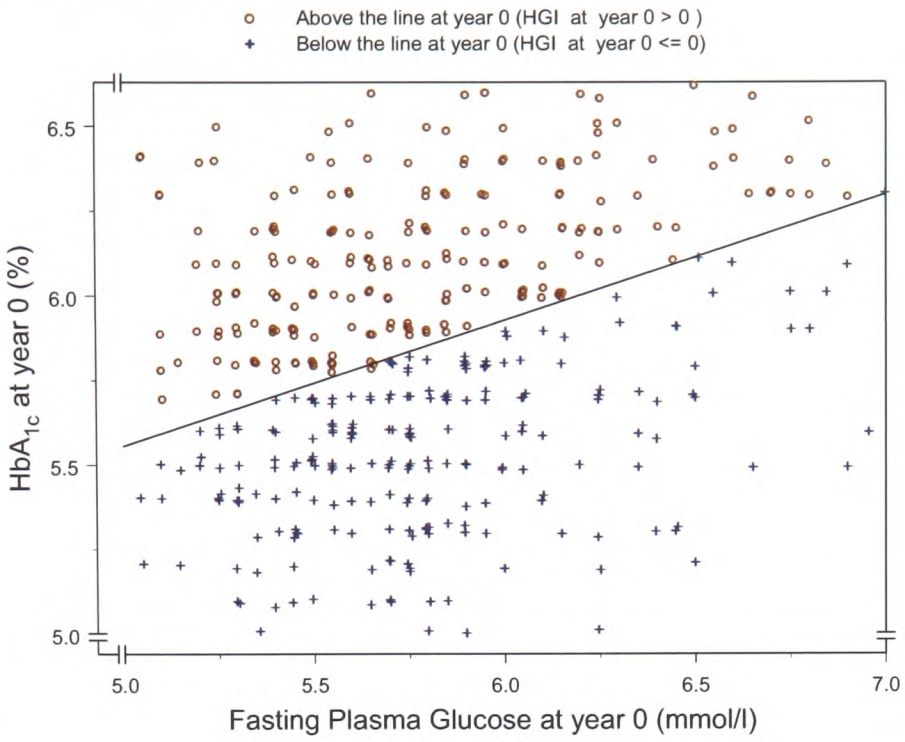
HGI values were calculated at year 0, year 3 and year 6. The median (1st, 3rd quartile) of HGI at year 0 was -0.0012 (-0.32, 0.27). The median (1st, 3rd quartile) of HGI at year 3 was 0.037 (-0.24, 0.30). The median (1st, 3rd quartile) of HGI at year 6 was 0.037 (-0.28, 0.30). 14%, 19% and 20% of subjects had observed HbA1c values more than 0.5% higher or lower than that expected for their FPG at year 0, year 3 and year 6, respectively. 3%, 3% and 2% of subjects had observed HbA1c values more than 1% higher or lower than that expected for their FPG at year 0, year 3 and year 6, respectively.

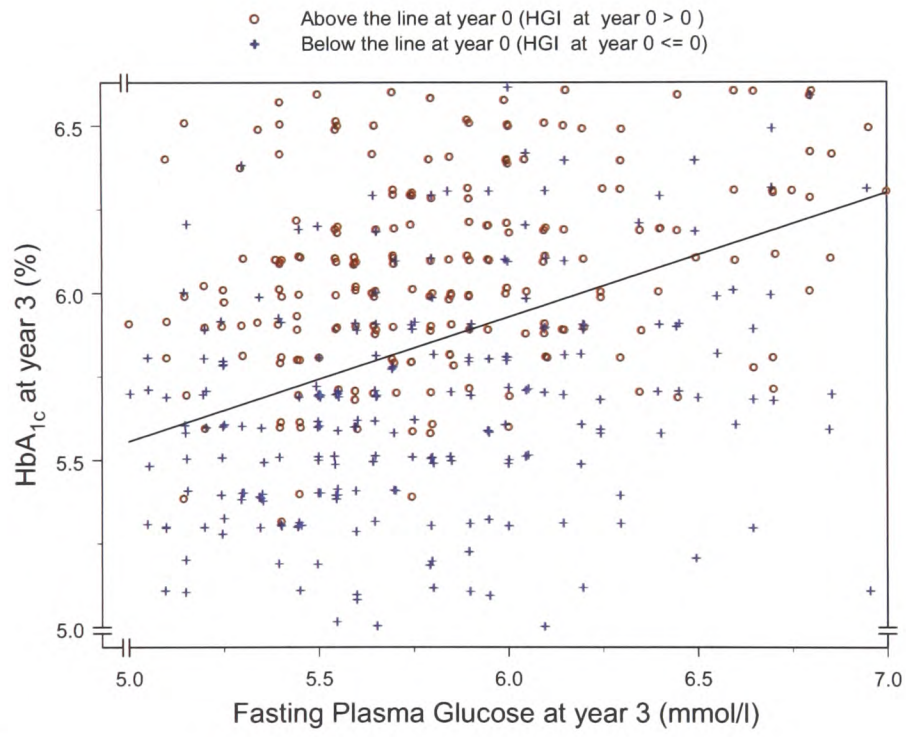
3.4 Reproducibility of HGI

3.4.1 Graphical and Categorical methods

To examine whether the HGI of a subject is reproducible over time, HGI was categorised as follows: let category = 1 if HGI > 0 (HbA1c lies above the regression line of HbA1c on FPG) and let category = 0 if HGI <= 0 (HbA1c lies below the regression line). The independence of category at year 0 and category at year 3 was tested with Pearson's chi-square test with Yate's correction factor. To demonstrate that this is not only a short-term effect, an additional analysis tested for independence of "category at year 0" and "category at year 6", using 297 subjects with data available at year 6.

Figure 3.2 HbA1c vs. FPG at year 0, year 3 and year 6 colour-coded by the position relative to the regression line at year 0





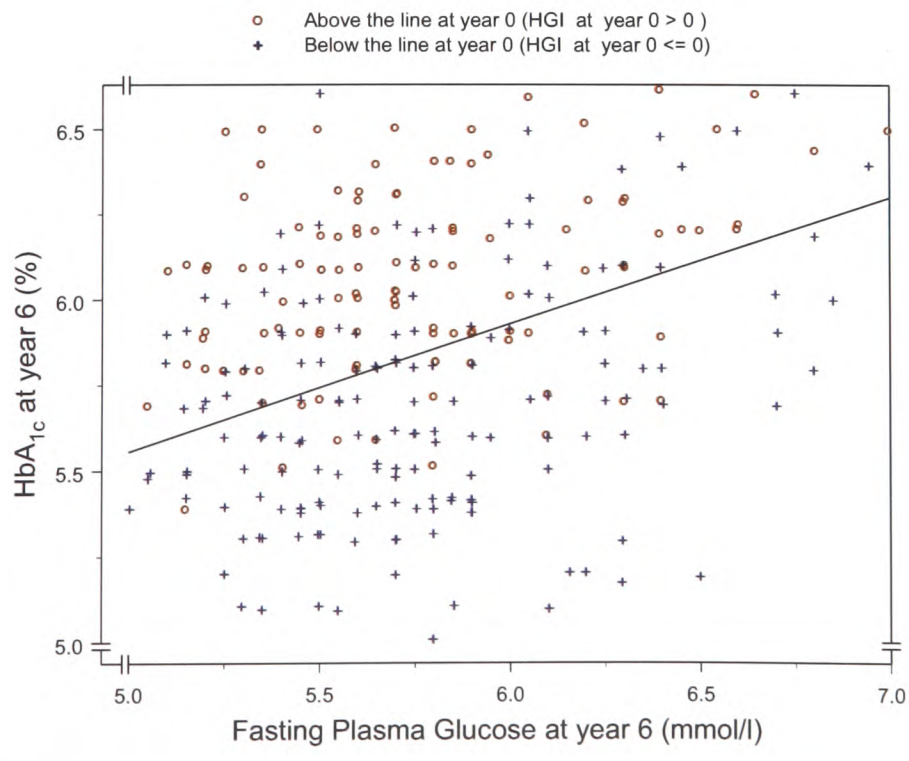


Figure 3.2 shows scatter plots of HbA1c against FPG at year 0, year 3 and year 6. The coloured symbols represent each subject's status at year 0; red circles denote people above the regression line at year 0 (HGI at year 0 > 0) and blue crosses denote people below the regression line at year 0 (HGI at year 0 ≤ 0). Random numbers from normal distribution with mean=0 and SD=0.0002 were added to both HbA1c and FPG at each year as 'jitter factors'. The majority of the subjects (74%) did not cross the line at year 3 ($p<0.0001$ by chi-square test). Likewise, the majority of the subjects (73%) did not cross the line at year 6 ($p<0.0001$ by chi-square test).

3.4.2 Graphical and Continuous methods

To confirm the reproducibility of HGI by more formal analysis, Pearson's correlation coefficients were calculated between HGI at year 0 and HGI at year 3 (n=474) or HGI at year 6 (n=297). Figure 3.3 displays the scatter plots of HGI at year 3 and HGI at year 6 against HGI at year 0. An association between HGI at year 0 and HGI at year 3 or HGI at year 6 was observed. Pearson's correlation coefficient between HGI at year 3 and HGI at year 0 was 0.71 ($p<0.0001$, 95% CI [0.66 to 0.75]). Pearson's correlation coefficient between HGI at year 6 and HGI at year 0 was 0.68 ($p<0.0001$, 95% CI [0.62 to 0.74]).

To investigate whether there is any systematic or non-linear relationship between HGI at different years, Bland-Altman difference plots of HGI at year 3 and HGI at year 6 against HGI at year 0 were drawn (Figure 3.4). These plots show that the difference is not associated with mean HGI and there is no heteroscedascity in the data.

Figure 3.3 HGI at year 3 vs. year 0 and HGI at year 6 vs. year 0

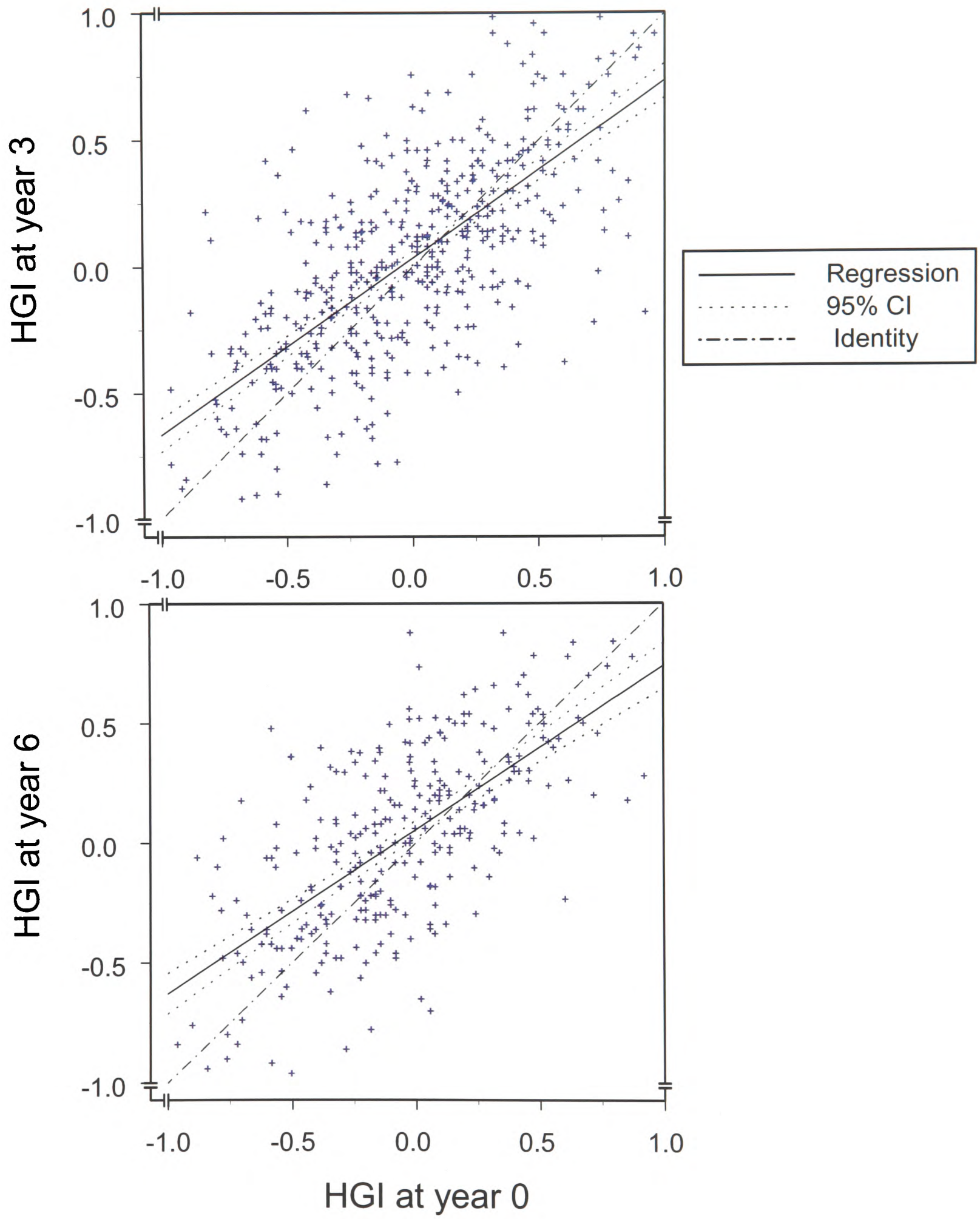
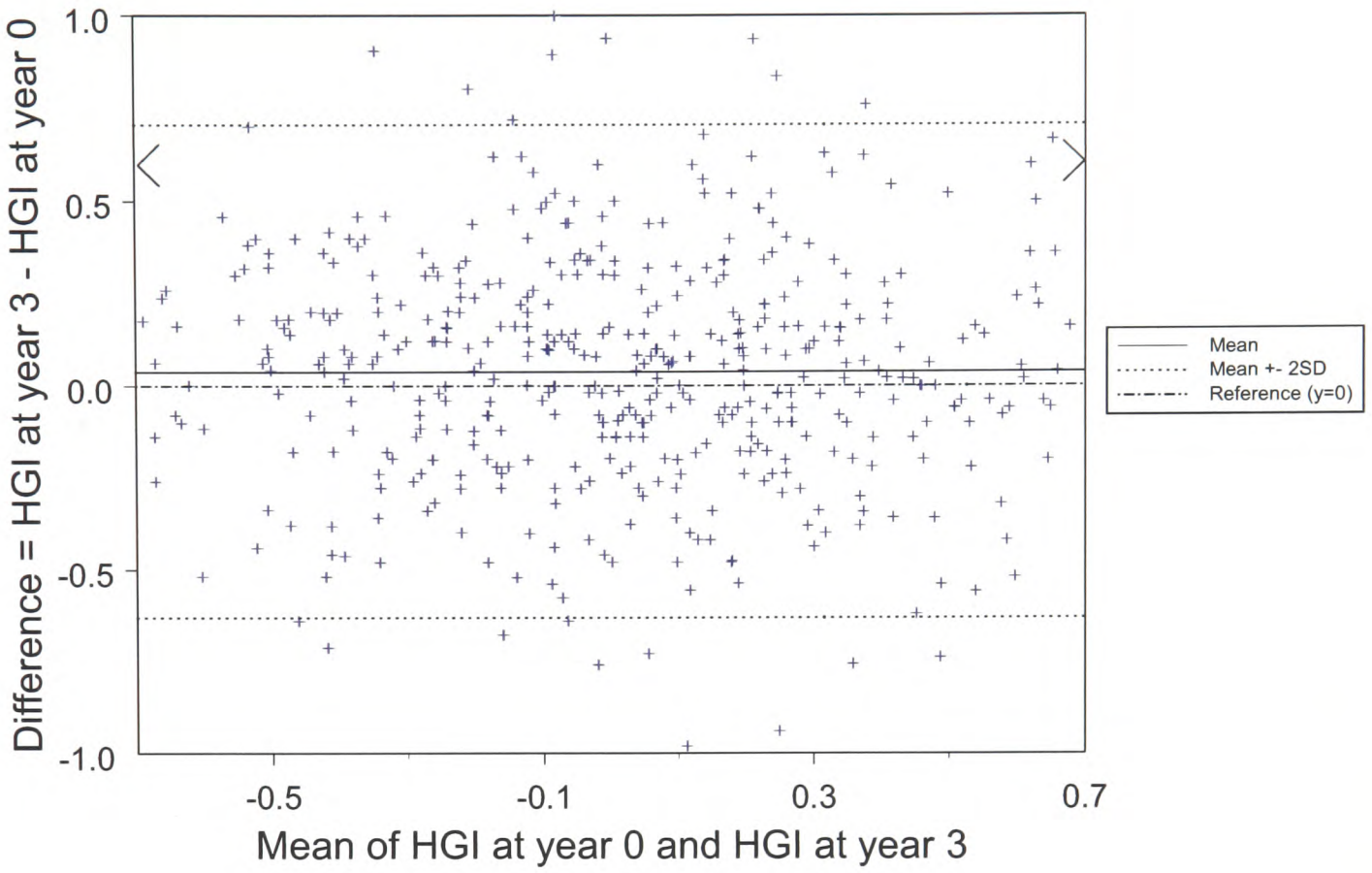
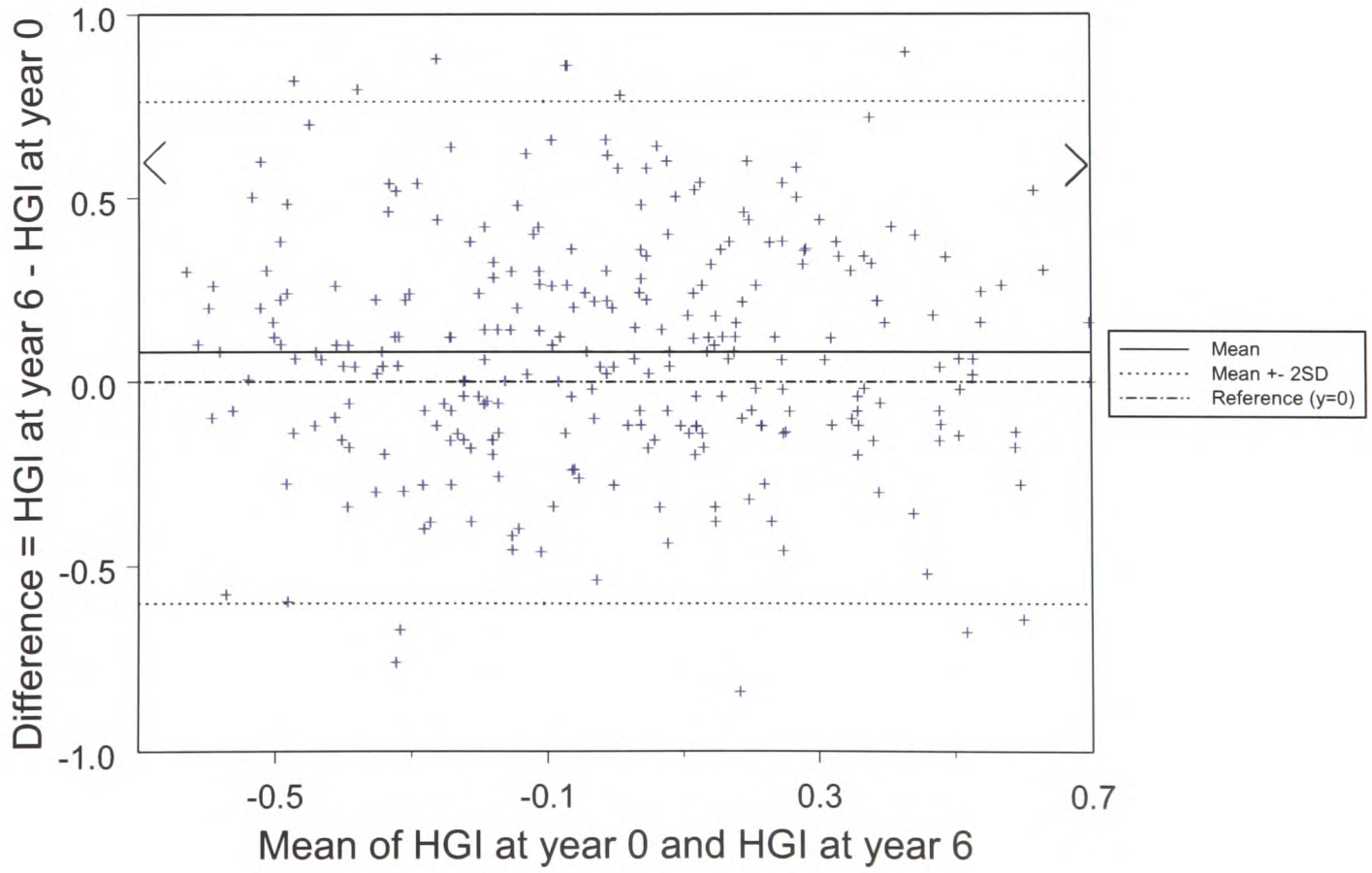


Figure 3.4 Difference plot of HGI at year 0 and HGI at year 3 or HGI at year 6





* Symbols in the graphs (<, >) represent there are some data points outside this range.

3.5 Discriminant ratio

To assess the reproducibility of HGI, the discriminant ratio (Levy, Morris et al. 1999) was calculated. The discriminant ratio (DR) is the ratio of the underlying between-subject standard deviation to within-subject standard deviation using duplicate measurements (please refer to Appendix 1 for the details). HGI at year 0 and year 3 were used for the calculation of the discriminant ratio of HGI. For comparison, the discriminant ratios for HbA1c, 2HPG and systolic blood pressure were also calculated (Table 3.2).

Table 3.2 Discriminant ratios of different measures

	Corrrected Between-Subject SD	Within-Subject SD	DR	95% CI	
HbA1c (%)	0.442	0.239	1.85	1.71	2.01
HGI (unit)	0.393	0.230	1.71	1.57	1.86
2HPG (mmol/l)	2.119	1.293	1.64	1.50	1.79
Systolic BP (mmHg)	1.259	0.996	1.26	1.14	1.40

Table 3.2 shows the discriminant ratios for different measures. Higher discriminant ratio indicates more reproducibility. HGI (DR=1.71) had similar DR to HbA1c (DR=1.85) and 2h post-challenge glucose (DR=1.64) suggesting HGI was as reproducible as these measures. HGI had higher DR than systolic blood pressure (DR=1.26), suggesting HGI was more reproducible than systolic blood pressure in this study. The discriminant ratio for FPG was not calculated since the range of FPG was restricted due to the study design.

3.6 Determinants of HGI

Given that HGI is reproducible, factors which might explain HGI were investigated. To determine whether subject characteristics can explain an individual's higher or lower HbA1c relative to that expected from their FPG, a multiple linear regression of

HGI at year 0 was fitted on potential confounders available at year 0: age, sex, body mass index (BMI), HDL and LDL cholesterol, triglycerides, systolic blood pressure, smoking status, randomisation, 30min, 60min, 90min and 2 hour post-challenge plasma glucose (2HPG) and area under the post-challenge glucose curve (AUC) from OGTT. A stepwise selection algorithm was used with a cut-off significance level of 5%.

Table 3.3 Regression of HGI at year 0 on covariates at year 0

Variables	Effect	95% CI		P-value
Age (per 10 years)	0.11	0.070	0.15	<0.0001
LDL cholesterol (per mmol/l)	0.075	0.032	0.12	0.00072
2HPG (per mmol/l)	0.019	0.0026	0.035	0.023
Smoking (vs.Never Smoked)	0.14	0.025	0.26	0.00032
Ex Smoking (vs.Never Smoked)	-0.10	-0.18	-0.016	0.00032
R-square		**0.13**		

Non-significant at 5% level: 30min, 60min, 90min PG, AUC, sex, body mass index, HDL cholesterol, triglycerides, systolic blood pressure, and randomisation

Table 3.3 shows the multiple linear regression result with age, LDL, 2h post-challenge plasma glucose (2HPG), current smoking and ex smoking being significantly associated with HGI at year 0 (all other variables were non-significant with $p > 0.2$ except for sex ($p = 0.062$) and 60 min PG ($p = 0.084$)).

Table 3.4 Regression of HGI at year 3 on HGI at year 0 and covariates at year 3

	Model 1			Model 2		
Variables	**Effect**	**95% CI**	**P-value**	**Effect**	**95% CI**	**P-value**
HGI at year 0 (per unit)	0.69	(0.63, 0.76)	<0.0001	0.67	(0.60, 0.73)	<.0001
Constant	0.034	(0.0056, 0.062)	0.019	-0.16	(-0.37, 0.047)	0.13
Age (per 10 years)				0.009	(-0.023, 0.040)	0.58
LDL Cholesterol at year 3(per mmol/l)				0.015	(-0.022, 0.051)	0.43
2HPG at year 3 (per mmol/l)				0.013	(0.0019, 0.024)	0.022
Smoking at year 3 (vs.Never Smoked)				0.01750	(-0.071, 0.11)	0.67
Ex Smoking at year 3 (vs.Never Smoked)				-0.021	(-0.082, 0.041)	0.67
R-square		**0.49**			**0.50**	

To examine whether HGI at year 3 is predicted by HGI at year 0, a regression model of HGI at year 3 was fitted on HGI at year 0 (Model 1, Table 3.4). This analysis duplicates the Pearson's correlation analysis but it was included since it is useful for comparison with the subsequent linear model. To determine whether the reproducibility of HGI could be attributed to the relationships found in the multiple linear regression model (Table 3.3), a further model considered HGI at year 0 in addition to the above covariates measured at year 3 as explanatory variables of HGI at year 3 (Model 2, Table 3.4). The R-squared statistic (proportion of variation explained by the model) was reported for each model. Normality and linearity assumptions were checked by graphical methods. HGI at year 0 was a significant predictor of HGI at year 3 ($p < 0.0001$) and the R-squared value was 0.49, suggesting HGI at year 0 explained 49% of total variation in HGI at year 3 (Model 1, Table 3.4). In a multiple linear regression model (Model 2, Table 3.4), HGI remained significant ($p < 0.0001$) after adjustment for the covariates previously found significant in Table 3.3. However, other covariates at year 3 became no longer significant except for 2HPG at year 3 ($p = 0.022$) after adjustment for HGI at year 0. The R-squared value was increased by 1% implying the variation in HGI at year 3 was mainly explained by HGI at year 0.

3.7 Repeated measures analyses (random effect models)

To verify the reproducibility of HGI was not a statistical artefact caused by a choice of a linear regression line to calculated HGI, two repeated measures linear regression models were fitted. In each model, a subject effect was modelled as a random effect and other covariates were modelled as fixed effects (for a details, refer to Appendix 2). In these models, the subject effect on HbA1c adjusting for FPG corresponds to

underlying mean HGI. In the first model, a subject effect on HbA1c was tested adjusting for FPG. In the second model, a subject effect on HbA1c was tested adjusting for FPG and potential confounders (chosen by stepwise selection with a 5% cut-off).

Table 3.5 Repeated measures linear regression of HbA1c on FPG with a random intercept

	Coef.	Std. Err.	P-value
Intercept	4.30	0.11	<0.0001
FPG (per mmol/l)	0.27	0.02	<0.0001
Variance of Subject Effect	0.16	0.012	<0.0001
Variance of Residual	0.049	0.0027	<0.0001

Table 3.5 shows the results of the first model. There was a significant subject effect on HbA1c ($p<0.0001$) with the variance 0.16 and residual variance 0.049 after adjustment for FPG. This suggests that each subject has clustered HbA1c levels over 6 year period and thus the variation of HbA1c levels within a subject is much smaller than the variation of HbA1c levels between different subjects after adjustment for FPG.

Table 3.6 Repeated measures linear regression of HbA1c on FPG and other subject characteristics with a random intercept

	Coef.	Std. Err.	P-value
Intercept	3.47	0.15	<0.0001
FPG (per mmol/l)	0.17	0.022	<0.0001
2HPG (per mmol/l)	0.027	0.0061	<0.0001
PG30 at year 0 (per mmol/l)	0.024	0.0079	0.0013
PG90 at year 0 (per mmol/l)	0.025	0.0078	0.0022
Age (per 10 years)	0.082	0.018	<0.0001
LDL cholesterol at year 0 (per mmol/l)	0.072	0.019	0.00013
Ex Smoking at year 0 (vs.Never Smoked)	-0.075	0.036	0.0014
Smoking at year 0 (vs.Never Smoked)	0.11	0.051	0.0014
Variance of subject effect	0.13	0.0092	<0.0001
Variance of residual	0.048	0.0025	<0.0001

Not significant: sex, body mass index, HDL cholesterol, triglycerides, fasting plasma insulin, systolic blood pressure, PG60, area under the curve, randomisation arms (all $p>0.3$)

Table 3.6 shows the result of the second model. HbA1c was significantly associated with FPG, 2h post-challenge glucose (2HPG), 30 minute post-challenge glucose at year 0 (PG30), 90 minute post-challenge glucose at year 0 (PG90), age, LDL cholesterol at year 0 and smoking status at year 0 (all $p<0.01$). These covariates were the same as those selected for the ordinary least square linear regression model (Table 3.3) except for 30 minute and 90 minute post-challenge glucose levels. Even after adjustment for these covariates, there was a significant subject effect on HbA1c ($p<0.0001$) with the variance 0.13 and residual variance 0.048. To preclude a possible clinical centre effect, I repeated the analyses using the largest centre only. When the analysis was restricted to the largest centre only (n=76), the subject effect on HbA1c adjusting for FPG remained significant for both models (all $p<0.0001$).

3.8 Discussion

Relationship between HbA1c and FPG in pre-diabetes

This chapter demonstrated that HGI, a measure of the variation in HbA1c not attributable to FPG, is a reproducible characteristic in people with pre-diabetes. Although an association between HbA1c and FPG is observed within the population, there is a wide variability in HbA1c at any given FPG. If a person has higher HbA1c level than expected for his or her FPG, this person is more likely to have higher HbA1c level after 3 or even 6 years. Likewise, if a person has lower HbA1c level than expected for his or her FPG, this person is more likely to have lower HbA1c level after 3 or even 6 years. In repeated measures models, the subject effect on HbA1c adjusting for FPG was significant even after adjustment for potential confounders. This suggests that the relationship between FPG and HbA1c in a subject is reproducible.

Weak association between HGI and 2h post-challenge glucose

HbA1c is widely accepted to reflect average glucose incorporating both fasting and postprandial glucose levels. The variation in HbA1c not attributable to FPG, captured by HGI, may be associated with 2h post-challenge glucose. However, this study found a weak significant association between HGI and 2h post-challenge glucose.

HGI vs. 'high and low glycators'

This study extends the findings of previous research in non-diabetic subjects (Yudkin, Forrest et al. 1990) and type 1 diabetes (Hempe, Gomez et al. 2002). Yudkin et al. (Yudkin, Forrest et al. 1990) classified subjects as "high and low glycators" comparing their 2h blood glucose ranking with HbA1c ranking. Their classification was consistent after 4 years. High and low glycators showed no difference in age, sex, body mass index, haemoglobin levels or smoking. However, this classification (high and low glycators) just tells us whether people have higher or lower HbA1c relative to their 2h blood glucose. In contrast, the HGI (Hempe, Gomez et al. 2002) can capture both the magnitude and the direction of the discordance between HbA1c and fasting blood glucose.

Linear regression equation for calculation of HGI

A linear regression equation for calculation of HGI can be fitted in different ways. For example, the authors who proposed HGI (Hempe, Gomez et al. 2002) fitted a linear regression equation employing all the available pairs of HbA1c and FBG during their study. In this chapter, a linear regression equation was fitted using all the available pairs at baseline. This method would probably cause drift in HGI values

over time. However, this method was selected since it is more practical for a clinician to obtain a linear regression equation using baseline covariates.

Methodological discussion

In this chapter, different statistical methods were applied to show the reproducibility of HGI. Of these, repeated measures linear regression seems most appropriate since it enables us to test the relationship between HbA1c and FPG taking account of the within-subject variation in HbA1c and FPG. Repeated measures linear regression can use the data from the subjects who may miss one or more of the measurements. The colour-coded plots seem more effective in a presentation since people can understand easily what is happening over time without any statistical knowledge. However, the scatter plots of HGI at different years contain more information compared to the colour-coded plots. The discriminant ratio, the ratio of the underlying between-subject standard deviation to within-subject standard deviation of a measure, is a useful tool to compare different measures. The coefficient of variation (CV), the standard deviation divided by the mean of a measure, is widely used to report the reliability of a measure but the CV is not appropriate for a measure which can have negative values such as HGI.

Possible additional determinants of the unexplained variation in HGI

HGI might be explained by non-glycaemic factors. One possibility is the factors affecting erythrocytes (red blood cells). For example, erythrocyte turnover rate has been suggested as a factor influencing HbA1c levels (Jiao, Okumiya et al. 1998). Suppose someone has a higher red blood cell turnover rate, then their HbA1c levels would be lower than their usual HbA1c levels since there is less time for red blood

cells to accumulate stable HbA1c. The authors who proposed HGI tested erythrocyte creatinine concentration which is inversely correlated with erythrocyte turnover rate (Hempe, Gomez et al. 2002) but found no significant effect of erythrocyte creatinine concentration on HGI in type 1 diabetes. Erythrocyte permeability (the ratio of intra-erythrocyte to plasma glucose concentration) and metabolism (erythrocyte 2,3-diphosphoglycerate, a catalyst of glycation) also have been suggested as explanatory variables for the difference between high and low glycators (Gould, Davie et al. 1997). Another possibility is the haemoglobin variants. Genetic variation in haemoglobin has been reported to cause disproportionately high or low HbA1c levels (Harano, Harano et al. 1985; Langdown, Davidson et al. 1992; Chico, Padros et al. 2004). For example, foetal haemoglobin has been reported to elevate HbA1c assays spuriously which might lead to spuriously high HGI (Villanueva, Zavala et al. 1995; Egede, Obah et al. 2000). Finally, an enzyme (fructosamine 3-kinase) involved in 'deglycation' has been suggested as a factor affecting HbA1c levels (Delpierre and Van Schaftingen 2003). More recently, the same authors (Delpierre, Veiga-da-Cunha et al. 2006) have found that the activity of this enzyme is associated with a gene and influences the glycation of specific site of the haemoglobins.

Potential limitations

There are several limitations in the present study. Firstly, this study could not examine all the factors which may affect HbA1c level. No markers for erythrocyte turnover rate, erythrocyte permeability and metabolism and the enzyme (fructosamine 3-kinase) involved in deglycation were available. Secondly, a gene analysis cannot be performed in this study although there might be some genes which affect the rate of glycation. Thirdly, there was a study which showed that a specific gene can influence

a certain type of HbA1c measurement method (HPLC) which was used in this study. However, it has been reported that this gene is rare in White-Caucasian population (Chico, Padros et al. 2004). Fourthly, 2h post-challenge plasma glucose used in the analysis may not be equivalent to blood glucose levels after meals as discussed in chapter 1. Finally, the study design has implications for the results. In this study, fasting glucose level was restricted to the range 5.0 to 7.0 mmol/l. Thus, the proportion of variation in HbA1c which is explained by FPG is likely to be underestimated.

Summary

This study suggests that HGI is reproducible in pre-diabetes, i.e. the discordance between fasting glucose and HbA1c in a subject is consistent over time. The reproducibility of HGI needs to be confirmed by a study which recruits people with a wider range of blood glucose level. In chapter 4, the relationship between fasting glucose and HbA1c over time will be examined in subjects with type 2 diabetes who had a wider range of fasting glucose.

4 Relationship between HbA1c and FPG values in patients with type 2 diabetes over time

4.1 Background and aim

The previous chapter showed that the haemoglobin glycation index (HGI), the variation in HbA1c not explained by FPG, was reproducible in patients with pre-diabetes. It was also shown that HGI was not explained by patient characteristics such as post-challenge glucose levels.

Figure 4.1 HbA1c levels in conventional and intensive therapy groups in the UKPDS

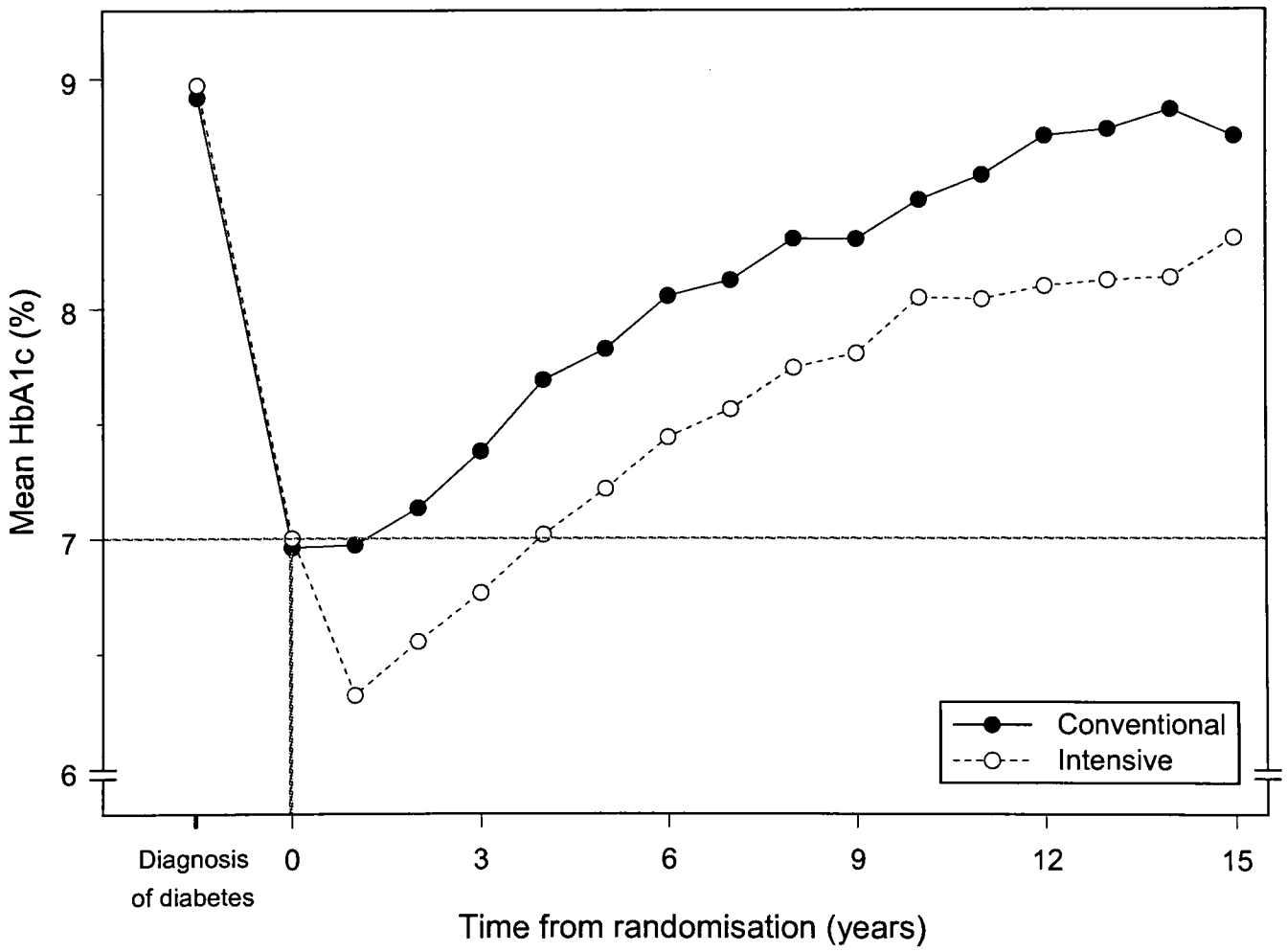


Figure 4.1 shows HbA1c levels over time in the UKPDS. As FPG levels shown in chapter 1, HbA1c levels decreased markedly during the dietary run-in period in both conventional and intensive therapy groups. After randomisation, HbA1c levels

increased steadily over time, regardless of therapy, although there was an initial decrease in HbA1c during the first year in the intensive group.

In this chapter, the relationship between HbA1c and FPG in patients with type 2 diabetes over time will be examined using data from the UKPDS. The aims of this chapter are to determine whether HGI is reproducible, i.e. is there a consistent relationship between HbA1c and FPG, in newly diagnosed type 2 diabetes, and to quantify HGI in this population. In the light of the results obtained in chapter 3, these questions will be addressed employing Pearson's correlation coefficients, Bland-Altman difference plots, the discriminant ratios and repeated measures linear regression models.

4.2 Study population

The UKPDS recruited 5102 individuals with newly diagnosed type 2 diabetes aged 25-65 years from 23 centres in the UK between 1977 and 1992. The study ran to 1997. The methods and main results of the study, designed primarily to evaluate the effect of improved glycaemic control on diabetic complications, have been published (UKPDS Group 1998). Briefly, after diagnosis of diabetes, patients entered a dietary run-in period lasting three or four months. After the dietary run-in period, 3867 patients meeting the inclusion criteria for the main randomisation were allocated to an 'intensive' or a 'conventional' blood glucose control policy. All 5,102 patients were monitored for fatal and nonfatal clinical endpoints whether or not they met the criteria for randomisation. Blood samples were taken annually after an overnight fast. HbA1c measurements were made on these samples by a central laboratory using methods aligned to an international standardisation program (Little, Rohlfing et al. 2001). FPG

was measured at each clinical centre, with comparability maintained by the UKPDS Glucose Quality Assurance Scheme (UKPDS Group 1994).

4.3 Baseline for the analyses

Figure 4.2 Study design of the UKPDS

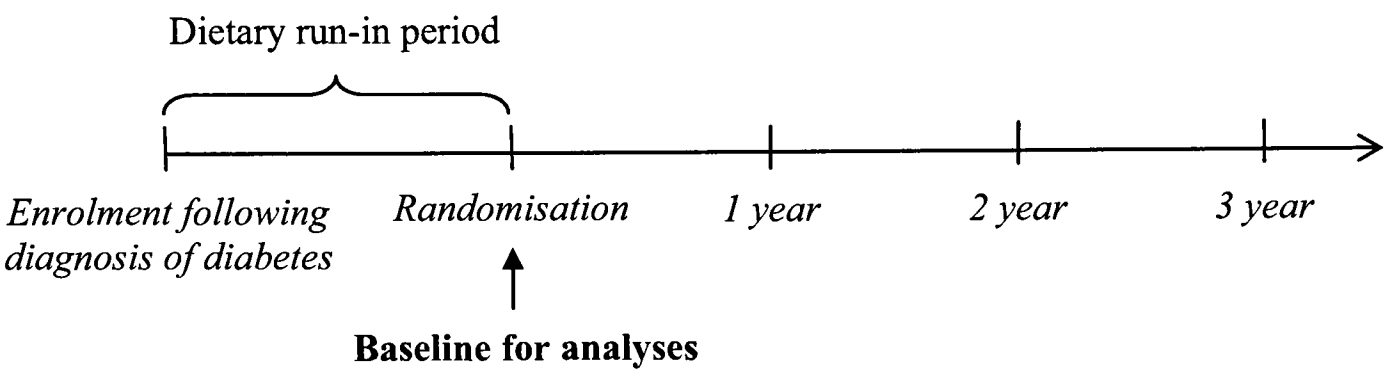


Figure 4.3 FPG at enrolment following the diagnosis and after the dietary run-in

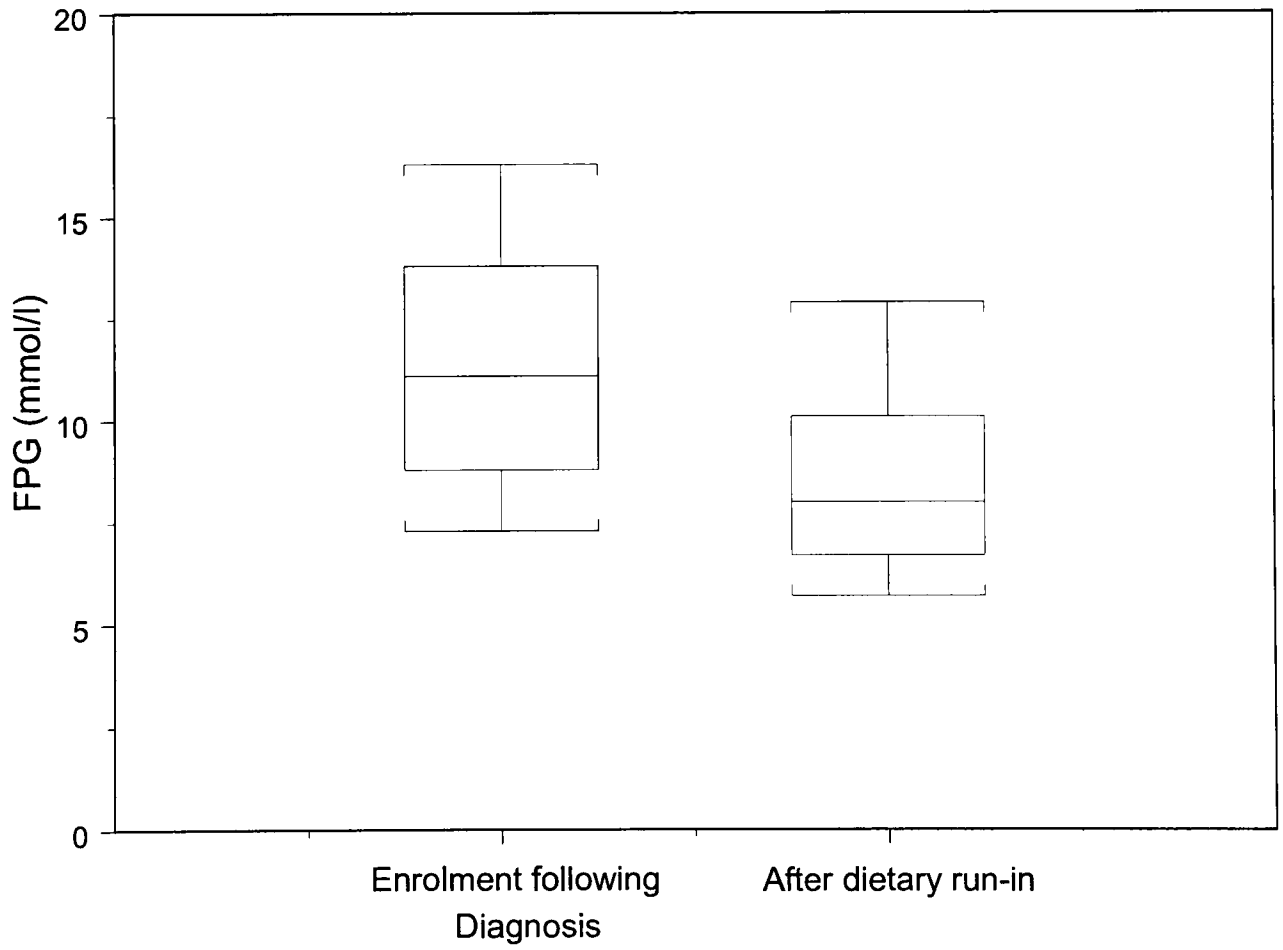


Figure 4.2 shows the study design of the UKPDS. For the UKPDS analyses presented in this thesis, the post-dietary run-in data was used as the baseline. As it is depicted in Figure 4.3, post-dietary run-in FPG (IQR 6.7 to 10.1) is less variable compared to

FPG at the enrolment for the study (IQR 8.8 to 13.8). Blood glucose level at the enrolment for the study is more variable due to the effect of factors such as inter-current infection which may have influenced glycaemic levels at the time of diagnosis of type 2 diabetes (Colagiuri, Cull et al. 2002). Thus, glucose levels following the dietary run-in were used in the following analyses.

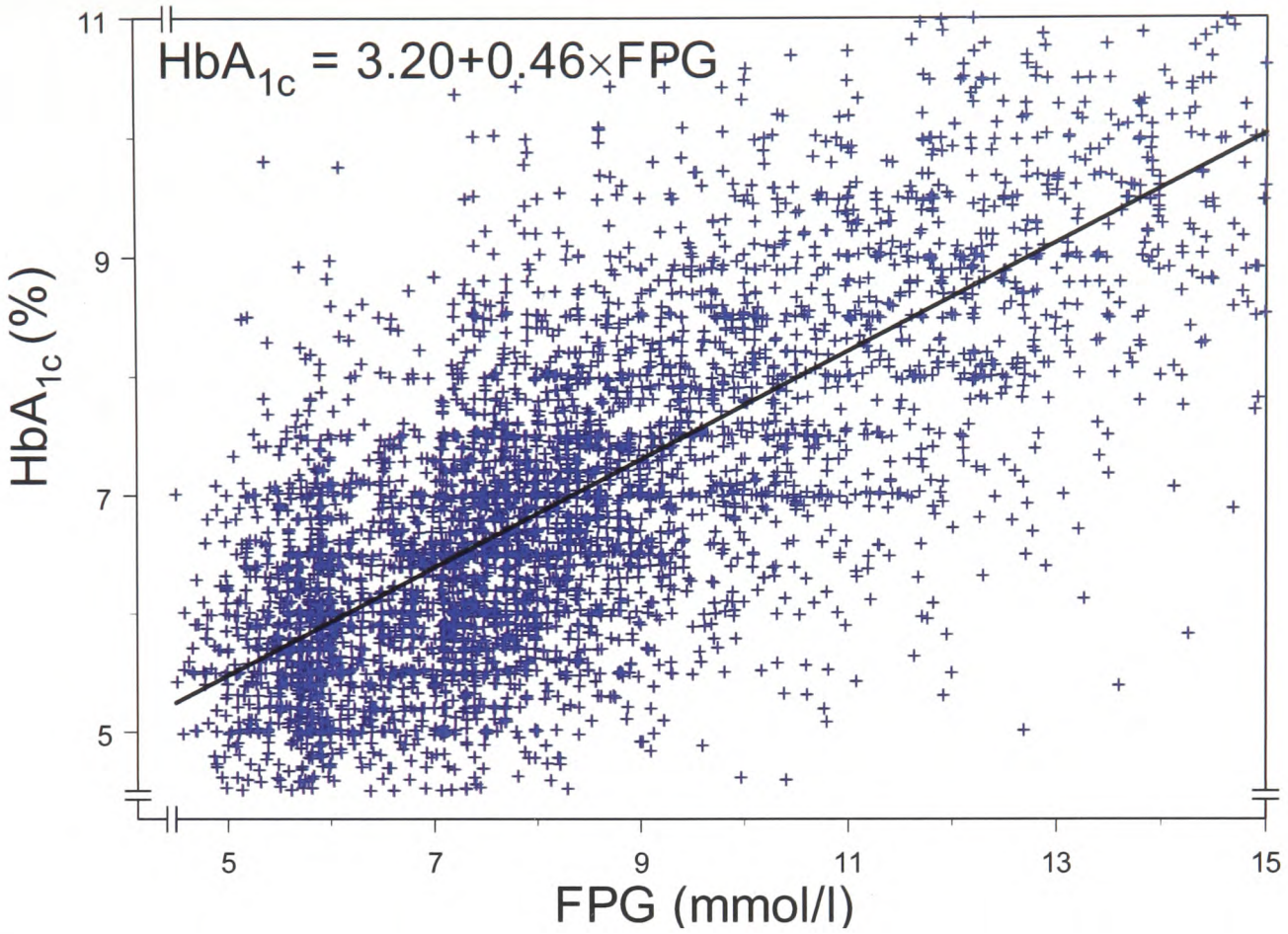
For this chapter, 3651 patients with the requisite data were included in the analysis after 1451 patients were excluded due to missing data at baseline (for a consort diagram, please refer to Appendix 3). Table 4.1 shows patient characteristics after the dietary run-in period (randomisation) and at year 5, year 10 and year 15. Smoking status and LDL cholesterol were not available at year 5, year 10 and year 15.

Table 4.1 Patient characteristics after dietary run-in period and at year 5, year 10 and year 15

After dietary run-in	Median (IQR) or %
FPG (mmol/l)	7.9 (6.6, 10.0)
HbA1c (%)	6.8 (5.9, 8.0)
Age (years)	54 (47, 60)
Male	60%
Ethnicity (White/Indian Asian/Afro-Caribbean)	82/ 10/ 8%
Smoker (never smoked/ex/current)	35/ 35/ 30%
Body mass index (kg/m^2)	26.7 (24.0, 30.4)
Systolic blood pressure (mm Hg)	130 (120, 149)
HDL cholesterol (mmol/l)	1.04 (0.91, 1.20)
LDL cholesterol (mmol/l)	3.4 (2.8, 4.1)
Triglycerides (mmol/l)	1.5 (1.1, 2.1)
Albuminuria (urine albumin ≥ 50 mg/l)	12%
Year 5	Median (IQR) or %
FPG at year 5 (mmol/l)	7.8 (6.1, 10.2)
HbA1c at year 5 (%)	7.0 (6.1, 8.4)
Body mass index at year 5 (kg/m2)	28.1 (25.3, 31.4)
Systolic blood pressure at year 5 (mm Hg)	135 (121, 150)
HDL cholesterol at year 5 (mmol/l)	1.05 (0.89, 1.24)
Triglycerides at year 5 (mmol/l)	1.5 (1.1, 2.2)
Albuminuria at year 5 (urine albumin ≥ 50 mg/l)	16%
Year 10	Median (IQR) or %
FPG at year 10 (mmol/l)	8.6 (6.6, 11.2)
HbA1c at year 10 (%)	8.0 (6.8, 9.4)
Body mass index at year 10 (kg/m2)	28.1 (25.3, 31.7)
Systolic blood pressure at year 10 (mm Hg)	139 (125, 151)
HDL cholesterol at year 10 (mmol/l)	1.06 (0.88, 1.26)
Triglycerides at year 10 (mmol/l)	1.4 (1.0, 2.1)
Albuminuria at year 10 (urine albumin ≥ 50 mg/l)	22%
Year 15	Median (IQR) or %
FPG at year 15 (mmol/l)	9.5 (6.6, 11.8)
HbA1c at year 15 (%)	8.0 (6.9, 9.9)
Body mass index at year 15 (kg/m2)	27.9 (25.2, 30.5)
Systolic blood pressure at year 15 (mm Hg)	140 (125, 133)
HDL cholesterol at year 15 (mmol/l)	1.09 (0.91, 1.33)
Triglycerides at year 15 (mmol/l)	1.4 (0.9, 2.2)
Albuminuria at year 15 (urine albumin ≥ 50 mg/l)	29%

4.4 Haemoglobin Glycation Index (HGI)

Figure 4.4 HbA1c vs. FPG at baseline (n=4353)



To calculate HGI, a linear regression of HbA1c on FPG at baseline was fitted, using all the available pairs of HbA1c and FPG at baseline, i.e. after 3 months dietary run-in period (n=4353). The R-square statistics was 0.58, indicating 58% of total variation in HbA1c is explained by FPG. Figure 4.4 shows the plot of HbA1c against FPG at baseline in the UKPDS. It is observed that patients with the same FPG can have much higher or lower HbA1c than expected for their FPG. The fitted regression equation was

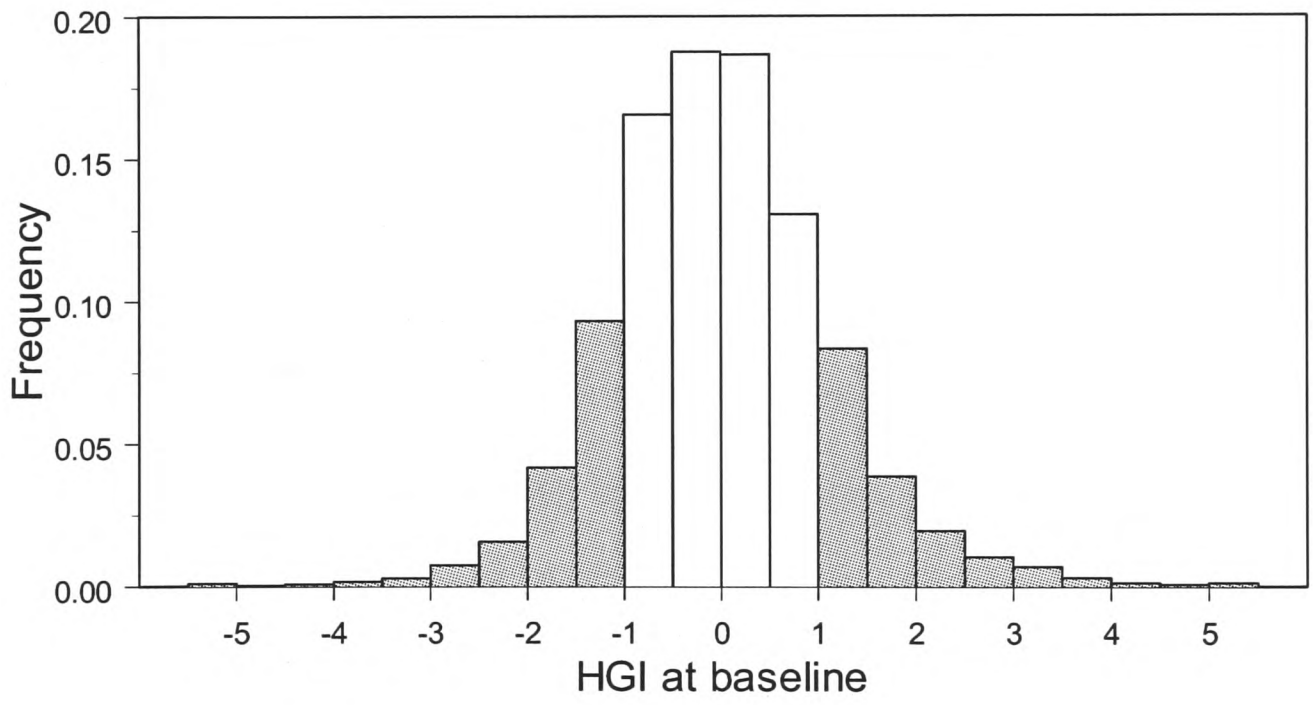
$$\text{HbA1c} = 3.20 + 0.46 \times \text{FPG} \qquad \text{(Equation 4.1)}$$

After that, predicted HbA1c is calculated, entering patients' FPG at any given time point into the linear equation (Equation 4.1). Then HGI is calculated subtracting predicted HbA1c from patients' actual HbA1c at any given time point.

HGI at baseline

Figure 4.5 shows the distribution of HGI at baseline in the UKPDS. HGI was approximately normally distributed with a median -0.03 and inter-quartile range of – 0.72 to 0.65. In these patients with a median (IQR) HbA1c of 6.9% (5.9 to 8.1), 33% had observed HbA1c more than 1% higher or lower than expected for their FPG (shaded area in Figure 4.5). 63% had observed HbA1c more than 0.5% higher or lower than expected for their FPG.

Figure 4.5 Histogram of HGI at baseline in the UKPDS



HGI and different treatments groups

Table 4.2 Mean (SD) of HGI at year 0, year 1, year 2, and difference in HGI values between year 0 and year 1 and between year 1 and year 2 by randomisation group (n=3405)

	N	year 0	year 1	year 2	year1 - year0	year2 - year1
Diet	913	-0.042 (1.06)	-0.14 (1.10)	-0.039 (1.13)	-0.10 (1.23)	0.13 (1.19)
Sulphonylurea	1272	-0.023 (1.11)	-0.22 (1.10)	-0.045 (1.18)	-0.20 (1.33)	0.17 (1.29)
Insulin	943	-0.059 (1.04)	-0.043 (1.11)	0.11 (1.13)	0.016 (1.26)	0.16 (1.32)
Metformin	277	0.018 (1.07)	-0.21 (1.07)	-0.078 (0.97)	-0.22 (1.24)	0.13 (1.20)

Table 4.2 shows HGI at baseline, year 1, year 2, difference between HGI values at year 1 and baseline and difference between HGI values at year 2 and year 1 by randomisation group. When HGI values were compared by randomisation group, there was no significant difference in HGI at baseline (p=0.71 by analysis of variance) whereas HGI at year 1 (p=0.0016) and year 2 (p=0.011) were significantly different. Difference between HGI values at year 1 and baseline (p=0.0005) was significantly different between randomisation groups whereas difference between HGI values at year 2 and year 1 (p=0.89) was not. When this analysis was repeated including those remaining on allocated therapy at year 1 (n=2639), similar results were found: there was no significant difference in baseline HGI (p=0.48) and difference in HGI values at year 2 and year 1 (p=0.70) whereas HGI at year 1 (p<0.0001), HGI at year 2 (p<0.0001) and difference in HGI values at year 1 and baseline (p<0.0001) were significantly different between treatment groups.

These results suggest that the relationship between HbA1c and FPG over time might be associated with therapies. It has been shown elsewhere that the regression coefficients between FPG and HbA1c (the intercept and slope) in those treated with insulin were significantly different from the other three groups over 9-year period (Cull, Manley et al. 1995).

Comparison of linear equations in different studies

Table 4.3 Intercepts and gradients of the linear equations in different studies

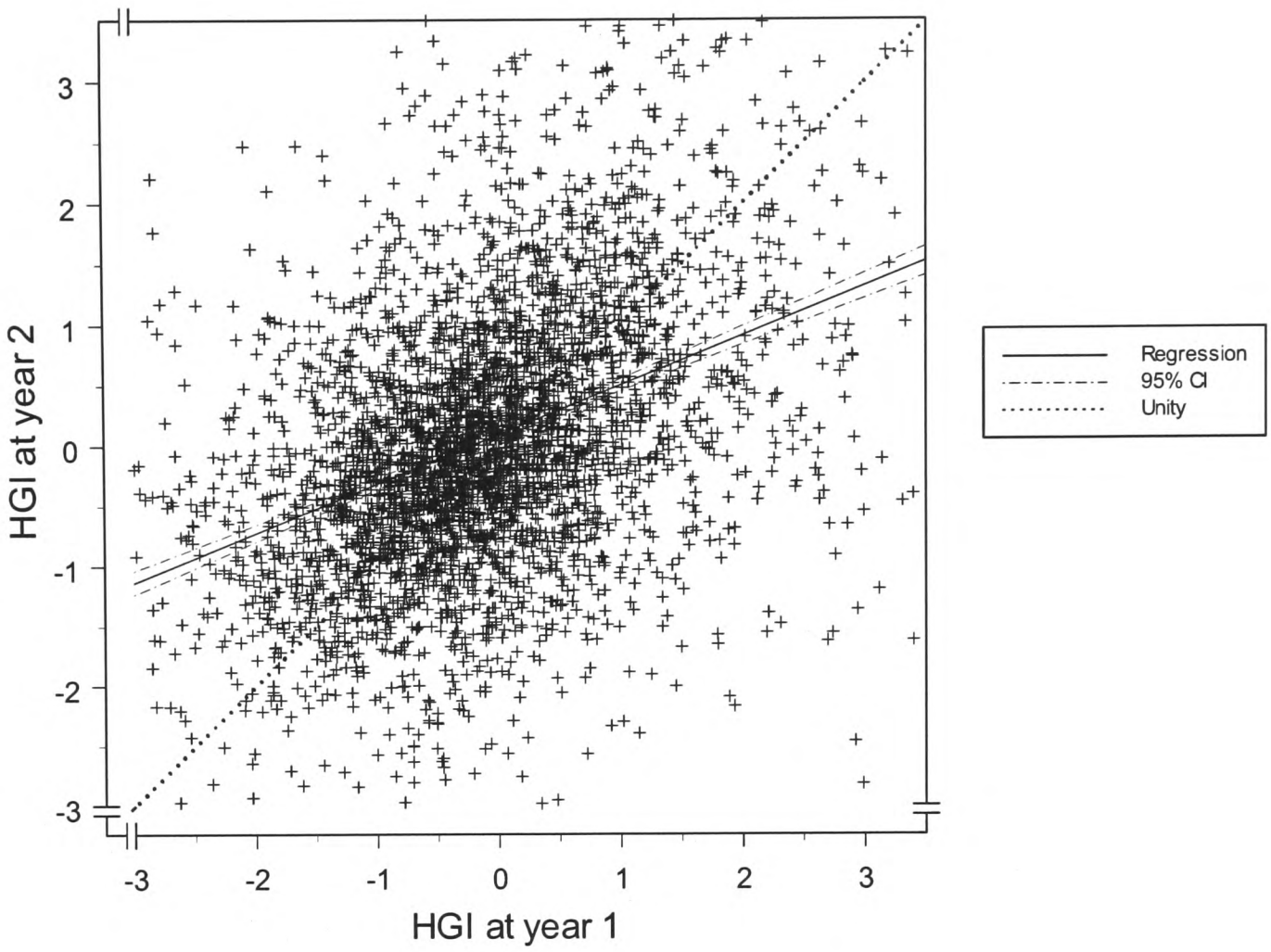
	Intercept (SE)	Gradient of FPG (SE)
UKPDS (Type 2 diabetes)	3.20 (0.055)	0.46 (0.0059)
EDIT (Pre-diabetes)	3.54 (0.16)	0.40 (0.027)
Type 1 diabetes (Hempe et al. (2002))	7.6 (N/A)	0.29 (N/A)

Table 4.3 shows the intercepts and gradients of the linear equations in different studies. To make the linear equations comparable, the gradient reported in type 1 diabetes was adjusted, converting the unit of fasting blood glucose (mg/dl) to mmol/l (divide by 18) and fasting blood glucose to FPG (multiply by 1.1). When the intercepts and gradients were compared between UKPDS and EDIT, there was no significant difference ($p=0.37$ for intercept, $p=0.35$ for gradient by analysis of covariance). The gradient and intercept in type 1 diabetes could not be compared to other studies by a formal test since patient-level data was not available.

4.5 Pearson's correlation coefficients and Bland-Altman's difference plots

To assess the reproducibility of HGI, Pearson's correlation coefficient between HGI at year 1 and year 2 was calculated. For this analysis, HGI at year 1 and year 2 were used instead of HGI at baseline and year 1 since patients were started their therapy after randomisation at baseline which might have affected their HbA1c levels.

Figure 4.6 Scatter plots for HGI at year 2 and HGI at year 10 against HGI at year 1



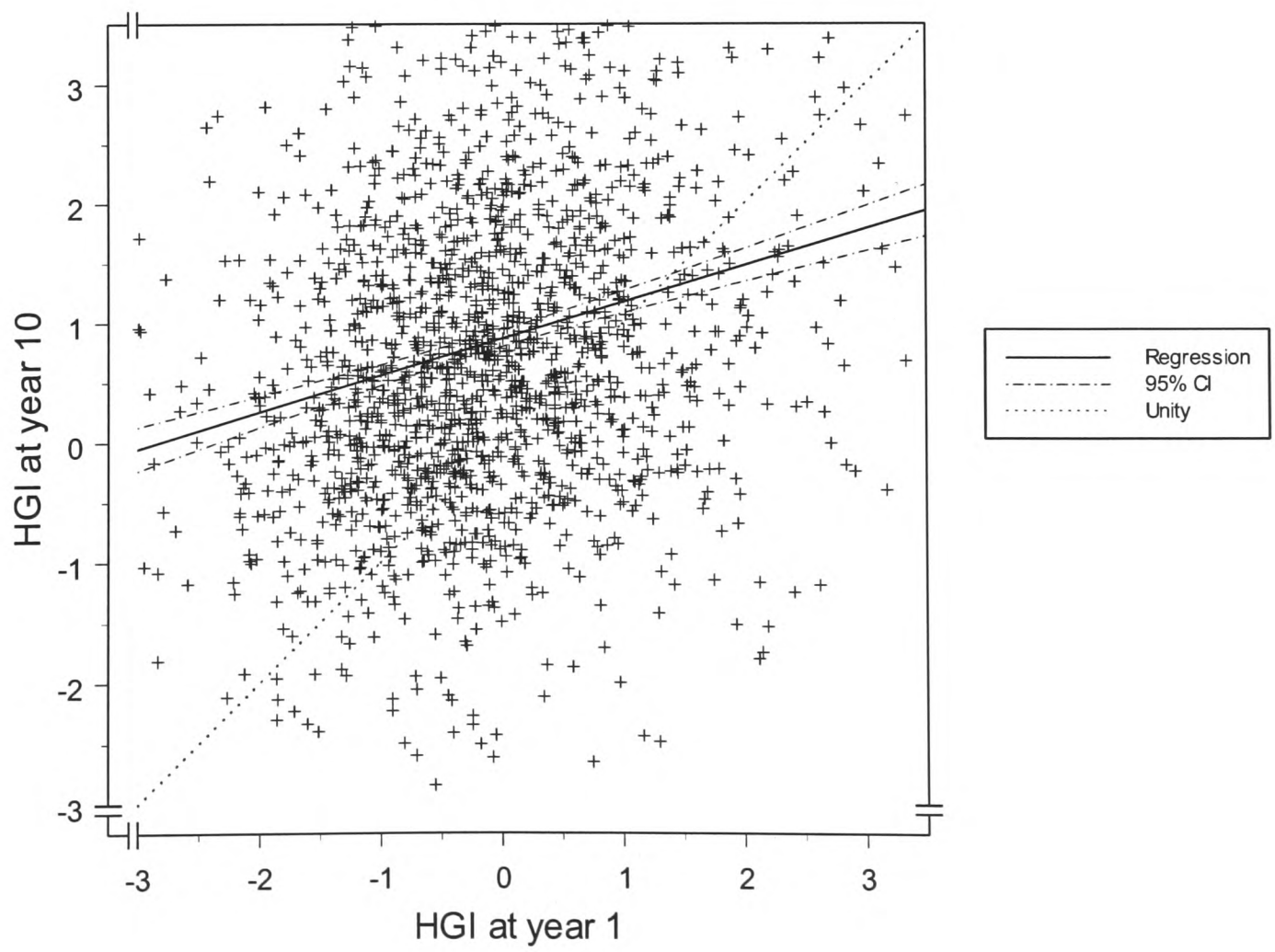
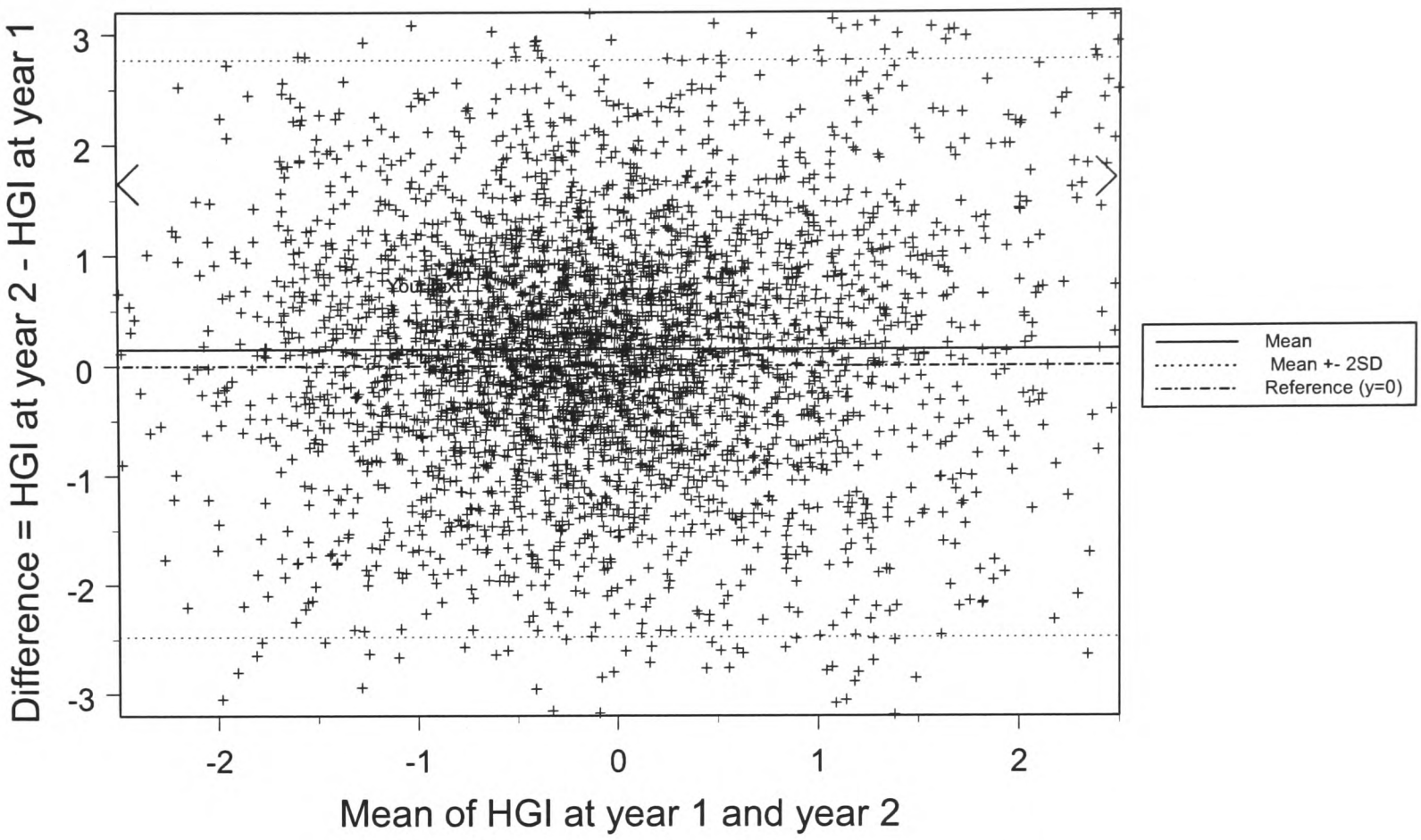


Figure 4.7 Difference plots for HGI at year 2 and HGI at year 10 against HGI at year 1



* Symbols in the graphs (<, >) represent there are some data points outside this range.

Figure 4.6 displays scatter plots for HGI at year 2 and HGI at year 10 against HGI at year 1. Scatter plots indicate that there is an association between HGI at year 1 and HGI at year 2 or HGI at year 10. Pearson's correlation coefficient between HGI at year 1 and year 2 was 0.38 (95% CI [0.35 to 0.41], $p<0.0001$) and Pearson's correlation coefficient between HGI at year 1 and year 10 was 0.36 (95% CI [0.32 to 0.43], $p<0.0001$). Figure 4.7 shows difference plots of HGI at year 2 and HGI at year 10 against HGI at year 1. The difference plot of HGI at year 2 against HGI at year 1 (upper pane) indicates that the difference was not associated with mean HGI and there was no pattern in the data whereas the difference plot of HGI at year 10 against HGI at year 1 (lower pane) suggests that there is a trend towards increases in HGI over time.

4.6 Discriminant ratio

To assess the reproducibility of HGI, the discriminant ratio (DR) was calculated. HGI at year 1 and year 2 were used to calculate DR. HGI at baseline and year 1 could not be used since patients were randomised at baseline and started treatments which could have altered blood glucose levels at year 1. For comparison, the discriminant ratios for HbA1c, FPG and systolic blood pressure were also calculated.

Table 4.4 Discriminant ratios of different measures

	Corrrected Between-Subject SD	Within-Subject SD	DR	95% CI	
Systolic BP (mmHg)	1.767	1.240	1.43	1.38	1.47
HbA1c (%)	1.188	0.973	1.22	1.18	1.27
FPG (mmol/l)	1.845	1.647	1.12	1.08	1.16
HGI (unit)	0.739	0.953	0.78	0.73	0.82

Table 4.4 shows discriminant ratios for different measures. Higher discriminant ratio represents better reproducibility. HGI (DR=0.78) had lower DR than systolic blood

pressure (DR=1.43), HbA1c (DR=1.22) and FPG (DR=1.12), suggesting HGI was less reproducible than these measures in this population.

It seems necessary to take a mean of repeated HGI values in order to obtain more reproducible HGI in type 2 diabetes. The mean HGI is more representative of underlying HGI for a patient, reducing errors in HGI values for the patient (within-subject SD of HGI). If we assume that each HGI measurement follows a normal distribution, SD of the mean HGI would be equal to within-subject SD divided by square root of the number of repeated HGI measurements. For example, if two HGI measurements are used to calculate mean HGI, within-subject SD of mean HGI would be reduced to 0.67 (=0.953/$\sqrt{2}$)

Table 4.5 Number of repeated HGI measurements used for taking a mean HGI and the discriminant ratio of HGI

Number of repated measurements of HGI	DR of HGI
2	1.10
3	1.34
4	1.55
5	1.73

Table 4.5 shows the number of repeated HGI values used for taking a mean and the discriminant ratio of HGI. A mean of two repeated HGI values (DR=1.10) could yield a similar discriminant ratio to FPG in the UKPDS and a mean of three repeated HGI values (DR=1.34) could produce similar discriminant ratio to HbA1c in the UKPDS.

4.7 Repeated measures analysis (random effect models)

Two repeated measures linear regression models were fitted using annual measurements of FPG and HbA1c over median 9.0 years period. In each model, a

subject effect was modelled as a random effect and other covariates were modelled as fixed effects. In these models, the subject effect on HbA1c adjusting for FPG corresponds to underlying mean HGI. In the first model, a subject effect on HbA1c was tested adjusting for FPG. In the second model, a subject effect on HbA1c was tested adjusting for FPG and other potential confounders (chosen by stepwise selection with a 5% cut-off).

Table 4.6 Repeated measures linear regression of HbA1c on FPG

	Coef.	Std. Err.	P-value
Intercept	4.33	0.027	<0.0001
FPG (per mmol/l)	0.36	0.0026	<0.0001
Variance of Subject Effect	0.71	0.021	<0.0001
Variance of Residuals	1.10	0.0095	<0.0001

Table 4.6 shows the results of the first model. There was a significant subject effect on HbA1c ($p<0.0001$) with the variance 0.71 and residual variance 1.10 adjusting for FPG. This result suggests that the variation in HbA1c levels within a subject is larger than the variation in HbA1c levels between different patients adjusting for FPG, i.e. DR<1.

Table 4.7 Repeated measures linear regression of HbA1c on FPG after adjustment for other patient characteristics and randomisation group

	Coef.	Std. Err.	P-value
Intercept	4.72	0.17	<0.0001
FPG (per mmol/l)	0.35	0.0027	<0.0001
Age (per 10 years)	-0.007	0.0020	0.00040
HDL Cholesterol (per mmol/l)	0.27	0.069	<0.0001
LDL Cholesterol (per mmol/l)	0.053	0.016	0.0011
Triglycerides (mmol/l)	0.049	0.019	0.010
Systolic blood pressure (mm Hg)	-0.029	0.0087	0.0008
Male (vs. Female)	-0.15	0.035	<0.0001
Afro-Caribbean (vs.White-Caucasian)	0.21	0.055	<0.0001
Indian Asian (vs.White-Caucasia)	0.33	0.061	<0.0001
Ex Smoking (vs.Never Smoked)	-0.035	0.041	0.0037
Smoking (vs.Never Smoked)	0.093	0.041	0.0037
Albuminuria (urine albumin ≥50 mg/l)	0.38	0.022	<0.0001
Randomisation group:			
Insulin (vs.Conventional)	0.018	0.043	<0.0001
Chlorpropamide (vs.Conventional)	-0.20	0.047	<0.0001
Glibenclamide (vs.Conventional)	-0.18	0.047	<0.0001
Metformin (vs.Conventional)	-0.13	0.062	<0.0001
Variance of Subject Effect	0.67	0.020	<0.0001
Variance of Residuals	1.08	0.0095	<0.0001

Not significant at the 5% level: body mass index (p=0.48)

Table 4.7 shows the results of the second model. HbA1c was significantly associated with FPG, age, HDL and LDL cholesterol, triglycerides, systolic blood pressure, sex, ethnicity, smoking status, presence of albuminuria and randomisation group (all $p<0.01$) but not with body mass index ($p=0.48$). Confounders reduced the variance of the subject effect from 0.71 to 0.67. The subject effect on HbA1c was still significant ($p<0.0001$) with the variance 0.67 and residual variance 1.08 after adjustment for FPG and other confounders listed above. These results confirm that different patients have different mean HGI.

The effect of clinical centres was not tested as a possible determinant of HGI since it is the policy of the UKPDS Group not to test for differences between their clinical centres. When the repeated measures analysis was restricted to the largest centre only

(n=305), the subject effect on HbA1c adjusting for FPG remained significant for both models (all $p<0.0001$).

4.8 Discussion

Relationship between HbA1c and FPG in type 2 diabetes

This chapter has demonstrated both the correlation between FPG and HbA1c within the diabetic population and the wide variability in HbA1c between individuals with the same FPG. In UKPDS, 33% had observed HbA1c more than 1% higher or lower than expected for their FPG, as shown in the distribution of HGI. This chapter also showed that HGI, a measure of the variation in HbA1c not attributable to FPG, is a reproducible characteristic in patients with type 2 diabetes. In the repeated measures models, the subject effect on HbA1c adjusting for FPG was significant even after adjustment for potential confounders, indicating that the relationship between FPG and HbA1c in a subject is consistent during a median 9 years follow up. This confirms and extends findings in normal patients (Yudkin, Forrest et al. 1990), pre-diabetes (chapter 3) and type 1 diabetes (Hempe, Gomez et al. 2002; McCarter, Hempe et al. 2004).

Comparison of HGI in type 2 diabetes and pre-diabetes

I found that there was no significant difference in the linear equations used for the calculation of HGI between type 2 diabetes (UKPDS) and pre-diabetes (EDIT). As in EDIT, HGI was not explained by other confounders. In the repeated measures model, more confounders were selected probably due to more statistical power from the large data. However, the additional confounders did not add much predictive power. HGI was reproducible in both pre-diabetes and type 2 diabetes but in EDIT, HGI had

similar DR to other covariates such as HbA1c whereas in UKPDS, HGI had lower DR than other covariates. It appears that particularly high reproducibility in pre-diabetes could be an artefact of a restricted range of FPG. In order to obtain more reproducible HGI in type 2 diabetes, it seems practical to take a mean of three HGI values repeated every three months which could yield a similar reproducibility to HbA1c. Based on the assumption that HbA1c reflects an average glucose over preceding 2-3 months, measuring HGI every three months could add extra information to mean HGI.

Potential Limitations

In addition to the limitations discussed in chapter 3, no postprandial glucose measure or surrogate was available in this chapter. However, this may not be that important as post-challenge glucose was only weakly associated with HGI in the previous chapter. HGI might be the results of effects of different therapies. Actual therapy was not tested in UKPDS but randomisation group did not explain much variation in HGI in the repeated measures model.

HGI and diabetic complications

My analysis showed that the variation in HbA1c not attributable to FPG, captured by HGI, was reproducible in patients with type 2 diabetes over median 9 years. Potential clinical importance of HGI is suggested by an analysis of the DCCT cohort which found HGI to be an independent risk factor for microvascular disease in type 1 diabetes (McCarter, Hempe et al. 2004). The association between HGI and diabetic complications in type 2 diabetes has not been investigated yet. Systematic differences between HbA1c and FPG may explain why people with relatively good glycaemic

control measured by HbA1c sometimes develop diabetic complications whereas people with apparently poor glycaemic control sometimes do not develop diabetic complications. Understanding these differences is potentially important to the management of diabetes as HbA1c becomes increasingly widely used as a standard of glycaemic control. In chapter 5, the association between HGI and diabetic complications in type 2 diabetes will be investigated using the UKPDS data.

5 Relationship between HGI and diabetic complications in type 2 diabetes

5.1 Background and aim

It is well-established that hyperglycaemia is associated with diabetic complications, and these have been associated with several different measures of glycaemia such as FPG, 2h post-challenge glucose and HbA1c (The DCCT Research Group 1993; Hanefeld, Fischer et al. 1996; UKPDS Group 1998; Decode Study Group on behalf of the European Diabetes Epidemiology Group 2001). Few studies, however, have investigated whether HbA1c and different measures of glycaemia make independent contributions to diabetic complications (Hanefeld, Fischer et al. 1996; Decode Study Group on behalf of the European Diabetes Epidemiology Group 2001; Cavalot, Petrelli et al. 2006). Given that the variation in HbA1c not attributable to FPG, captured by HGI, is reproducible in people with type 2 diabetes, as shown in chapter 4, it is reasonable to ask whether HGI may also contribute independently to diabetic complications.

In type 1 diabetes, the glycosylation gap (GG) has been shown to be an independent risk factor for nephropathy after adjustment for either HbA1c or fructosamine (Cohen, Holmes et al. 2003). HGI has been also reported as an independent risk factor for retinopathy and nephropathy in type 1 diabetes after adjustment for MBG using the data from the DCCT (McCarter, Hempe et al. 2004). However, McCarter et al's finding was criticised by Genuth et al. (Genuth, Lachin et al. 2005) since HGI, a residual of HbA1c, is highly correlated with HbA1c and thus the results just showed

that diabetic complications are associated with HbA1c. They commented that HGI need to be tested after adjustment for HbA1c to determine whether HGI is an independent risk factor of diabetic complications. To date, there has been no study which investigated the relationship between HGI and diabetic complications in type 2 diabetes.

In this light, I have examined whether HGI is an independent risk factor for macrovascular or microvascular complications in type 2 diabetes, after adjustment for either FPG or HbA1c, using data from the UKPDS.

5.2 Study population

The UKPDS has been described in chapter 4. All 5,102 patients in the UKPDS were monitored for fatal and nonfatal clinical endpoints whether or not they met the criteria for randomisation. For this analysis, 3,651 patients with requisite data were included (for patient characteristics at baseline and at year 5, year 10 and year 15, please refer to Table 4.1)

5.3 Outcome measures

Table 5.1 Numbers and types of complications for "any diabetes-related endpoint", macrovascular complications and microvascular complications

Any diabetes-related endpoint	n	%
Myocardial infarction	331	28.1
Retinal photocoagulation	251	21.3
Angina	212	18.0
Cataract extraction	149	12.6
Stroke	112	9.5
Heart failure	75	6.4
Sudden death	17	1.4
Lower extremity amputation	15	1.3
Renal failure	8	0.7
Vitreous haemorrhage	5	0.4
Death from hyperglycaemia or hypoglycaemia	3	0.3
Death from peripheral vascular disease	1	0.1
Total	1179	100%
Macrovascular Complications	n	%
Fatal MI	167	21.8
Fatal Stroke	31	4.0
Fatal Sudden	23	3.0
Non-fatal IHD	235	30.7
Non-fatal MI	210	27.4
Non-fatal Stroke	100	13.1
Total	766	100%
Microvascular Complications	n	%
Fatal Renal Failure	3	1.0
Non-fatal Retinopathy	290	92.7
Non-fatal Renal Failure	13	4.2
Non-fatal VH	7	2.2
Total	313	100%

Three UKPDS pre-defined aggregate outcomes were examined. These were: 1) "any diabetes-related endpoint", defined as the first event to occur of myocardial infarction (MI), sudden death, angina, stroke, renal failure, lower extremity amputation or death from peripheral vascular disease, death from hyperglycaemia or hypoglycaemia, heart failure, vitreous haemorrhage, retinal photocoagulation and cataract extraction; 2) macrovascular complications, defined as the first event to occur of fatal or non-fatal MI, fatal or non-fatal stroke, non-fatal ischemic heart disease (IHD) and sudden death; and 3) microvascular complications, defined as the first event to occur of retinopathy requiring photocoagulation, vitreous haemorrhage, and fatal or non-fatal renal failure.

5.4 Statistical methods

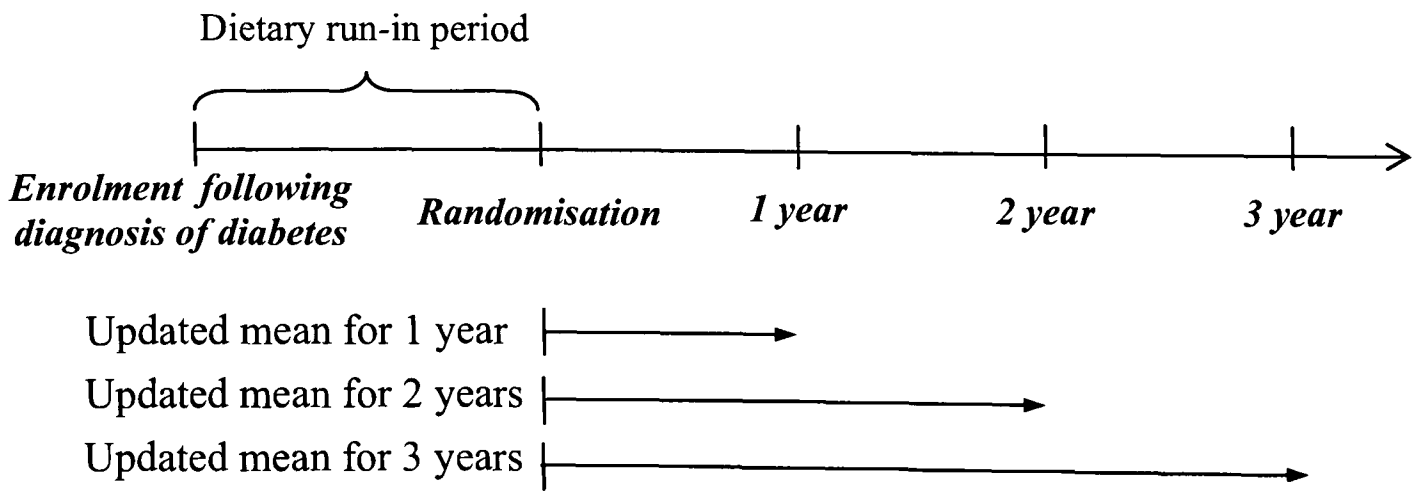
Cox models for survival data

To determine which measure of glycaemia is associated with microvascular or macrovascular complications, Cox proportional hazard modelling was employed. As HGI is calculated from HbA1c and FPG values, the following three Cox models are all equivalent:

- A Cox model with HbA1c and FPG
- A Cox model with FPG and HGI
- A Cox model with HbA1c and HGI

Thus, if the hazard ratios of FPG and HbA1c are obtained from the Cox model, the hazard ratio of HGI adjusting for FPG or HbA1c can be calculated (please refer to the Appendix 3 for the calculations). Thus, to verify whether HGI is independently associated with diabetic complications in type 2 diabetes, it is sufficient to determine whether FPG and HbA1c are independently associated with diabetic complications in type 2 diabetes. In this way, the problem with McCarter et al's analyses can be avoided.

Figure 5.1 Calculation of updated mean in the UKPDS



To determine whether FPG and HbA1c were independently associated with any diabetes-related endpoint, macrovascular or microvascular complications, two sets of Cox models for time to each outcome were fitted. In the first set, baseline FPG and HbA1c values were tested whilst the second set used time-dependent covariates, with updated mean FPG and HbA1c. Updated means were calculated from annual measurements, for each individual from baseline to each year of follow-up (Figure 5.1). As stipulated in chapter 4, post-dietary run-in data was used as the baseline for analyses presented here.

In additional analyses, the above Cox models were repeated adjusting for randomisation arms (n=3177). FPG and HbA1c at enrolment for the study (diagnosis of diabetes) were tested, replacing FPG and HbA1c after dietary run-in. For microvascular complications, FPG and HbA1c were tested together again after adjustment for the presence of retinopathy at diagnosis of diabetes since microvascular complications comprise retinopathy.

All Cox models in this chapter were adjusted for the following potential confounders measured after dietary run-in: HDL and LDL cholesterol, triglycerides, systolic blood pressure, sex, age, ethnic group, smoking status (current/ex/never) and presence of albuminuria (urine albumin $\geq$ 50 mg/l). Models were fitted in SAS and p-values were calculated by likelihood ratio tests.

Effects of missing values on model fitting

Since a Cox model with FPG and HbA1c, a Cox model with HGI and FPG and a Cox model with HGI and HbA1c are all equivalent, these three Cox models have the same

likelihood in theory. When three Cox models were fitted with baseline covariates, all three models had the same likelihood as it was expected. However, when three Cox models were fitted with time-dependent covariates, three models had different likelihood values. This disparity was due to the missing values in FPG or HbA1c. To make three Cox models with time-dependent covariates comparable, if there was a missing value in either FPG or HbA1c at any time point, both FPG and HbA1c were treated as missing values at that time point.

5.5 *Any diabetes-related endpoint*

Table 5.2 Relationship between "the outcome 'any diabetes-related endpoint'" and FPG and HbA1c at baseline and as updated means

Main Analyses	HR	95% CI	P-value
Model with baseline covariates			
FPG after dietary run-in (mmol/l)	1.10	1.07 1.13	<0.0001
HbA1c after dietary run-in (%)	1.00	0.95 1.04	0.85
Model with time-dependent covariates			
Updated mean FPG (mmol/l)	1.09	1.05 1.13	<0.0001
Updated mean HbA1c (%)	1.15	1.08 1.22	<0.0001
Additional Analyses	HR	95% CI	P-value
Models after adjustment for randomisation			
Model with baseline covariates			
FPG after dietary run-in (mmol/l)	1.15	1.11 1.20	<0.0001
HbA1c after dietary run-in (%)	1.01	0.95 1.07	0.77
Model with time-dependent covariates			
Updated mean FPG (mmol/l)	1.12	1.07 1.17	<0.0001
Updated mean HbA1c (%)	1.15	1.07 1.23	<0.0001
Model with FPG and HbA1c at enrolment for the study			
FPG at enrolment for the study (mmol/l)	1.04	1.02 1.06	0.0002
HbA1c at enrolment for the study (%)	1.03	1.00 1.07	0.055

All adjusted for sex, age, ethnic group, smoking status, HDL and LDL cholesterol, triglycerides, systolic blood pressure, and presence of albuminuria after dietary run-in

There were 1179 patients with "the outcome 'any diabetes-related endpoint'" during the median 9.2 years follow-up. Table 5.2 shows the relationship between "the outcome 'any diabetes-related endpoint'" and FPG and HbA1c at baseline and as

updated means after adjustment for potential confounders. In the model with baseline covariates, FPG ($p<0.0001$) but not HbA1c ($p=0.85$) emerged as an independent risk factor for "the outcome 'any diabetes-related endpoint'". In the model with time-dependent covariates, both updated mean FPG ($p<0.0001$) and updated mean HbA1c ($p<0.0001$) were significantly associated with "the outcome 'any diabetes-related endpoint'" after adjustment for potential confounders at baseline.

In the additional analysis, adjusting for randomisation arms, the results did not change materially. In the model with baseline covariates, FPG ($p<0.0001$) but not HbA1c ($p=0.77$) was associated with "the outcome 'any diabetes-related endpoint'". In the model with time-dependent covariates, both updated mean FPG ($p<0.0001$) and updated mean HbA1c ($p<0.0001$) were associated with "the outcome 'any diabetes-related endpoint'". When FPG and HbA1c values at enrolment for the study replaced FPG and HbA1c measured after dietary run-in, the result for FPG was similar ($p=0.0002$) whereas HbA1c approached conventional significance ($p=0.055$).

5.6 Macrovascular complications

Table 5.3 Relationship between macrovascular complications and FPG and HbA1c at baseline and as updated means

Main Analyses	HR	95% CI	P-value
Model with baseline covariates			
FPG after dietary run-in (mmol/l)	1.06	1.02 1.10	0.0035
HbA1c after dietary run-in (%)	0.97	0.91 1.03	0.32
Model with time-dependent covariates			
Updated mean FPG (mmol/l)	1.04	0.99 1.09	0.15
Updated mean HbA1c (%)	1.12	1.03 1.21	0.0056
Additional Analyses	HR	95% CI	P-value
Models after adjustment for randomisation			
Model with baseline covariates			
FPG after dietary run-in (mmol/l)	1.08	1.03 1.13	0.0009
HbA1c after dietary run-in (%)	0.98	0.91 1.05	0.52
Model with time-dependent covariates			
Updated mean FPG (mmol/l)	1.03	0.97 1.09	0.40
Updated mean HbA1c (%)	1.16	1.06 1.26	0.0009
Model with FPG and HbA1c at enrolment for the study			
FPG at enrolment for the study (mmol/l)	1.02	0.99 1.04	0.27
HbA1c at enrolment for the study (%)	1.02	0.98 1.07	0.31

All adjusted for sex, age, ethnic group, smoking status, HDL and LDL cholesterol, triglycerides, systolic blood pressure, and presence of albuminuria after dietary run-in

There were 766 patients with the macrovascular complication endpoint during median 9.7 years follow-up. Table 5.3 shows the relationship between macrovascular complications and FPG and HbA1c at baseline and as updated means after adjustment for potential confounders. In the model with baseline covariates, as seen with "any diabetes-related endpoint", FPG (p=0.0035) but not HbA1c (p=0.32) was associated with macrovascular complications. In the model with time-dependent covariates, unlike the result with "any diabetes-related endpoint", updated mean HbA1c (p=0.0056) but not updated mean FPG (p=0.15) was an independent risk factor for macrovascular complications after adjustment for potential confounders at baseline.

In the additional analysis, adjusting for randomisation arms, the results did not change materially. In the model with baseline covariates, FPG (p=0.0009) but not HbA1c (p=0.52) was associated with macrovascular complications. In the model with time-dependent covariates, both updated mean HbA1c (p=0.0009) but not updated mean FPG (p=0.40) were associated with macrovascular complications. When FPG and HbA1c at enrolment for the study replaced FPG and HbA1c after dietary run-in, the result for HbA1c was similar (p=0.31) whereas FPG became no longer significant (p=0.27).

5.7 Microvascular complications

Table 5.4 Relationship between microvascular complications and FPG and HbA1c at baseline and as updated means

Main Analyses	HR	95% CI		P-value
Model with baseline covariates				
FPG after dietary run-in (mmol/l)	1.17	1.12	1.22	<0.0001
HbA1c after dietary run-in (%)	1.06	0.98	1.15	0.17
Model with time-dependent covariates				
Updated mean FPG (mmol/l)	1.16	1.09	1.24	<0.0001
Updated mean HbA1c (%)	1.30	1.16	1.44	<0.0001
Additional Analyses	HR	95% CI		P-value
Models after adjustment for randomisation (n=3177)				
Model with baseline covariates				
FPG after dietary run-in (mmol/l)	1.35	1.27	1.44	<0.0001
HbA1c after dietary run-in (%)	1.04	0.94	1.15	0.46
Model with time-dependent covariates				
Updated mean FPG (mmol/l)	1.31	1.21	1.42	<0.0001
Updated mean HbA1c (%)	1.21	1.06	1.37	0.0036
Model with FPG and HbA1c at enrolment for the study				
FPG at enrolment for the study (mmol/l)	1.07	1.03	1.11	0.0009
HbA1c at enrolment for the study (%)	1.08	1.02	1.15	0.012
Models after adjustment for retinopathy at baseline				
Model with baseline covariates				
FPG after dietary run-in (mmol/l)	1.17	1.11	1.23	<0.0001
HbA1c after dietary run-in (%)	1.03	0.93	1.14	0.60
Model with time-dependent covariates				
Updated mean FPG (mmol/l)	1.16	1.08	1.25	<0.0001
Updated mean HbA1c (%)	1.25	1.10	1.42	0.0006

All adjusted for sex, age, ethnic group, smoking status, HDL and LDL cholesterol, triglycerides, systolic blood pressure, and presence of albuminuria after dietary run-in

There were 313 patients with the microvascular complications endpoint during median 10.0 years follow-up. Table 5.4 shows the relationship between microvascular complications and FPG and HbA1c at baseline and as updated means after adjustment for potential confounders. In the model with baseline covariates, similar to the relationship seen with "any diabetes-related endpoint" and macrovascular complications, FPG ($p<0.0001$) but not HbA1c ($p=0.17$) was significantly associated with microvascular complications. In the model with time-dependent covariates, in

contrast to the relationship seen with macrovascular complications, both updated mean FPG ($p<0.0001$) and updated mean HbA1c ($p<0.0001$) were significantly associated with microvascular complications after adjustment for potential confounders at baseline.

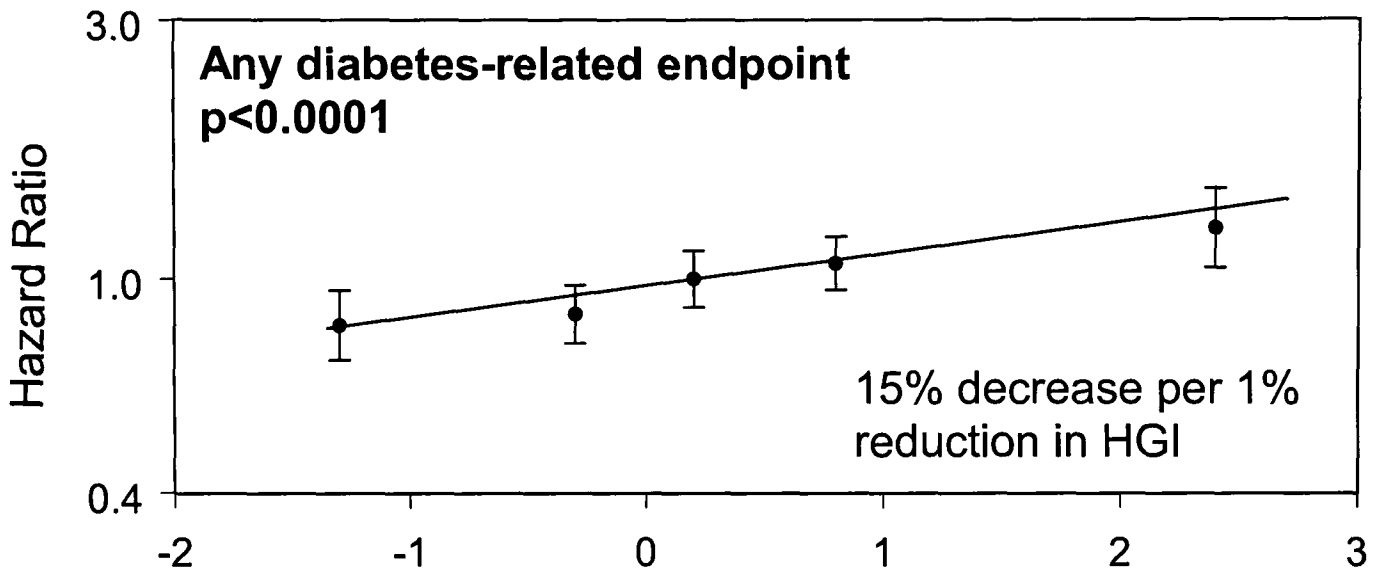
In the additional analysis, adjusting for randomisation arms, the results did not change materially. In the model with baseline covariates, FPG ($p<0.0001$) but not HbA1c ($p=0.46$) was associated with microvascular complications. In the model with time-dependent covariates, both updated mean FPG ($p<0.0001$) and updated mean HbA1c ($p=0.0036$) were associated with microvascular complications. When FPG and HbA1c at enrolment for the study replaced FPG and HbA1c after dietary run-in, the result for FPG was similar ($p=0.0009$) whereas HbA1c became significant ($p=0.012$). Adjusting for the presence of retinopathy at diagnosis of diabetes did not change the results materially. In the model with baseline covariates, FPG ($p<0.0001$) but not HbA1c ($p=0.60$) was associated with microvascular complications; both updated mean FPG ($p<0.0001$) and updated mean HbA1c ($p=0.0006$) were associated with microvascular complications

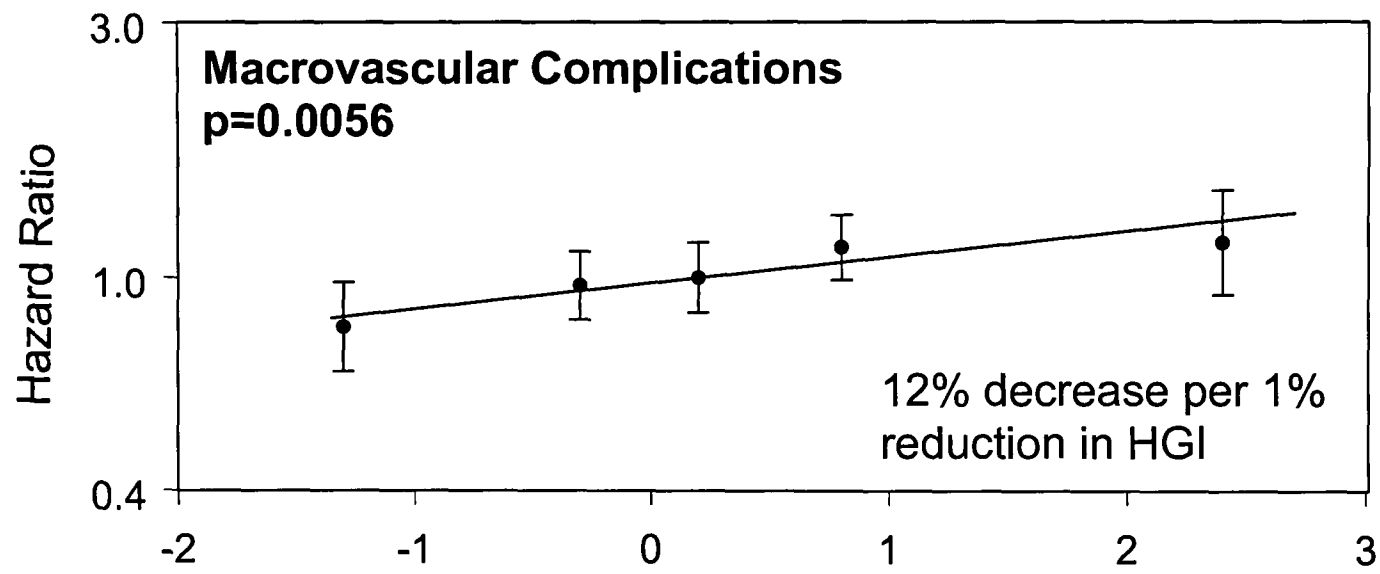
5.8 Relationship between HGI and macrovascular and microvascular complications

Relation of diabetic complications to HGI after adjustment for FPG

A Cox model with FPG and HbA1c is equivalent to a Cox model with FPG and HGI as discussed before. In fact, HGI has the same hazard ratio as HbA1c after adjustment for FPG (please refer to Appendix 4). Thus, the hazard ratio of updated mean HGI after adjustment for updated mean FPG could be obtained from the estimated hazard ratio of updated mean HbA1c for each outcome. The percentage decrement in risk for each 1% reduction in updated mean HGI was calculated as 100% minus the reciprocal of the hazard ratio of updated mean HGI. To calculate hazard ratios associated with quintiles of updated mean HGI, a Cox model for each outcome was fitted with the quintiles using the middle category as a reference category. The Cox models were adjusted for updated mean FPG, sex, age, ethnic group, smoking status (current/ex/never), HDL and LDL cholesterol, triglycerides, systolic blood pressure, and presence of albuminuria (urine albumin $\geq$ 50 mg/l). These hazard ratios were plotted against the mean of each quintile. 95% confidence intervals were estimated as floating absolute risk (Easton, Peto et al. 1991).

Figure 5.2 Association between quintiles of updated mean HGI and diabetic complications. Reference category (hazard ratio 1.0) is the middle category with log-linear scale. P-value reflects the contribution of updated mean HGI to multivariate model. Data adjusted for updated mean FPG and other potential confounders.





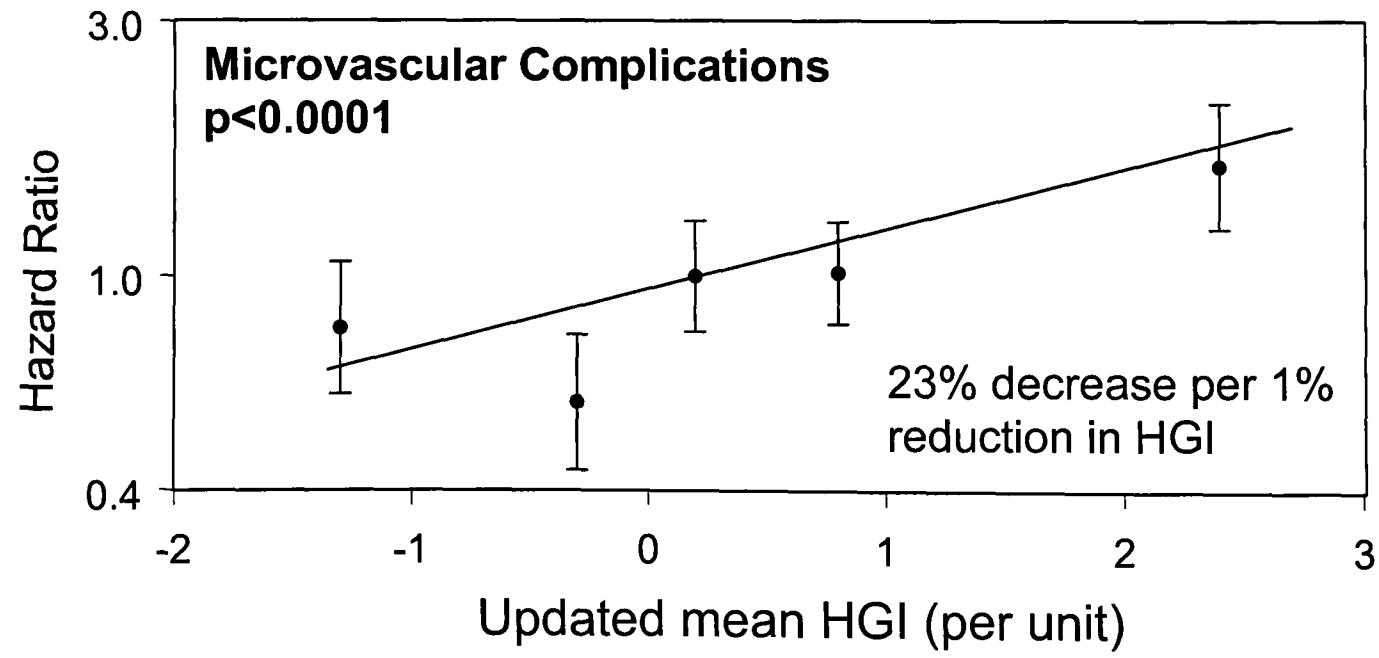


Figure 5.2 shows the association between each of three clinical outcomes and quintiles of updated mean HGI. The risk of complications rose with increasing updated mean HGI after adjustment for updated mean FPG and other potential confounders. It also shows the decrement in risk for each 1% absolute reduction in updated mean HGI. The decrement was 15% for "any diabetes related endpoint", 12% for macrovascular complications and 23% for microvascular complications.

Relation of diabetic complications to HGI after adjustment for HbA1c

The hazard ratio of updated mean HGI after adjustment for updated mean HbA1c can be calculated from the estimated hazard ratio of updated mean FPG for each outcome. The hazard ratio of updated mean HGI after adjustment for update mean HbA1c was 0.83 ($p<0.0001$) for "any diabetes-related endpoint", 0.92 ($p=0.15$) for macrovascular complications and 0.72 ($p<0.0001$) for microvascular complications. In contrast to the relationship between HGI and macrovascular complications adjusting for FPG ($p=0.0056$), updated mean HGI did not make an independent contribution to macrovascular complications after adjustment for update mean HbA1c ($p=0.15$). Thus, updated mean HGI can be regarded as an independent risk factor for microvascular complications but not for macrovascular complications.

Figure 5.3 Effect of HGI for microvascular complications risk after adjustment for FPG or HbA1c (illustrative data)

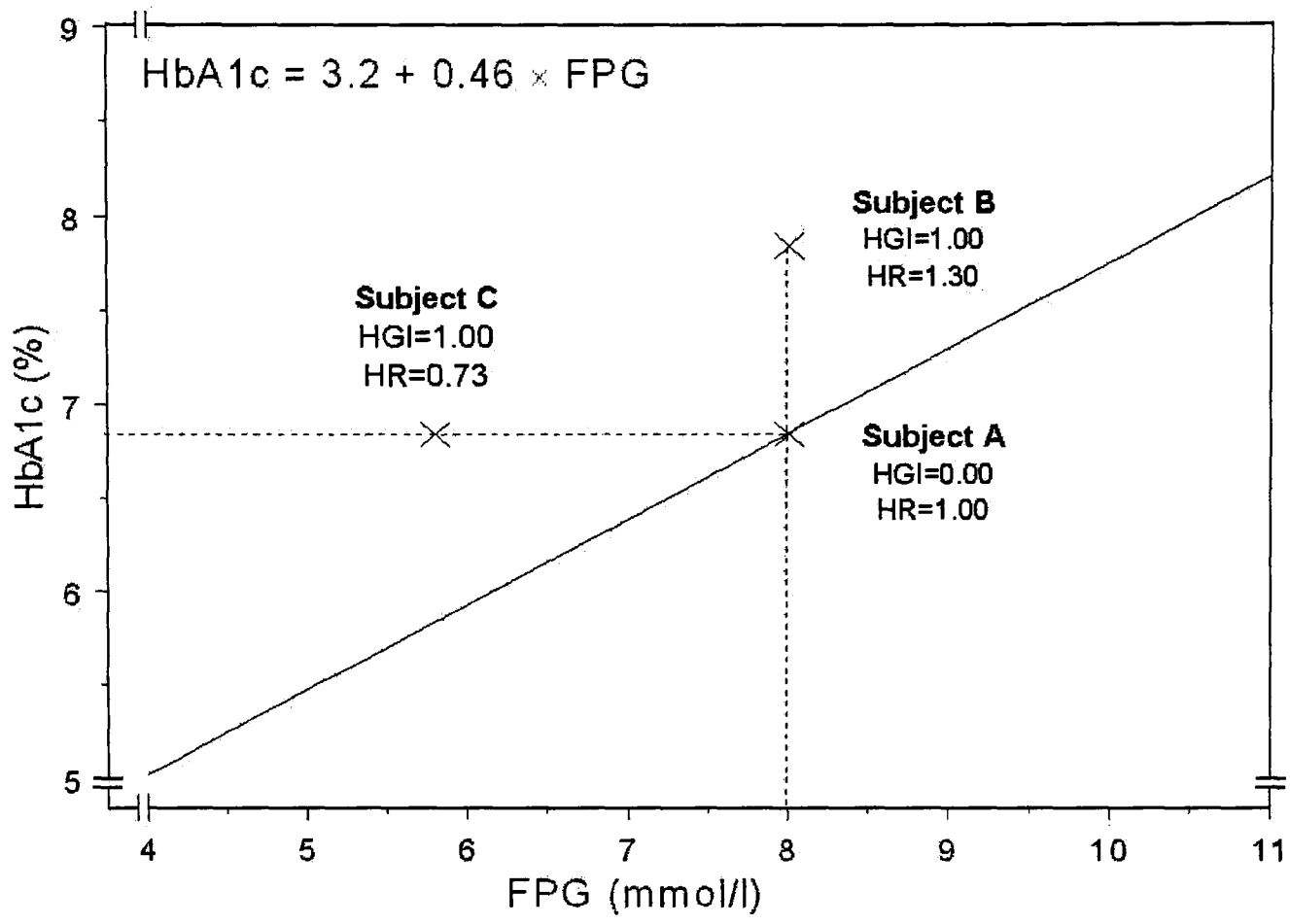


Figure 5.3 illustrates different effects of HGI after adjustment for either FPG or HbA1c. The corresponding HGI value for Subject A being on the linear regression line was 0.00 reflecting the fact that his actual HbA1c level equals that which would be expected for his FPG. Subject B and C had the same HGI value of 1.00, reflecting the higher observed HbA1c than that which would be expected for their FPG. Subject A and B had the same FPG but differing HGI values, and according to the model of Table 5.4, Subject B had a higher risk of microvascular complications. Subject A and C had the same HbA1c but differing HGI values, and according to the model of Table 5.4, Subject C had a lower risk of microvascular complications. Although Subject B and C had the same HGI value, Subject B had a higher risk compared to Subject A whereas Subject C had a lower risk compared to Subject A. This shows that HGI acts as a risk factor for microvascular complications after adjustment for FPG whereas HGI acts as a protective factor after adjustment for HbA1c.

5.9 Discussion

Relationship of FPG and HbA1c to macrovascular complications

Previously, no study has investigated the relationship between macrovascular complications and HGI in either type 1 or type 2 diabetes. For macrovascular complications, HbA1c but not FPG was an independent risk factor after adjustment for other potential confounders. This association remained after adjustment for randomised therapy. This result was consistent with the findings in previous small studies (Florkowski, Scott et al. 1998; Roselli della Rovere, Lapolla et al. 2003) but contradicts the result of a study (Lehto, Ronnemaa et al. 1997) which found FPG but not HbA1c as a predictor of CVD mortality or CVD events. However, the authors (Lehto, Ronnemaa et al. 1997) used FPG and HbA1c as categorical variables in the multivariate analysis which could reduce statistical power (Altman and Royston 2006). This chapter also showed that HGI is not independently associated with macrovascular complications after adjustment for HbA1c.

Relationship of FPG and HbA1c to microvascular complications

This observational analysis showed that for microvascular complications, both FPG and HbA1c were independent risk factors after adjustment for other potential confounders. These associations remained after adjustment for randomisation groups. This result was different from those in previous studies (Knuiman, Welborn et al. 1986; Liu, Pettitt et al. 1993) which found HbA1c but not fasting or non-fasting blood glucose made an independent contribution to retinopathy. However, these studies included people who were not newly diagnosed type 2 diabetes, had relatively small data compared to UKPDS and performed baseline covariates analyses only. This chapter also showed that HGI was independently associated with microvascular

complications after adjustment for either FPG or HbA1c. These results were consistent with the findings in type 1 diabetes (Cohen, Holmes et al. 2003; McCarter, Hempe et al. 2004). However, HGI has an opposing effect on micorvascular complications depending on whether it is adjusted for FPG or HbA1c. It is because higher HGI represents higher HbA1c after adjustment for FPG whereas higher HGI represents lower FPG after adjustment for HbA1c as depicted in Figure 5.3. Thus, HGI need to be interpreted carefully when it is used to calculate risk for microvascular complications in clinical practice.

HGI and diabetic complications

Conceptually, factors influencing HbA1c can be divided into two groups – glucose related factors and non-glucose related factors. Suppose that FPG represents glucose related factors and HGI represents non-glucose related factors. Since my analyses found that both FPG and HGI make independent contributions to diabetic complications, non-glucose related factors perhaps affect diabetic complications. For example, non-enzymatic glycation is involved with both HbA1c and advance glycation end-products (AGEs) (Bucala, Cerami et al. 1995). AGEs have been suggested to be involved in the development of microvascular and macrovascular complications (Furth 1997; Brownlee 2005).

One possible clinical explanation why HGI is independently associated with microvascular but not with macrovascular complications after adjustment for HbA1c might be that HGI is associated with erythrocyte factors. For example, erythrocyte deformability could affect microvascular but not macrovascular complications (Shevkoplyas, Yoshida et al. 2006). This explanation is also applicable to the

association between the glycosylation gap (GG) and microvascular complications in type 1 diabetes (Cohen, Holmes et al. 2003).

One statistical possibility which cannot be ruled out is that only HbA1c is significant for macrovascular complications as a result of a statistical artefact. When HbA1c and FPG have very similar hazard ratios for macrovascular complications, HbA1c alone can deliver all the information. The statistical power for macrovascular complications would be weaker compared with microvascular complications since the association between glycaemia and macrovascular complications is weaker (Barrett-Connor 1997; Duckworth, McCarren et al. 2001).

Baseline vs. time-dependent covariates analyses

In the time-dependent covariates analyses, for both microvascular and macrovascular complications, HbA1c but not FPG was an independent risk factor. In the baseline covariate analyses, for both microvascular and macrovascular complications, FPG but not HbA1c was a predictor. FPG may be more informative after dietary run-in as it could be a surrogate for the duration of undiagnosed diabetes (Colagiuri, Cull et al. 2002). In contrast, HbA1c, a measure of long-term glycaemia, may be more informative than FPG during the study.

Comparison with methodology in McCarter et al.'s analyses

Genuth et al. (Genuth, Lachin et al. 2005) argued that since HGI is heavily dependent on HbA1c, McCarter et al.'s finding in type 1 diabetes (McCarter, Hempe et al. 2004) just showed that the risk of diabetic complications is associated with HbA1c. They

said that the question not addressed is whether HGI is a risk factor of retinopathy or nephropathy independent of the observed HbA1c.

In fact, there are several flaws in McCarter et al.'s analyses. Firstly, they did not show that HGI is independently associated with retinopathy or nephropathy after adjustment for HbA1c as Genuth et al criticised. Secondly, the calculation of mean HGI needs attention. They used all HGI values during the course of study to calculate mean HGI. However, once retinopathy or nephropathy happens, the person's blood glucose level could be affected by the diabetic complications. Thus to test the association between mean HGI and diabetic complications, it seems more appropriate to include HGI values before the occurrence of retinopathy and nephropathy. In my analysis, update means as time-dependent covariates were calculated using FPG and HbA1c measured before the development of diabetic complications. Thirdly, they converted continuous variables to categorical variables, using tertiles of HGI and MBG. However, categorisation of continuous variables can lead to several problems (Altman and Royston 2006). It reduces the statistical power to detect the significant association between the variable and patient outcome although McCarter et al. found a significant association between HGI and microvascular complications probably due to the large data of the DCCT. It also characterises people who have similar HGI values close to a cut point but belong to different HGI groups very differently rather than very similarly. In my analyses, FPG, HbA1c and HGI were all used as continuous variables. Nevertheless, since my analyses showed that HGI was independently associated with microvascular complications in type 2 diabetes after adjustment for either FPG or HbA1c, their findings in type 1 diabetes still seem valid.

For the calculation of HGI, I fitted a linear regression model using all the available pairs at baseline whereas McCater et al. fitted a linear regression line using all the available pairs during the study. Indeed, HGI can be calculated using different linear equations. However, the association between HGI and microvascular complications is not due to the choice of the linear equation since both FPG and HbA1c are independently associated with microvascular complications.

Potential Limitations

Microvascular outcome was predominantly retinopathy. The relationship between HGI and other microvascular complications such as nephropathy and neuropathy needs to be confirmed. Actual therapy might explain the association between HGI and diabetic complications but this could not be investigated in detail in this thesis. However, including randomisation arms in the analyses did not change the results materially.

Implications of HGI

This chapter confirms and extends the findings in type 1 diabetes (Cohen, Holmes et al. 2003; McCarter, Hempe et al. 2004) to type 2 diabetes and has three major implications. Firstly, for macrovascular complications, HbA1c is a satisfactory glycaemic measure. This validates the use of HbA1c as a glycaemic measure in a risk calculator for macrovascular complications such as the UKPDS risk engine (Stevens, Kothari et al. 2001; Kothari, Stevens et al. 2002). Secondly, for microvascular complications, HbA1c is not a sufficient measure and additional information is needed. It suggests the use of other blood glucose measures such as fasting glucose in addition to HbA1c in a risk calculator for microvascular complications. Thirdly, the

independent association between HGI and diabetic complications suggests that not only glucose levels but also other factors affecting HbA1c may be related to diabetic complications in type 2 diabetes.

6 Conclusions

Overview

In chapter 2, I have demonstrated that the mean blood glucose (MBG) and blood glucose peaks equally predict glycated haemoglobin (HbA1c) in type 2 diabetes, using blood glucose profiles from continuous glucose monitoring. This result verifies that the finding from previous studies, that HbA1c reflects average blood glucose, remains valid when more complete glucose profiles are used. In chapter 3 and 4, I have shown that the variation of HbA1c not attributable to fasting glucose, captured by the haemoglobin glycation index (HGI), is reproducible and not explained by other confounders in pre-diabetes and in type 2 diabetes. This result confirms and extends the finding in non-diabetic people (Yudkin, Forrest et al. 1990) and in people with type 1 diabetes (Hempe, Gomez et al. 2002; McCarter, Hempe et al. 2004). To date, the relationship between HGI and diabetic complications in type 2 diabetes has never been investigated. In chapter 5, I have shown that for microvascular complications, HGI makes an independent contribution after adjustment for either FPG or HbA1c whereas for macrovascular complications, HGI does not make an independent contribution after adjustment for HbA1c in type 2 diabetes.

HbA1c is a reasonable single measure which reflects overall blood glucose levels. However, my work showed that there can be marked differences in individual HbA1c levels relative to their fasting plasma glucose values which remained consistent over time. As HbA1c is increasingly accepted as the standard on which many clinical decisions are made, HbA1c levels should be interpreted carefully. HGI may play a role in clinical practice, informing the healthcare professionals of the likely

magnitude and direction of the difference between observed HbA1c levels and those expected from blood glucose levels.

Relationship between HbA1c and derived blood glucose measures from blood glucose profiles captured by continuous glucose monitoring

Previously, it was found that HbA1c reflects average blood glucose over the preceding 2-3 months (Nathan, Singer et al. 1984; Meigs, Nathan et al. 1996). However, this result was based on incomplete blood glucose profiles from infrequent sampling. In chapter 2, I sought to find the best predictor of HbA1c by relating HbA1c to different blood glucose measures derived from more complete glucose profiles provided by continuous glucose monitoring. In my analyses, MBG or peaks of blood glucose were more strongly associated with HbA1c than fasting glucose and standard deviation of glucose profile. However, I could not establish whether MBG or peaks of blood glucose is a better predictor of HbA1c. Thus, the results from previous studies, that HbA1c reflects average blood glucose over preceding 2-3 months, still seem applicable when more complete glucose profiles are employed since both MBG and blood glucose peaks equally predict HbA1c.

Reproducible variation in HbA1c not attributable to fasting glucose is captured by HGI

Although there is a correlation between HbA1c and other blood glucose measures, the variation in HbA1c not attributable to other blood glucose measures has been recognised (Yudkin, Forrest et al. 1990; Hempe, Gomez et al. 2002; Cohen, Holmes et al. 2003; McCarter, Hempe et al. 2004). To capture this variation in HbA1c, the haemoglobin glycation index (HGI) has been proposed as a measure of whether a

subject's HbA1c is higher or lower than expected for their blood glucose levels (Hempe, Gomez et al. 2002). HGI is a vertical offset from a linear regression line when a linear regression of HbA1c is fitted on MBG or fasting blood glucose. My analyses found the presence of variation in HbA1c distinct from that attributable to fasting glucose. I have demonstrated this result in people with pre-diabetes (chapter 3) and in people with type 2 diabetes (chapter 4). As measured by HGI, individual tendency toward higher or lower HbA1c relative to one's fasting glucose was consistent over time and was not explained by other patient characteristics examined in both populations. This evidence of individual variation in HbA1c confirms and extends the results in non-diabetic subjects (Yudkin, Forrest et al. 1990) and in people with type 1 diabetes (Hempe, Gomez et al. 2002; Cohen, Holmes et al. 2003; McCarter, Hempe et al. 2004).

Relationship of fasting glucose and HbA1c to macrovascular complications in type 2 diabetes

The relation of macrovascular complications to HGI has never been investigated previously. In chapter 5, I found that HbA1c but not fasting plasma glucose is an independent risk factor for macrovascular complications in type 2 diabetes. This verifies the findings in previous small studies (Florkowski, Scott et al. 1998; Roselli della Rovere, Lapolla et al. 2003). I also showed that HGI is not an independent risk factor for macrovascular complications after adjustment for HbA1c in type 2 diabetes.

Relationship of fasting glucose and HbA1c to microvascular complications in type 2 diabetes

For microvascular complications, HGI has been reported as an independent risk factor after adjustment for MBG in type 1 diabetes (McCarter, Hempe et al. 2004). I found that both fasting plasma glucose and HbA1c make independent contributions to the risk of microvascular complications in type 2 diabetes. Therefore, HGI is an independent risk factor for microvascular complications adjusting for either fasting glucose or HbA1c. Although the result in type 1 diabetes (McCarter, Hempe et al. 2004) was criticised by Genuth et al. (Genuth, Lachin et al. 2005), I used a method which overcomes this criticism.

My analyses suggest that HbA1c is a sufficient single measure of glycaemic control for macrovascular complications whereas we need additional information besides HbA1c for microvascular complications.

HGI for research and clinical practice

HGI can play an important role in research. Although several studies (The DCCT Research Group 1993; UKPDS Group 1997; The DCCT/ EDIC Study Research Group 2005) have shown that hyperglycaemia, measured by HbA1c, is associated with diabetic complications, the mechanisms involved remain uncertain (Peppa, Uribarri et al. 2003). In fact, as discussed in chapter 3, there exist other factors, apart from blood glucose levels, that may affect HbA1c. In this context, HGI may emerge as a means of capturing non-glucose sources of population variation in diabetic complications. Given the findings in pre-diabetes (chapter 3) and type 1 diabetes (McCarter, Hempe et al. 2004) showing that HGI is not determined by post-challenge

or postprandial glucose levels, it follows that HGI may reflect a fraction of HbA1c that is not explained by FBG or MBG. While my work cannot completely rule out the possibility that post-challenge or postprandial glucose might contribute to diabetic complications in some patient groups, it does suggest a potential role for HGI in the identification of patient risk.

Since HGI is a person-specific property which indicates whether a subject's HbA1c is higher or lower relative to their blood glucose level routinely, it also has important implications for the management of diabetic patients. HbA1c has been increasingly used as the standard based on which treatment decisions are made and the efficacy of treatment is assessed (Kilpatrick 2000; Jeffcoate 2004; Saudek, Derr et al. 2006). In fact, a new reward system has been introduced recently in the U.K wherein general practitioners are financially encouraged to lower their patients' HbA1c below a target level (Department of Health 2001). However, my analyses suggest that treatment decisions solely based on HbA1c levels could be inappropriate. If some patients have normal fasting glucose levels but relatively high HbA1c levels, treatment decisions solely based on HbA1c could lead to setting unattainable blood glucose goals and might increase risk of hypoglycaemia. Likewise, if some patients have normal HbA1c levels but relatively high fasting glucose levels, treatment decisions based on HbA1c might lead to acceptance of the HbA1c level as evidence of good glycaemic control while leaving high blood glucose levels untreated. This suggests that HGI can be useful as a clinical tool which informs healthcare professionals of the likely direction and magnitude of the discordance between observed HbA1c and that which would be expected based on fasting glucose in a diabetic subject.

If HGI is to be widely used as a clinical tool, several steps need to be taken. Firstly, a linear equation of HbA1c on a blood glucose measure available at the clinic is needed. This equation could be either from other studies based on a population relevant to the patient seen in the clinic or from a calculation using the data available at the clinic. Considering the similar gradients and intercepts of the linear equations in pre-diabetes (chapter 3) and in type 2 diabetes (chapter 4), we can speculate that equations from different studies or clinics may not be significantly different. Secondly, HbA1c needs to be measured by the same method or a standardised method. In the past, several assays were used to measure HbA1c, leading to significant differences in HbA1c results between different laboratories (Little, Wiedmeyer et al. 1991; Hoshine, Okahashi et al. 1997). However, a national standardisation program such as the National Glycohaemoglobin Standardisation Program (NGSP) (Little, Rohlfing et al. 2001) has improved the comparability of HbA1c results from different laboratories. An international standardisation has been developed by the International Federation of Clinical Chemists (IFCC) and the approved IFCC reference method for measuring HbA1c is now available (Jeppsson, Kobold et al. 2002). Thirdly, HGI needs to be calculated again if a new treatment is added (especially starting on insulin) as discussed in chapter 4. Finally, use of the mean of three three-monthly measurements for HbA1c and the blood glucose measure is recommended to obtain good reproducibility.

There are several limitations with respect to using of HGI as a clinical tool. The reproducibility of HGI was lower in type 2 diabetes compared to that in pre-diabetes. However, reproducibility in type 2 diabetes can be improved by taking the mean of three or more measurements of HbA1c and blood glucose level. No postprandial

glucose measure was available in the UKPDS and thus its association to HGI could not be examined in type 2 diabetes. In pre-diabetes, the association between HGI and 2h post-challenge glucose was weak. Finally, different treatments can change the relationship between HbA1c and fasting glucose over time.

As a possible future project extending the work of this thesis, repeated measures models could be extended to examine the relationship between HGI and other factors such as postprandial glucose in type 2 diabetes. These models could also test for differences in the HGI relationships at different time points. Survival analysis models could be extended to assess whether the effect of HGI is similar for single endpoints or after adjustment for actual therapy. It might be also useful to investigate possible clinical management options to control HGI such as life-style intervention or drugs.

My analyses show that the same HbA1c level does not necessarily have the same clinical meaning in two different subjects. The reproducible variation in HbA1c which may not attributable to blood glucose, captured by HGI, shows in part why patients with the same HbA1c level do not have the same risk of developing diabetic complications. Therefore, HbA1c levels should be interpreted with caution and treatment decisions need to be made considering individual glycaemic control rather than solely based on the target HbA1c level.

Appendix 1. Discriminant ratio

The discriminant ratio (DR) (Levy, Morris et al. 1999), developed to compare different tests of a continuous physiological variable, is the ratio of the underlying between-subject standard deviation (SD) to within-subject standard deviations (SD), using duplicate measurements. Comparing between- and within-subject standard deviation, the discriminant ratio can be used to compare reproducibility. A higher discriminant ratio indicates more reproducibility.

The DR is expressed as follows:

$$\mathrm{DR} = \frac{\text{underlying between - subject SD}}{\text{within - subject SD}} = \frac{\sqrt{[(MS_b - MS_w)/k]}}{\sqrt{MS_w}}$$

where MS_b is the between-subject mean square, MS_w is the within-subject mean square and k is the number of replicate measurements in each subject from an analysis of variance (ANOVA).

$(1+1/\mathrm{DR}^2)$ can be used as a correction factor for the regression dilution bias (Frost and Thompson 2000).

Appendix 2. Random effects model for HGI

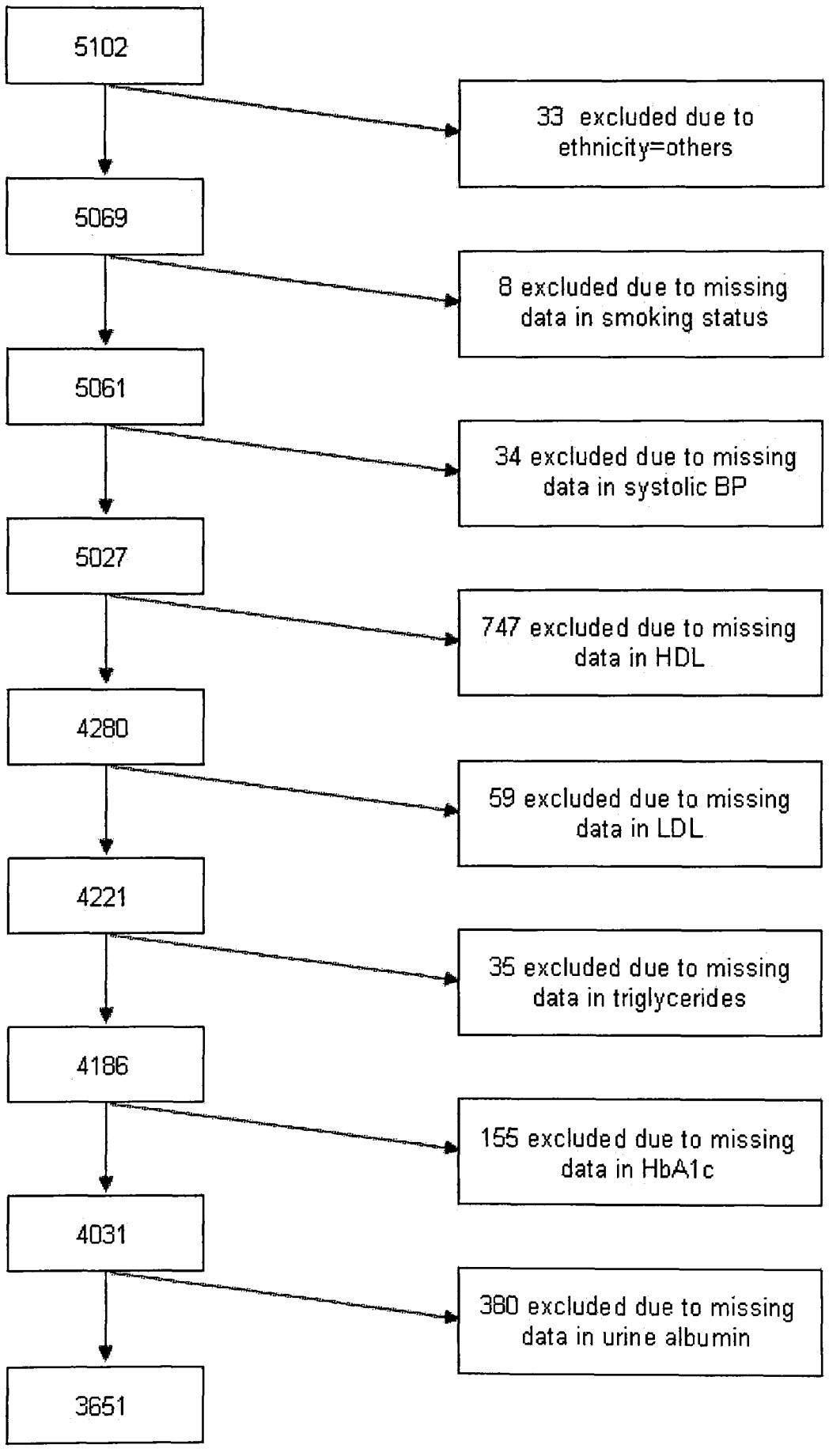
The repeated measures linear regression model (Singer 1998; Vittinghoff, Glidden et al. 2005) fitted in chapter 3 can be written as follows:

$$HbA1c_{ij} = \beta_0 + \beta_1 FPG_{ij} + \text{Subject Effect}_i + \varepsilon_{ij}$$

$$\text{with Subject Effect}_i \sim \text{i.i.d N } (0, \sigma_u^2),\ \varepsilon_{ij} \sim \text{i.i.d N } (0, \sigma_\varepsilon^2)$$

$HbA1c_{ij}$ and FPG_{ij} represent HbA1c and FPG of subject i at time j. Subject Effect_i represents variation in intercepts between subjects and ε_{ij} represents residuals of subject i at time j. It is assumed that Subject Effect_i and residuals (ε_{ij}) are independent. The variance in HbA1c for any given FPG is assumed to be $\sigma_u^2 + \sigma_\varepsilon^2$. This structure is known as compound symmetry. The covariance of HbA1c for any given two FPG measurements in a single subject is σ_u^2. The covariance of HbA1c for any given two FPG measurements in different subjects is 0. Residual plots were checked whether there is any departure from the assumptions.

Appendix 3. Consort diagram for UKPDS data



Appendix 4. Comparison of different Cox models

As HGI is calculated from HbA1c and FPG, a Cox model with HGI and FPG or a Cox model with HGI and HbA1c is equivalent to a Cox model with FPG and HbA1c. This can be shown as follows. From chapter 4,

$$HGI = HbA1c - 3.20 - 0.46\ FPG \qquad \text{(Equation 5.1)}$$

Suppose α and β are coefficients for FPG and HbA1c from a Cox model. Then the proportional hazards function for the *i*'th individual can be written as follows (Collett 1994):

$$h_i(t) = \exp(\alpha\ FPG_i + \beta\ HbA1c_i) \times h_0(t) \qquad \text{(Equation 5.2)}$$

If we take natural logarithm and substitute HbA1c in equation 5.2 using the equation 5.1, we can obtain log hazard function with FPG and HGI (the subscript *i* is suppressed).

$$\begin{aligned}
\ln h(t) &= \alpha\ FPG + \beta\ HbA1c + \ln h_0(t) \\
&= \alpha\ FPG + \beta\ (HGI + 3.20 + 0.46\ FPG) + \ln h_0(t) \\
&= (\alpha + 0.46\ \beta)\ FPG + \beta\ HGI + 3.20\ \beta + \ln h_0(t) \\
&= \alpha^*\ FPG + \beta^*\ HGI + 3.20\ \beta + \ln h_0(t) \qquad \text{(Equation 5.3)}
\end{aligned}$$

where $\alpha^* = \alpha + 0.46\ \beta$, $\beta^* = \beta$

We can see that after adjustment for FPG, the coefficient of HGI is the same as HbA1c.

Likewise, if we substitute FPG in Equation 5.2 using Equation 5.1, we can obtain log hazard function with HbA1c and HGI.

$$\ln h(t) = \alpha\, FPG + \beta HbA1c + \ln h_0(t)$$

$$= \alpha\,(HbA1c - HGI - 3.20) / 0.46 + \beta\, HbA1c + \ln h_0(t)$$

$$= (\alpha / 0.46 + \beta)\, HbA1c - \alpha\, HGI / 0.46 - 3.20\, \alpha / 0.46 + \ln h_0(t)$$

$$= \alpha^{**}\, HbA1c + \beta^{**}\, HGI - 3.20\, \alpha / 0.46 + \ln h_0(t) \qquad \text{(Equation 5.4)}$$

where $\alpha^{**} = \alpha / 0.46 + \beta$, $\beta^{**} = -\alpha / 0.46$

We can see that after adjustment for HbA1c, the coefficient of HGI has a negative sign compared to that of FPG.

Appendix 5. List of published abstracts

Kim, J., R. J. Stevens, R. R. Holman. (2005). "FPG and HbA1c are independent risk factors for microvascular but not macrovascular complications in newly diagnosed type 2 diabetes" Diabetologia 48 (Suppl): A33.

Kim, J., R. J. Steven, R. R. Holman. (2005). "The Haemoglobin Glycation Index Is an Independent Risk Factor for Microvascular Complications in UKPDS Patients with Newly Diagnosed Type 2 Diabetes." Diabetes 54 (Suppl): A244

Kim, J., R. J. Stevens, R. R. Holman. (2004). "The haemoglobin glycation index is reproducible in dysglycaemic individual but is not explained by post-challenge plasma glucose levels." Diabetologia 47 (Suppl): A122.

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