Hypoglycaemia in older people with diabetes

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ABSTRACT

Diabetes prevalence is increasing in our ageing and increasingly obese society. Diabetes is a heterogeneous condition, and challenges remain in all aspects of its management - from diagnosis through to optimising treatment, to managing complications. Increasing age brings altered physiological responses to disease, treatments and complications - and there may be more wide-ranging considerations such as dietary, mobility, dependency or cognition, to name just a few. Hypoglycaemia is one of the most important potential side-effects of insulin-therapy, and elderly adults are at particular risk from its consequences.

Insulin-treated patients may have long-standing Type 1 diabetes, or have Type 2 diabetes which has progressed to requiring insulin treatment, due to progressive beta cell deficiency. Even within this group of patients, there is heterogeneity, and assessment of risks can be challenging.

Endogenous insulin levels can be assessed by measuring C-peptide. Recent advances in this has meant this is much more practical, enabling assessment of endogenous levels in large numbers of patients more feasible, and hence allowing important questions to be addressed. In the context of older patients, particularly interesting questions are whether patients with long-standing Type 2 diabetes can develop severe insulin deficiency, and whether absolute/severe endogenous insulin levels have an impact on treatment or complications of diabetes within insulin-treated cohorts – such as hypoglycaemia. This may thence raise the question of whether C-peptide measurement could potentially be used as an extra clinical tool for risk assessment in a patient population which can be tricky to manage at times.

The aim of this thesis is thus to explore some of the issues around management of diabetes in the elderly: in particular hypoglycaemia, and use of C-peptide to more fully assess patients and consider a possible role for it in routine clinical care of some patients.

Chapter 1 puts the thesis in context, firstly reviewing hypoglycaemia in the elderly in general, and then considering aspects of endogenous insulin levels and C-peptide measurement.

Chapter 2 addresses the problem of recognition of hypoglycaemia in an elderly population, using primary care records and documented symptoms at consultations. Are we missing hypoglycaemia in this population?

Accurate diagnosis of diabetes is crucial for getting people on the right treatment guidelines, and can be challenging. Chapter 3 uses a spot urine measure of C-peptide to test for the first time the accuracy of the UK Practical Classification Guidelines (published by the Royal College of General Practitioners and NHS Diabetes).

Progressive insulin deficiency in Type 2 diabetes is the main reason people with long-standing Type 2 diabetes may eventually require insulin treatment. Chapter 4 uses the spot urine measure of C-peptide as a screening tool to assess if insulin-treated people with a clinical diagnosis of Type 2 diabetes may develop absolute insulin deficiency.

Even more practical than a spot urine test to measure C-peptide, could be a random non-fasting blood measure of C-peptide, which could thus be measured when patients have their routine blood tests done in the community or outpatient appointments. Chapter 5 looks at how such a measure correlates with the gold-standard mixed meal tolerance test C-peptide measure.

Severe insulin deficiency in Type 1 diabetes has been correlated with increased complications including hypoglycaemia, but the impact of endogenous insulin levels has not been assessed greatly in Type 2 diabetes. Chapter 6 reports a study looking into this possible relationship, using hypoglycaemia questionnaire responses from a large number of community-dwelling insulin-treated adults (of both diagnoses), in the context of their clinical diabetes diagnosis and their random non-fasted blood C-peptide levels.

Chapter 7 assesses in more detail the rates of hypoglycaemia in a small group of insulin-treated patients with a clinical diagnosis of Type 2 diabetes, selected on the basis of their endogenous C-peptide levels. As well as subjective assessment of their hypoglycaemia experience using questionnaires, continuous glucose monitoring was used to objectively assess their rates of hypoglycaemia and glucose variability.

Chapter 8 pulls all the above chapters together, summarising them in the context of other research, discussing their limitations and possible areas for future research, and their implications for now for clinical practice.

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ABBREVIATIONS

ACCORD	Action to Control Cardiovascular Risk in Diabetes trial
ADA	American Diabetes Association
ADVANCE	Action in Diabetes and Vascular disease: preterAx and diamicroN MR Controlled Evaluation study
AUC	Area Under The Curve
CGM	Continuous Glucose Monitoring
CI	Confidence Interval
CRF	Clinical Research Facility
DCCT	Diabetes Control and Complications Trial
EASD	European Association for the Study of Diabetes
eGFR	Estimated Glomerular Filtration Rate
fCP	Fasting C-peptide
GAD	Glutamic Acid Decarboxylase antibodies
HbA1c	Haemoglobin A1c/Glycosylated Haemoglobin
HR	Hazard ratio
IA2	Islet Antigen 2 antibodies
ICA	Islet Cell Antibodies

INTERVAL	INdividualised Treatment targets for ElderRly patients with type 2 diabetes using Vildagliptin Add-on or Lone therapy study
IQR	Interquartile Range
K^+ EDTA	Potassium Ethylenediaminetetraacetic acid
LADA	Latent Autoimmune Diabetes in Adults
MMTT	Mixed Meal Tolerance Test
NHS	National Health Service
NIHR	National Institute of Health Research
OHA	Oral Hypoglycaemic Agent
OR	Odds ratio
r	Correlation coefficient
RCGP	Royal College of General Practitioners
rCP	Random non-fasting C-peptide
ROC	Receiver Operating Characteristic
rUCPCR	Random non-fasting Urine C-peptide Creatinine Ratio
sCP	Stimulated C-peptide
SD	Standard deviation
sSCP	Stimulated Serum C-peptide
SU	Sulphonylurea
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TIA	Transient ischaemic attack

UCPCR	Urine C-peptide Creatinine Ratio
UKPDS	United Kingdom Prospective Diabetes Study

CHAPTER 1

INTRODUCTION

CHAPTER 1 – INTRODUCTION

Structure

This chapter is divided into 3 sections.

Part 1 states the structure and aims of this thesis.

Part 2 sets the scene with an overall review of clinical aspects of hypoglycaemia in older people with diabetes; this was published as the Feature Article in the 2013 June edition of the online journal Diabetic Hypoglycemia.

Part 3 introduces the other key concepts used in the thesis, namely the clinical implications of endogenous insulin levels altering over time in those with diabetes - particularly in terms of hypoglycaemia risk, and C-peptide as a useful measure of endogenous insulin levels.

Chapter 1: Table of contents

Part 1:
Structure and aims of the thesis

This thesis aims to explore some of the diverse aspects of the challenging field of management of diabetes in the elderly, and in particular hypoglycaemia.

A novel theme developed through the thesis is that of the use of C-peptide measurement to more fully assess patients with diabetes, and a possible role for this in the routine clinical care of some older patients with diabetes is considered.

Chapter 1 - Introduction

This is in two parts. Firstly an overview of hypoglycaemia in the elderly, with exploration of some of the key issues facing those with or looking after elderly patients with insulin-treated diabetes. The second part discusses the relevance of endogenous insulin levels in diabetes, and introduces C-peptide as a useful measure of endogenous insulin levels.

The first part (hypoglycaemia in the elderly review) is a published article in the online journal, Diabetic Hypoglycaemia.

Chapter 2 – Recognising hypoglycaemia

This chapter attempts to assess in real clinical practice, the notion raised in Chapter 1 that hypoglycaemia is an under-recognised phenomenon in the older population, partly by virtue of the non-specific nature of symptoms associated with it. A systematic approach was used to go through primary care consultation records looking for consultations with non-specific symptoms which may have represented hypoglycaemia. This short report has been submitted to the primary care journal, Family Practice.

Chapter 3 – Accurate diagnosis of diabetes type

Treatment guidelines/pathways in diabetes rely on having a correct starting diagnosis, and thus accurate diagnosis is crucial. Being on the right management pathway will affect treatment and education, as well as recognition by healthcare professionals of factors of relevance for a particular individual, such as their risk of hypoglycaemia. However accurate diabetes diagnosis is surprisingly challenging, and in the past there has been a dearth of diagnosis guidelines. In response to this gap the UK Practical Classification Guidelines were published in 2010, and this chapter systematically assesses the accuracy of them, against a "gold-standard" definition which incorporates a measure of endogenous insulin levels, urinary C-peptide creatinine ratio (UCPCR). This paper has been accepted for publication by the British Journal of General Practice.

Chapter 4 – Insulin deficiency in Type 2 diabetes

Progressive insulin deficiency in Type 2 diabetes results in people with long-standing Type 2 diabetes often requiring insulin treatment. Chapter 4 uses UCPCR as a screening tool to identify people with a clinical diagnosis of Type 2 diabetes who may have developed absolute insulin deficiency. Stimulated blood C-peptide measured in a mixed meal tolerance test (gold-standard C-peptide measure) was then used to confirm findings. This chapter is composed of an article published in Diabetic Medicine.

Chapter 5 – Random non-fasting blood C-peptide

Even more practical than a spot urine test to measure C-peptide, could be a random non-fasting blood measure of C-peptide, which could thus be measured when patients have a routine blood test. The aim of Chapter 5 was to look at how such a measure might correlate with the aforementioned gold-standard mixed meal tolerance test measure of C-peptide. This has been accepted for publication as a Short Report by Diabetic Medicine.

Chapter 6 – Endogenous insulin levels and hypoglycaemia risk

Chapter 6 makes use of this random non-fasting blood C-peptide measure in further evaluating risk of hypoglycaemia in insulin-treated patients. Lower endogenous insulin levels are well-known to be associated with increased frequency of hypoglycaemia – and hypoglycaemia unawareness – in patients with Type 1 diabetes, but there has been limited research on this in Type 2 diabetes. This study thus evaluated self-reported hypoglycaemia frequency and awareness in almost 500 patients with Type 1 or insulin-treated Type 2 diabetes using standard hypoglycaemia questionnaires, and evaluated results according to their random non-fasting C-peptide levels. An earlier analysis of these results was presented as a poster at the Diabetes UK Annual Professional Conference in 2015, and the full paper is to be submitted shortly.

Chapter 7 – Endogenous insulin levels and hypoglycaemia risk

Chapter 7 addresses whether stratifying insulin-treated patients with Type 2 diabetes, matched by their clinical characteristics and differing only by C-peptide level, results in different objective and subjective levels of hypoglycaemia. Seventeen patients with insulin-treated Type 2 diabetes and very low C-peptide levels were matched by gender and HbA1c 1:1 with seventeen patients with higher C-peptide levels. Hypoglycaemia frequency was compared using data from continuous glucose monitoring and hypoglycaemia questionnaires. These results were presented as an oral presentation at the Diabetes UK Annual Professional Conference 2016, and the full paper is to be submitted shortly.

Chapter 8 - Discussion

The studies presented in the above chapters are summarised and discussed especially in terms of their strengths and limitations, their possible implications for clinical practice, and possible areas for future research.

Part 2: Overview of hypoglycaemia in the elderly

2.1 Introduction

In an ageing population where the prevalence of diabetes is increasing, a vast increase in the number of elderly people with diabetes is expected. Landmark diabetes trials have suggested glycaemic control is one of the keys to preventing long-term complications from diabetes, and monitoring of HbA1c as the usual clinical measure of glycaemic control, is used as a marker of success, and deterioration in HbA1c levels often used as the sign to increase intensity of treatment.

Type 1 diabetes is recognised as a disease of absolute insulin deficiency, whereas Type 2 diabetes is a more heterogeneous condition, comprising people with mainly insulin resistance, to those with more severe insulin deficiency. Over time insulin deficiency becomes more important, and people with long-standing Type 2 diabetes often need insulin treatment eventually, in order to control blood glucose levels.

The most serious (and feared) complication of insulin treatment, is hypoglycaemia. All patients on insulin have the potential to experience hypoglycaemia, though it appears some suffer more than others. Those with Type 1 diabetes have higher recognised rates than those with Type 2 diabetes. Recognition of hypoglycaemia however cannot always be straightforward, and particularly in the elderly population, where symptoms can be non-specific and interpreted as other conditions.

The following review introduces hypoglycaemia in the elderly in more detail, and some of the key issues surrounding it.

Hypoglycaemia in the elderly

Suzy V Hope and W David Strain

2.2 Abstract

Hypoglycemia is a common, under-recognized complication of the management of type 2 diabetes. Elderly individuals have a higher burden of co-morbidities, cognitive impairment, physical dysfunction and frailty, which makes them more vulnerable to complications of hypoglycemia, such as falls, fractures, cognitive impairment and cardiovascular events, than younger patients. Furthermore, with ageing comes impairment of autoregulatory responses, which means the symptoms of hypoglycemia are often less specific, and are therefore either missed or incorrectly diagnosed as transient ischemic attacks or other cerebrovascular events. Older adults with diabetes have a greater risk of hypoglycemia associated with the physiological decline of ageing, and the extended duration of diabetes and insulin treatment. The elderly are also more prone to the effects of hypoglycemia such as the increased risk of accidents, falls and fractures, hospitalizations, in-hospital mortality, and long-term impairment of cognition. Using individualized treatment targets to base treatment strategies around individual circumstances may reduce the risk of hypoglycemia.

2.3 Introduction

Type 2 diabetes is one of the most common chronic conditions in older adults, and the number of elderly individuals with diabetes is growing worldwide. For example, of the 2.6 million people in the UK with diabetes, at least half are over 65 years old (1). The prevalence of diabetes in the elderly is more than 10% compared with 4.1% in the general adult population (2), and approaches 25% in care home residents (1). The management of elderly patients presents unique challenges. Episodes of hypoglycemia are a major complication of the treatment of diabetes with insulin and some oral medications. The consequences of hypoglycemia may be much greater in the frail older population than in younger adults.

This older population with diabetes represents a heterogeneous group, ranging from those who have been diagnosed recently (mainly with type 2 diabetes) to those with longstanding type 2 diabetes or type 1 diabetes, and from fit and active people to frail institutionalized individuals. Treatment of elderly patients also varies considerably. Once diabetes is established, the principal aims of 'good diabetes care' comprise blood glucose lowering, managing cardiovascular risk and identifying and treating long-term complications (3). As glycemic control tends to deteriorate with disease progression, stepwise intensification of treatment is usual. This often includes prescription of sulfonylureas (SUs) and insulin, the agents most likely to precipitate hypoglycemia.

The utilization of these agents in order to achieve strict glycemic control is facing increasing scrutiny. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study (4) demonstrated increased mortality with intensive treatment using strategies based around the use of SUs and insulin. Other studies have not shown this increased mortality with stricter glycemic control, but they have also failed to show any improvement in all-cause mortality (5-7). Meta-analysis of five relevant randomized controlled trials (4-6, 8, 9) that examined the effect of intensive glycemic control on major outcomes in type 2 diabetes, has demonstrated that stricter glycemic control (an average of 0.9% reduction in HbA1c maintained over 5 years from a mean baseline of 7.8%) can lead to a 17% reduction in events of non-fatal myocardial infarction, and a 15%

reduction in events of coronary heart disease (7). In the Action in Diabetes and Vascular disease: preterAx and diamicroN MR Controlled Evaluation (ADVANCE) study (5), severe hypoglycemia was associated with increased risk of macrovascular events, microvascular events, and death from both cardiovascular and non-cardiovascular causes (10), although not in a clear exposure-outcome or dose-response manner. The link between hypoglycemia and other conditions, which is also relevant to quality of life in older patients, such as diminishing cognitive function, necessitates a better understanding of the precipitants of hypoglycemia and its avoidance in older people with diabetes.

This review examines how hypoglycemia can affect an older population, and highlights the need for increased attention to avoid hypoglycemia completely in elderly people.

2.4 Prevalence

The true prevalence of hypoglycemia in the elderly is unknown. Most studies that have tried to address this question rely on recall of hypoglycemic episodes by participants. Accurate recall of hypoglycemia is notoriously difficult in any age group, and none more so than in an elderly population. For epidemiological purposes ‘severe’ hypoglycemia is usually defined as that requiring external assistance for treatment. This is easier to measure in terms of prevalence as it is usually more dramatic and accuracy of recall is more robust for up to a year in type 1 and type 2 diabetes (11, 12). Episodes of severe hypoglycemia can also be corroborated with documentary evidence from the medical emergency services.

The difficulties in accurate patient recall of episodes of hypoglycemia was addressed by a carefully designed prospective observational study over 9–12 months in the UK (13). Participants were required to return a data-collection sheet every time they experienced a severe hypoglycemia episode. The annual prevalence of SU-associated severe hypoglycemia was 7%, similar to that observed in people with type 2 diabetes treated with insulin for <2 years. This compared to a prevalence of 25% in patients with type 2 diabetes who had

received insulin treatment for >5 years, and 46% in those with long-standing type 1 diabetes (>15 years). However, the highest mean age of any of the subgroups included in this study was only 62 years and all had good glycemic control (HbA1c <8%). In the retrospective assessment of an older population over the age of 70 years, taking oral glucose-lowering agents, which relied on participant recall, Bramlage *et al* (14) found that only 1% reported episodes of symptomatic hypoglycemia external assistance had been required.

Different definitions, varying ability to recognize hypoglycemia and varying ability to recall preceding episodes all contribute to disparate estimates between studies. Mild episodes of hypoglycemia – usually defined as those that can be self-treated – are much more difficult to estimate. It has been shown that recall of mild episodes is unreliable beyond one week in people with type 1 diabetes (15). It may be poorer still in the older population with type 2 diabetes in whom cognitive function is often diminished. In the year prior to inclusion in their study, Bramlage and colleagues found that 12.8% of the participants aged over 70 years and on oral treatment reported any episode of hypoglycemia, compared with 10.1% aged 60–69 years, and 9% aged under 60 years (14). Over one year in the prospective UK Hypoglycaemia Study (13) with its intensive concurrent data collection, 39% of those with SU-treated type 2 diabetes reported at least one episode of mild (self-treated) hypoglycemia, compared with 64% in those with type 2 diabetes who had been treated with insulin for >5 years, and 85% of those with long-standing type 1 diabetes. Furthermore, this represented a middle-aged cohort, so the prevalence might well differ in an older population, and it may be lower if clinicians are more pragmatic with glycemic targets and choice of treatment. However, in the INdividualised Treatment targets for ElderRly patients with type 2 diabetes using Vildagliptin Add-on or Lone therapy (INTERVAL) study, where clinicians were encouraged to set individualized treatment targets for elderly patients, taking account of age, frailty, and co-morbidities, physicians still set HbA1c targets in the region of 7.0% (55 mmol/mol)(16), making this premise unlikely. Conversely, more hypoglycemia might be anticipated in elderly patients who eat less and are not confident about altering the dose of their medications. In addition, hypoglycemia may be missed in older patients when their non-specific symptoms are

attributed to other age-related ailments, or the neurological symptoms are misinterpreted as transient ischemic attacks (TIAs) or other cerebrovascular events (17).

Knowledge of symptoms of hypoglycemia in patients of this age group is often poor (18, 19). Mild episodes of hypoglycemia are under-recognized by patients, relatives or carers, and their healthcare providers. Studies have shown poor correlation between recall of hypoglycemia by relatives and patients (20-22), with relatives tending to recall more episodes. Furthermore, the recognition of mild hypoglycemia is made more difficult as the hypoglycemia symptom profile changes with age, as do the glycemic thresholds for symptom generation and cognitive impairment (23). Even if hypoglycemia is recognized, patients are known to under-report this to their doctors (24).

2.5 Symptoms, physiology and recognition

The symptoms of hypoglycemia derive from the physiological response to the change in glucose (25). Although symptoms may differ between people, in the younger adult these are usually easy to perceive. The Edinburgh Hypoglycaemia score was developed from analyzing the most common symptoms reported by people experiencing hypoglycaemia (26, 27), and comprises autonomic symptoms (such as sweating and pounding heart), neuroglycopenic symptoms (such as confusion and light-headedness), and non-specific symptoms (such as malaise). Considerable variability in symptoms occurs between hypoglycemic events, even within the same person (28). In older people, the symptoms of hypoglycemia are notably less intense during hypoglycemia than in younger adults (17, 29), and there is an overall reduced subjective awareness of hypoglycemia with increasing age (30). In younger people, autonomic symptoms of hypoglycemia tend to be more prominent than neuroglycopenic symptoms, although the latter also occur. These autonomic symptoms of hypoglycemia become less prominent with increasing duration of diabetes and also in older patients with diabetes (29, 31). It has been postulated that this change in symptoms may be related to a reduced end-organ response in older people (29). The attenuation of autonomic symptoms, and change in glycemic threshold at which they are generated, crucially restricts the ‘protective

window' for action between the recognition of symptoms and the onset of cognitive dysfunction (23, 32). This may be particularly dangerous in an elderly person, who may therefore progress to severe neuroglycopenia.

The Edinburgh Hypoglycaemia score can be adapted to include the neurological symptoms that are common in older people (studied in those aged over 70 years); light-headedness and unsteadiness were found to be particularly frequent (17). The non-specific nature of symptoms and their lower intensity in the elderly person with diabetes can make self-recognition of hypoglycemia difficult; a non-specific episode of confusion can be caused by numerous conditions prevalent in older patients, such as infection, early cognitive impairment, cerebral hypoperfusion resulting from postural hypotension or a TIA. If an episode is not thought to be significant enough to 'worry the doctor' it may not be recorded as a hypoglycemic event or even treated, and even if it is mentioned to medical attendants, the chances of it being recognized as hypoglycemia are not high, because of conflicting differential diagnoses. The treatment of the patient's diabetes may therefore remain unchanged, and unrecognized hypoglycemia may continue to occur. As with younger patients with diabetes, repeated episodes of hypoglycemia can lead to impaired hypoglycemia awareness (27, 33). In insulin-treated people with type 2 diabetes, patients with impaired hypoglycemia awareness had a 17-fold higher frequency of severe hypoglycemia events than those with normal awareness (33). Furthermore, newer methodologies, such as continuous glucose monitoring (CGM), have demonstrated that hypoglycemia is more common than previously appreciated (34).

As symptoms of hypoglycemia are varied and non-specific in the older population (33), the most pertinent pragmatic question is how to identify those at greatest risk?

2.6 Risk factors – including comorbidities and frailty

Elderly people have multiple potential risk factors for hypoglycemia. These risk factors are similar to those observed in young adults, but in people of advanced age these risk factors are cumulative and have a greater impact.

In type 1 diabetes, duration of insulin therapy, loss of endogenous insulin secretion, and a previous history of severe hypoglycemia are predictors for an increased risk of severe hypoglycaemia (17, 35). Other than treatment with insulin and SUs (8), the predictors for an increase in incidence of hypoglycemia in type 2 diabetes are more varied, consistent with the heterogeneity of type 2 diabetes, and advanced age increases the potential for serious morbidity. One important risk factor is the duration of insulin treatment (8, 22, 27, 36, 37). Other observed associations vary between studies, and include older age (38), longer duration of diabetes per se (22, 27), increased comorbidities (especially chronic kidney disease) (38, 39), impaired hypoglycemia awareness (22, 27, 33), intensive therapy and strict glycemic control (27, 40), and behavioural factors, such as irregular eating (41), exercise(39, 42), and errors in timing of medication (42).

The observed association between increased frequency of hypoglycemia with increased duration of diabetes is linked with increasing age and increasing loss of endogenous insulin secretion (43). Certainly in type 1 diabetes, the Diabetes Care and Complications Trial (among others) showed that the lower the C-peptide the higher the rate of severe hypoglycaemia (17, 35, 44). Surprisingly, few studies have examined the role of endogenous insulin secretion in type 2 diabetes, and results have been conflicting: the UK Hypoglycaemia Study Group found an association with frequency of hypoglycemia and C-peptide levels (13), whereas a Danish study by Akram et al (22) did not.

There is increasing evidence that people with cognitive impairment may be at higher risk of experiencing hypoglycaemia (45-49). Of the 11,140 patients with type 2 diabetes in the ADVANCE study (45), 212 were classed as having ‘severe’ cognitive impairment (scoring <24/30 on the Mini-Mental State Examination), and this subgroup had double the risk of severe hypoglycemia (hazard ratio [HR] 2.10, 95% CI 1.14–3.87; p=0.018), than those with ‘mild’ or no cognitive impairment. Similarly, in 497,900 veterans with diabetes aged 65 years or over (46), the adjusted odds ratios for experiencing hypoglycemia that required medical assistance over the course of 1 year were 1.58 (95% CI 1.53–1.62) for those with dementia. Over a median 3.25 years of follow-up, post-hoc analysis of 2,956 patients with type 2 diabetes aged over 55 years in the

ACCORD trial (47), showed that poorer scores on a battery of cognitive tests were predictive of a first episode of hypoglycemia requiring medical assistance (HR 1.13, 95% CI 1.08–1.18). Yaffe at al (49) have recently demonstrated that over 12 years of follow-up, 14.2% of patients with diabetes who developed dementia, subsequently experienced an episode of severe hypoglycemia, compared with 6.3% of those who did not develop dementia (multivariate-adjusted HR 3.1, 95% CI 1.5–6.6; p<0.001).

People with diabetes who live in residential homes, where the estimated prevalence of diabetes is 20–25% (1, 50), are perhaps at particular risk. Reasons for this include advanced age (38), duration on insulin treatment (8, 22, 27, 36, 37), comorbidities (38, 39), reduced ability to manage their food consumption (41), reduced cognition (45-49), impaired mobility, limited facilities to resolve fluctuations in glucose levels, and progressive impairment of hypoglycemia awareness (22, 27, 33). Holstein *et al* (38) found that 34% of German patients with type 2 diabetes who required emergency medical services for severe hypoglycemia were nursing home residents or were being cared for by home nursing services. The residential home population has not, however, been systematically evaluated (33, 51). Education about diabetes among care home staff is often patchy or absent (52).

2.7 Effects of hypoglycemia on quality of life, morbidity and mortality

So why does it matter that older people are exposed to hypoglycemia? Hypoglycemia has a major adverse impact on quality of life (53-55), which has been under-appreciated by healthcare professionals for many years (56). Patients fear hypoglycemia more than the long-term consequences of diabetes (57). Hypoglycemia has been linked to poor outcomes pertinent to an older population: increased risk of accidents (58), falls and fractures (58-60), hospitalizations (58), in-hospital mortality (61), frailty (62), long-term impairment of cognition (48, 63) and a two-fold increased risk of developing dementia (49, 64). It is also associated with electrophysiological changes, particularly

prolongation of the QT interval, a known precipitant of cardiac dysrhythmia, which may persist for up to 48 hours after the hypoglycemic event (65, 66).

The risk of accidents resulting in hospital visits among people with type 2 diabetes on medications excluding insulin, was assessed retrospectively in a large US health insurance database (58). Hypoglycemia was associated with significantly increased hazards for any accident (HR 1.39, 95% CI 1.21–1.59; p<0.001), accidental falls (HR 1.36, 95% CI 1.13–1.65; p<0.001) and motor vehicle accidents (HR 1.82, 95% CI 1.18–2.80; p=0.007). Diabetes *per se* is associated with an increased risk of osteoporosis (67) and a large retrospective observational study in the USA (60) of more than 360,000 patients with type 2 diabetes aged over 65 years, found 4.7% who had a documented hypoglycemic episode over the course of 1 year (resulting in an outpatient medical claim) had a 70% higher chance of having a fall-related fracture (odds ratio 1.7, 95% CI 1.58–1.83); the odds still remained high even after correcting for potential confounders, such as presence of diabetic peripheral neuropathy.

These far-reaching and still under-estimated consequences of hypoglycemia have many financial as well as human costs, which are difficult to quantify. Hospital admissions resulting from hypoglycemia in type 2 diabetes are longer than those with type 1 diabetes, reflecting older age, more comorbidities and polypharmacy (68).

It has been repeatedly observed that dementia is more common in those affected by diabetes (69-72), although the precise mechanism(s) are still not established. Acute hypoglycemia impairs many aspects of cognition: immediate verbal and visual memory, working memory, delayed memory, visual-motor skills, visual-spatial skills, and global cognitive dysfunction (71, 73, 74). It has been suggested that this transient impairment is associated with long-term cognitive defects. Severe hypoglycemia could result in neuronal cell death, which might conceivably accelerate the development of dementia (75). One might postulate that an episode of severe hypoglycemia may be more likely to have a long-term effect on cognition in an older and more vulnerable brain, or that repeated episodes of hypoglycemia (even if apparently less severe) may have a deleterious effect.

One large scale epidemiological study that suggested severe hypoglycemia may lead to dementia, was a longitudinal cohort study in the USA by Whitmer and colleagues (64). They found a graded increase in risk of dementia with increasing numbers of previous hypoglycemic events requiring hospitalization – even after adjustment for age, education, comorbidities, duration of diabetes, diabetes treatment, years on insulin, and 7-year mean HbA1c. This was based on the electronic hospital records of 16,667 patients with a diagnosis of type 2 diabetes (mean age 65 years): 8.8% (1,465) had documented at least one episode of severe hypoglycemia (requiring hospitalization) between 1980 and 2002, and 11% (1,822) had a diagnosis of dementia by follow-up. The fully adjusted HR for dementia having had one episode of hypoglycemia requiring hospitalization was 1.26 (95% CI 1.1–1.49), having had two episodes was 1.8 (95% CI 1.37–2.36), and for three or more episodes was 1.94 (95% CI 1.42-2.64). Similar HRs were found when considering emergency department admissions for hypoglycemia. This appeared to amount to a 2.3% increase in absolute risk of dementia per year of follow-up for patients with a history of severe hypoglycemia.

In a broadly similar study design based on the Taiwanese National Health Insurance Research Database, Lin and Sheu (76) found that of over 15,000 people with type 2 diabetes, mean age of 64.2 years and no documentation of a dementia diagnosis at recruitment, 7.2% developed dementia over 7 years of follow-up. From coding (hospital or ambulatory episodes), approximately 2% of the cohort were found to have an episode of hypoglycemia recorded over a 3-year period. An episode of hypoglycemia predicted an almost 3-fold increase in the risk of dementia (29.9 people developing dementia per 1,000 person-years [95% CI 22.1–39.2] versus 11.1 per 1,000 person-years [95% CI 10.3–11.8]), giving a crude risk ratio of 2.76 (95% CI 2.06–3.70; $p<0.001$). After adjustment for age and sex the risk ratio for developing dementia after hypoglycemia was 1.60 (95% CI 1.19–2.14; $p=0.002$), and this was a graded increase in risk according to the number of episodes of hypoglycemia experienced.

Both of these studies (64, 76) can be criticized for potential selection bias, lack of correction for certain potentially significant confounders, and inability to accurately assess for cognitive function (77). By only recording the most severe

hypoglycemic events (those requiring medical assistance), when most episodes of hypoglycemia are self-treated in the community, those patients identified may be those least able to look after their own diabetes and potentially may be those most at risk of being cognitively impaired (eg, with subclinical cerebrovascular disease) at the time of their severe hypoglycemia episode, which could neither be measured nor corrected for. Other potentially significant comorbid conditions such as a history of alcoholism, epilepsy, psychiatric illness or head injury could also not be corrected for (77). Additionally, patients who experience hypoglycemia needing hospitalization are often considered to be an atypical group of patients; they are often severely ill (eg, with sepsis), which may provide other causes precipitating subsequent cognitive decline (77). The authors considered that because the sub-analysis of data from emergency department attendances was as robust as that from hospital episodes, this made this scenario less likely – plus the up-to 15 year lag from hospital episode of hypoglycemia to diagnosis of dementia was likely to dispel the effect of any other comorbid conditions from the hospital admission (78). While it is acknowledged by the authors (78) that no observational study can completely eliminate all confounders, the strength of the data raises legitimate concerns that hypoglycemia may precipitate the onset of dementia. This calls for some circumspection when treating frail elderly to strict glycemic targets – and calls for the need for prospective studies in this area.

The Edinburgh Type 2 Diabetes Study avoided some of the methodological concerns of the above study (64) and also supported the suggestion of an association between severe hypoglycemia and subsequent development of dementia (48). In this study, a cross-sectional methodology was used, with 1,066 participants aged 60–75 who had type 2 diabetes, being asked to complete a validated questionnaire to assess their frequency of severe hypoglycemia in the previous year, and over their lifetime. Cognitive function was assessed both at the time of the study (using age-sensitive psychological tests to derive a ‘late-life cognitive ability factor’), and projected prior cognitive ability (using vocabulary tests that are stable during ageing). In those reporting at least one severe hypoglycemic event (113 patients, 10.6%), a slightly lower mean vocabulary score was observed, but was not statistically significant

(p=0.13), ie, there was seemingly no major difference in premorbid cognitive ability. However, a clear difference was found in their 'late-life general cognitive ability factor' (p<0.001), and this difference persisted even after adjustment for various potential confounders such as duration of diabetes, smoking, HbA1c and vascular disease. Additionally, although those having experienced severe hypoglycemia scored higher on the Hospital Anxiety and Depression Scale, the cognitive associations remained significant after being corrected for this. The temporal relationship between hypoglycemia and cognitive decline cannot be determined accurately with this cross-sectional design (48), and it is interesting to note that 76% of the patients reporting at least one episode of hypoglycemia had experienced an episode in the year preceding recruitment. However, on analysis no significant difference was observed in the overall strength of the association with cognition when hypoglycemia in the year preceding recruitment was used versus lifetime history.

The Fremantle Diabetes Study in Australia (79) found an association between previous severe hypoglycemia and subsequent cognitive impairment and dementia, when the cognition of 302 individuals was assessed and their previous exposure to severe hypoglycemia estimated retrospectively. However, a small prospective arm was included in an attempt to address the question of temporal decline. The study was probably underpowered to answer this question, and the authors did not find an association between severe hypoglycemia and evidence of premature dementia in 205 individuals over 70 years old without cognitive impairment who were followed over a comparatively short period of 4 years.

A recently published prospective study by Yaffe *et al* (49) provides more convincing evidence and lends weight to the causality of dementia in relation to hypoglycemia exposure. The authors found a two-fold increased risk of developing dementia in people who had experienced an episode of severe hypoglycemia requiring hospitalization. A total of 783 older adults (mean age 74 years) with diabetes but no evidence of cognitive impairment at recruitment (determined by a baseline Modified Mini-State Examination), were followed for 12 years. During this time, 7.8% (61 patients) had a severe hypoglycemic event requiring hospitalization, and 18.9% (148 patients) developed dementia

(determined by a dementia-related hospital event or prescription of a dementia medication, and confirmed by cognitive assessment). Of those who had experienced a hypoglycemic event, 34.4% developed dementia, compared with 17.6% who did not ($p<0.001$), with a multivariate-adjusted HR of 2.1 (95% CI 1.0–4.4). A bidirectional association was observed; those who developed dementia had a greater risk of subsequently experiencing hypoglycemia (14.2%) compared with those who did not develop dementia (6.3%, multivariate-adjusted HR 3.1, 95% CI 1.5–6.6; $p<0.001$).

Overall, an increasing body of evidence could support a putative association between hypoglycemia and dementia – in bidirectional fashion – and given the increasing prevalence and burden both of dementia and of diabetes in the older population, this is an area that deserves much more attention. Causes of dementia are still poorly understood; if reducing hypoglycemic events in the older population can help to reduce the likelihood of the development of dementia, physicians should tangibly address this possibility.

2.8 HbA1c targets

Strict glycemic control and intensive therapy are associated with an increased incidence of severe hypoglycaemia (5, 6, 80). A meta-analysis of randomized controlled trials (81) – which included over 28,600 patients with type 2 diabetes – found the relative risk of severe hypoglycemia was increased by 30% in the groups undergoing strict glycemic control. The frequency of mild hypoglycemia is also likely to be increased, as are acute daily glucose fluctuations, which are increasingly recognized as being associated with poor outcomes, such as effects on cognition (63). A retrospective cohort study using data from nearly 28,000 patients over the age of 50 years and with type 2 diabetes sourced from the UK General Practice Research Database, found a U-shaped association between HbA1c and all-cause mortality and cardiac events (82), with the lowest risk at an HbA1c of 7.5%.

Targets for glycemic control in elderly patients have become more controversial and pragmatic (83). Guidelines are starting to reflect a need for more individualized treatment, but the evidence base is very limited. To date only one

clinical study has even attempted to utilize individualized treatment targets (16), and no study has used clinically meaningful outcomes for elderly patients, such as falls, progression of frailty and quality of life. Disappointingly, in the INTERVAL study, despite being asked to individualize glycemic targets around patients' age, frailty and co-morbidities, the participating physicians only considered baseline HbA1c and gender, and they set a HbA1c target of 7.0%. Individualizing the treatment target was associated with lower than anticipated side effects, including hypoglycemia, and good tolerability of the strategy. Indeed, 27% of the population achieved their targets with nothing more than lifestyle change and increased contact with the care-providers. The American Diabetes Association (ADA) and the European Association for the Study of Diabetes have issued a joint position statement suggesting a more patient-centered approach for the treatment of type 2 diabetes (84). For older adults, they have relaxed the HbA1c target to <7.5% or even 8% if stricter targets are more difficult to achieve. In 2012, the ADA and American Geriatrics Society also issued a consensus report on diabetes in older adults (85), which suggested more pragmatic glycemic targets for older adults than those previously published (Table 1).

However, more relaxed HbA1c targets do not eradicate hypoglycemia. Munshi *et al* (86) used CGM to estimate the frequency of hypoglycemia in an older population (>69 years) with a 'more relaxed' glycemic target of HbA1c of >8%, and found that 65% experienced at least one episode of hypoglycemia (glucose <70mg/dl; 3.9 mmol/l) during the 72 hours of monitoring. They concluded that relaxing glycemic control to >8% was not necessarily sufficient to prevent hypoglycemia in this population. They did not compare the frequency of hypoglycemia events in this population with the frequency of events in patients with stricter glycemic targets; the frequency of hypoglycemia in patients with strict glycemic targets would be expected to be even higher.

Table 1. *The ADA/American Geriatrics Society consensus guidelines for setting HbA1c targets based on patient baseline characteristic (85)*

Patient type	Examples of patient features	HbA1c target
"Healthy"	Few coexisting chronic illnesses; cognitive & functional status intact	<58.5 mmol/mol (7.5%)
"Complex" or "intermediate"	Multiple coexisting chronic illnesses; >2 activities of daily living impairments; mild-to-moderate cognitive impairment	<64 mmol/mol (8%)
"Very complex" or "in poor health"	Long-term condition/end-stage chronic illnesses; moderate-to-severe cognitive impairment; >2 activities of daily living dependencies	<69 mmol/mol (8.5%)

In practice, inadequate recognition of hypoglycemia may not alert patients or clinicians to the need to re-evaluate individual treatment targets. With the increased recognition of the adverse effects of hypoglycemia and glucose variability, an increasing number of older people on insulin, and continued strict glycemic targets, hypoglycemia will become increasingly important. A stronger evidence base for individualized treatment is needed.

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Part 3: Endogenous insulin, C-peptide and hypos

3.1 Introduction

In our ageing population, diabetes – and long-standing diabetes - is becoming ever more prevalent, and the numbers of people who are insulin-treated ever more. As described previously, hypoglycaemia in the older person is important, and can be difficult to recognise.

3.2 Physiology of hypoglycaemia

Those with Type 1 diabetes are known to have higher rates of hypoglycaemia than those with insulin-treated Type 2 diabetes (1); the key difference contributing to this being endogenous insulin levels. Homeostasis of blood glucose levels is complex, but in summary, when blood glucose levels fall, the usual first-line of defence (where possible, ie in those without diabetes, or those with diabetes but with retained endogenous insulin levels), is to reduce endogenous insulin secretion (Figures 1 & 2). With ongoing reduction in blood glucose levels other defence mechanisms come into play: glucagon is released from the alpha-cells in the pancreas, which in turn stimulates glucose release from stores particularly in the liver; and gluconeogenesis is also stimulated. Nervous system sensing that blood glucose levels are falling also results in adrenaline release, which as well as stimulating the liver, gives rise to the characteristic autonomic symptoms prominent particularly in younger people experiencing hypoglycaemia – which alerts the person to eat food or treat themselves.

Figure 1: *Glycaemic thresholds for secretion of counter-regulatory hormones and onset of physiological, symptomatic, and cognitive changes in response to hypoglycaemia in the non-diabetic human (2)*

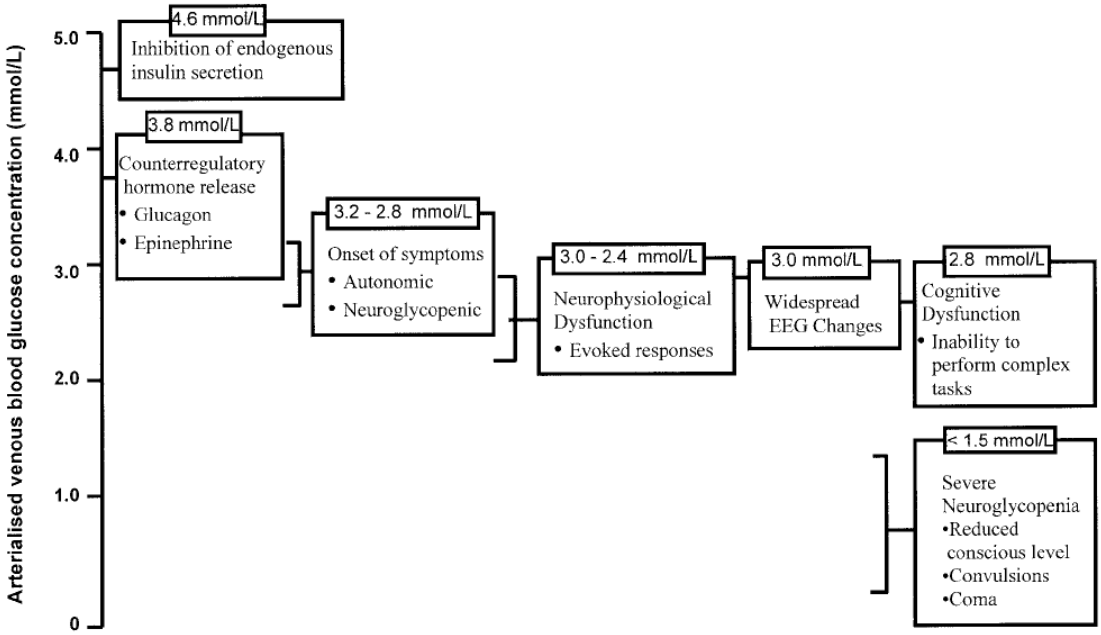
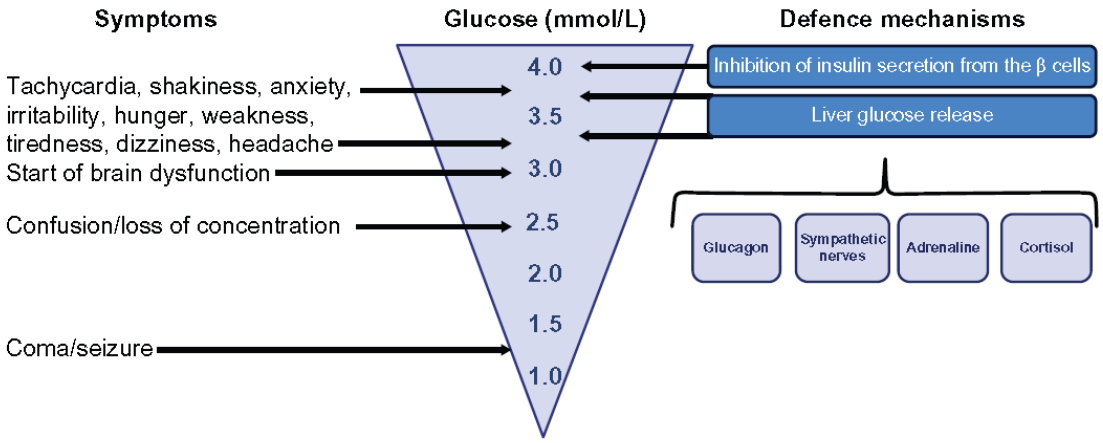


Figure 2: *Symptoms and defence mechanisms in relation to glucose levels in the subnormal range (3)*



In Type 1 diabetes, and “advanced” Type 2 diabetes, these defence mechanisms falter. Firstly, with absent or minimal endogenous insulin levels, there is limited capacity to reduce any effect of endogenous insulin by stopping its secretion. Secondly, being on insulin treatment, exogenous insulin will be “in the system”, and unless on an insulin pump, there is no possibility of stopping its ongoing action. Thirdly, it has been shown that glucagon release is diminished, and thus there is limited capacity for its stimulating action on other organs (4-6). These patients are thus heavily dependent on adrenaline secretion for their defence against hypoglycaemia – for the symptoms resulting in behavioural response, and for the effect of adrenaline on stimulating glycogenolysis and gluconeogenesis (6).

However over time in patients with Type 1 and advanced Type 2 diabetes, adrenaline response to falling blood glucose levels has been shown to diminish, and this seems to be associated particularly with repeated episodes of hypoglycaemia, the so-called “hypoglycaemia-associated autonomic failure” (6, 7). This is associated with a reduction in symptoms, giving the clinically recognised phenomenon of “hypoglycaemia unawareness”. Hypoglycaemia unawareness (and partly the reduced adrenaline component of defective glucose counter-regulation - but not the absent glucagon component) can be reversed by as little as two weeks of strict avoidance of hypoglycaemia (7-9) – but this relies on recognition that frequent hypoglycaemia is occurring. Regular night-time hypoglycaemia may be even less likely to be picked up on.

3.3 Recognition of symptoms in the elderly

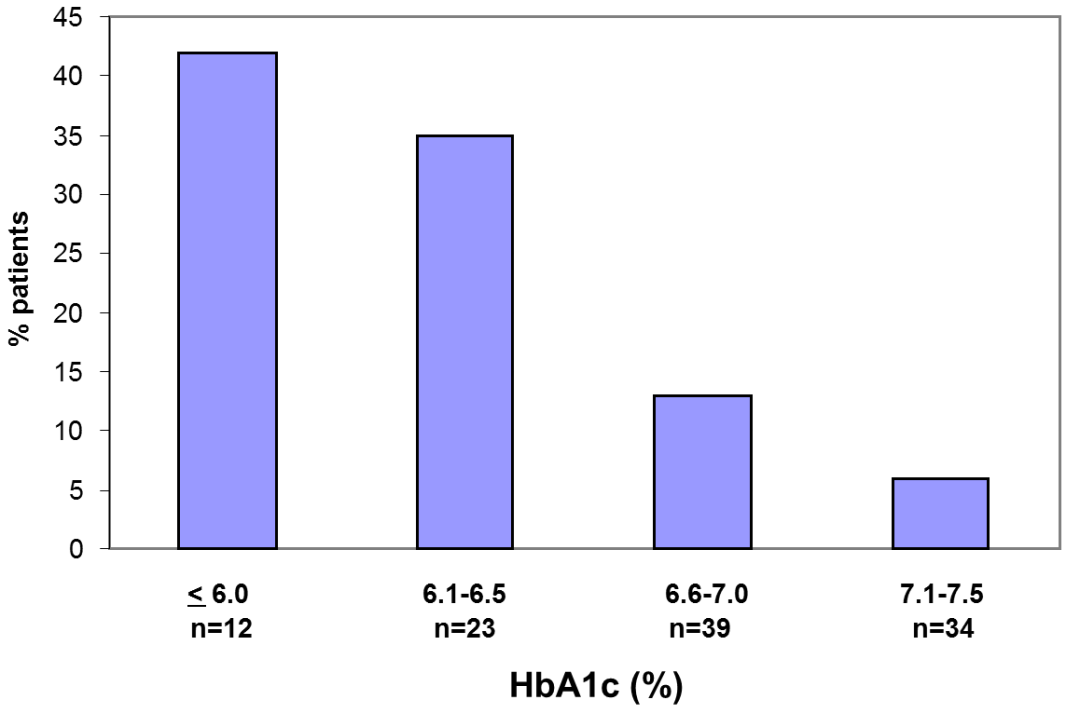
An additional, potentially increasingly significant, problem of reliance on the adrenaline response to hypoglycaemia, is that elderly people even without diabetes have reduced autonomic symptoms compared to younger people (10-12), and those with diabetes have even more pronounced changes (13). Thus the characteristic warning symptoms of hypoglycaemia are reduced, those that are present are less “intense” – and given their non-specific nature, can easily be attributed to other conditions of older age. This may be especially so in those

with Type 2 diabetes who may not have had much education on or experience of hypoglycaemia. Additionally this reduction in “warning” symptoms means there is a smaller window of opportunity to effect behavioural change (eat), before more severe symptoms resulting from direct neuroglycopenic effects on the brain, such as confusion, drowsiness and eventually coma, may occur.

In general, symptoms of hypoglycaemia are pretty consistent, both in terms of those reported by patients in clinic, and in physiological studies. As alluded to above, analyses of symptoms in patients with Type 1 diabetes have found them to fall into the general categories of autonomic (eg sweating, palpitations, shaking and hunger), general malaise (eg headache and nausea), and neuroglycopenic (eg confusion, odd behaviour, speech difficulty and drowsiness) (14). This spectrum of symptoms, developed into the Edinburgh Hypoglycaemia Questionnaire, has been found to be similar in those with Type 2 diabetes treated with insulin (15). Jaap et al (16) looked at symptoms experienced during daytime episodes of hypoglycaemia by elderly people with insulin-treated Type 2 diabetes, and found the frequency and classification of symptoms to be different from those seen in younger patients treated with insulin. Neurological symptoms were more common, particularly impairment of co-ordination and articulation, and light-headedness and dizziness – symptoms which may easily be misinterpreted as being due to cerebrovascular or cardiovascular causes.

In view of the non-specific nature of symptoms of hypoglycaemia especially in the elderly, a pilot study was done in a Devon GP practice in 2008. This comprised a retrospective review of primary care consultations over the previous year in all patients on insulin or sulphonylurea treatment, with tight glycaemic control (HbA1c <7.5%, 58.5mmol/mol) (Hope, Taylor & Hattersley, unpublished). This amounted to 106 patients in total. Any consultation where one of the classic hypoglycaemic symptoms (a “hypo clue”) was reported was logged, and Figure 3 shows the proportion in each HbA1c group with at least one “hypo clue” consultation. Of note, none of these consultations had documented hypoglycaemia as having been considered as a possible explanation, and thus no changes made to medications had been made.

Figure 3: *Proportion of patients in each HbA1c group with at least one "hypo clue" consultation (Hope, Taylor & Hattersley, unpublished)*



Subsequently, a general international appreciation that tight glycaemic control may bring more risks than benefits in the more frail and elderly population has brought about changes in international and national guidelines, with more "relaxed" – or individualised - HbA1c targets in those who are deemed "frail", or with multiple co-morbidities (17-21). There is little guidance however on how to do this however, with just one study to date specifically attempting to set individualised treatment targets (22), and finding clinicians reluctant to deviate from traditional glycaemic targets even in the frail elderly.

Recognition of those most at risk of hypoglycaemia is obviously a good starting point, and given it is well-recognised that those with Type 1 diabetes are much more at risk of having hypos than those with Type 2, accurate diagnosis is key.

3.4 Diagnosis of Type 1 or Type 2 diabetes

The key difference in terms of clinically managing Type 1 or Type 2 diabetes, is a difference in endogenous insulin levels. Type 1 diabetes is generally accepted as a condition of absolute insulin deficiency, due to an autoimmune process which ultimately leads to loss of pancreatic beta-cell function. The time taken to develop absolute insulin deficiency varies between individuals with Type 1 diabetes, but ultimately all those affected need treatment with exogenous insulin for survival.

Type 2 diabetes is a more heterogeneous condition, and perhaps reflecting this, has somewhat imprecise definitions, all on a variation of the theme: a "*form of diabetes, which accounts for ~90-95% of those with diabetes, previously referred to as non-insulin-dependent diabetes… individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency. … There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of B-cells does not occur, and patients do not have any of the other causes of diabetes listed…*". (23).

Getting the right diagnosis is crucial, as all treatment guidelines depend on having selected a diagnosis, and differ in terms of education, monitoring and treatment. However it has only been recently that some practical diagnosis guidelines from the Royal College of General Practitioners and NHS Diabetes have been published (24).

3.5 Endogenous insulin levels in Type 2 diabetes

Early in the natural history of Type 2 diabetes, endogenous insulin secretion increases (Figure 4). Individuals differ in the amount of contribution from insulin resistance and (relative) insulin deficiency in the loss of their glucose control. In this phase, weight loss (and thus reduction in insulin resistance) can help, some people will respond well to "insulin sensitisers" (metformin), and later to "insulin secretagogues" (sulphonylureas). But over time, response to these diminishes,

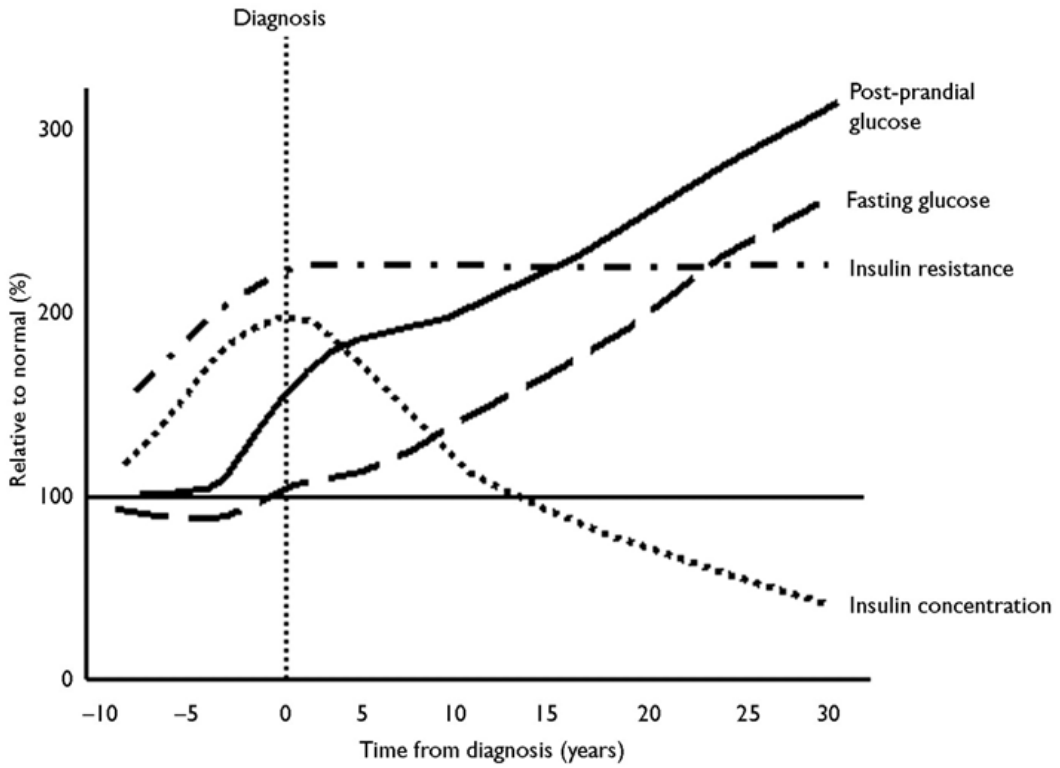
and insulin deficiency becomes more prominent – eventually resulting in a need for insulin treatment in order to maintain glycaemic control. The physiological reason for this decline of endogenous insulin levels is poorly understood, and the rapidity of decline varies hugely. Thus even amongst patients with Type 2 diabetes on insulin, there is clearly a wide spectrum of residual levels of insulin.

Some of those with a clinical diagnosis of Type 2 diabetes, and a more rapid decline in endogenous insulin levels, have some features in common with those with Type 1 diabetes, namely pancreatic autoantibodies. When tested, approximately 10% of patients with “Type 2 diabetes” are shown to have islet autoantibodies, and lower C-peptide levels than most people with T2D. These people are sometimes described as having “latent autoimmune diabetes in adults”, or “LADA” (25, 26). A definition of LADA has been proposed: development of diabetes >35 years of age, not initially (within the first 12 months of diagnosis) insulin requiring, but developing the need to start insulin treatment relatively soon (usually within 3-5 years), and autoantibody positive (26).

Borg et al (27) performed a prospective 12 year study looking at pancreatic autoantibody status and subsequent course of beta-cell failure (defined as fasting serum C-peptide <0.1nmol/L) in newly presenting adults (>20 years old) with diabetes in Sweden. ICA, IA2 and GAD autoantibodies were assessed at diagnosis, 3 years, 5 years and 12 years, and 107 subjects completed the 12 year follow-up. They found only patients with significant titres of islet antibodies developed beta-cell failure over the 12 years: almost all of those with GAD and/or ICA at diagnosis, with 91% being on insulin treatment by this point. Those with GAD positivity only at diagnosis tended to a slower onset of beta-cell failure – usually with some preserved function at 5 years, but all reached their criteria of beta-cell failure by 12 years. None of the subjects who were autoantibody-negative at diagnosis developed complete beta-cell failure by 12 years, although one third were on insulin-treatment by this point. Interestingly their fasting C-peptide levels were not significantly different from their levels at diagnosis. The fasting C-peptide levels of those patients with IA2 antibodies only at diagnosis were also not significantly changed by 12 years.

However in routine clinical practice, in those thought clinically to have Type 2 diabetes at presentation, pancreatic autoantibodies are rarely measured, and clinical management is based on clinical characteristics and responding to deterioration in glycaemic control or side effects of medications. Certainly in the UK therefore, the diagnosis of “LADA” is not widely used, and given the variation in the rate of loss of endogenous insulin even in those with known pancreatic autoantibodies, if a convenient and inexpensive measure of how much insulin someone is producing was possible at certain clinical decision-points, this may prove to be more useful than knowing there is a risk of developing severe insulin deficiency at some unknown point in the future.

Figure 4. *Natural history of insulin resistance and insulin secretion in Type 2 diabetes (28)*



3.6 Measuring endogenous insulin levels

Clinical management of diabetes tends to respond to levels of glycaemia and side effects of medications, rather than taking account of the endogenous insulin levels directly. Although endogenous insulin levels can be measured directly, insulin is rapidly metabolised by the liver, and as such its measurement has mainly been limited to research settings, where samples could be collected on ice and immediately processed.

Endogenous insulin levels can also be assessed using C-peptide. This is secreted in equimolar quantities to insulin following cleavage of the prohormone proinsulin. C-peptide has the distinct advantage that it can also be measured in people who are being insulin-treated (29), in contrast to insulin, where assays are not always able to distinguish endogenous from exogenous insulin. Fasting, glucagon-stimulated, and mixed meal test-stimulated blood C-peptide levels have thus all been used in research settings, but again C-peptide was thought to be relatively rapidly degraded by serum proteases and thus not practical for routine clinical use. However the concept of measuring endogenous insulin levels using C-peptide levels has not been lost, with international agreement on gold-standard stimulated C-peptide measures being designated as suitable outcome measures for intervention studies in Type 1 diabetes (30).

C-peptide metabolism occurs largely in the kidneys, and the total quantity of C-peptide excreted in the urine per day represents 5%–10% of pancreatic secretion (31). 24 hour urinary C-peptide measurements (31, 32), and spot urine measurements for urinary C-peptide creatinine ratio, UCPCR (33), have consequently been shown to accurately assess beta-cell secretory capacity. They have also been shown to also correlate well with fasting and stimulated serum insulin and C-peptide levels (32, 34). The development of the spot urine test in 2009 in particular has heralded significant practical advantages for use in clinical settings, with it having been shown to be stable in a boric acid container for 3 days (33). It subsequently has been demonstrated as a practical outpatient tool for differentiating genetic causes of diabetes from Type 1 diabetes (35), and for helping in monitoring of success of islet transplants (36).

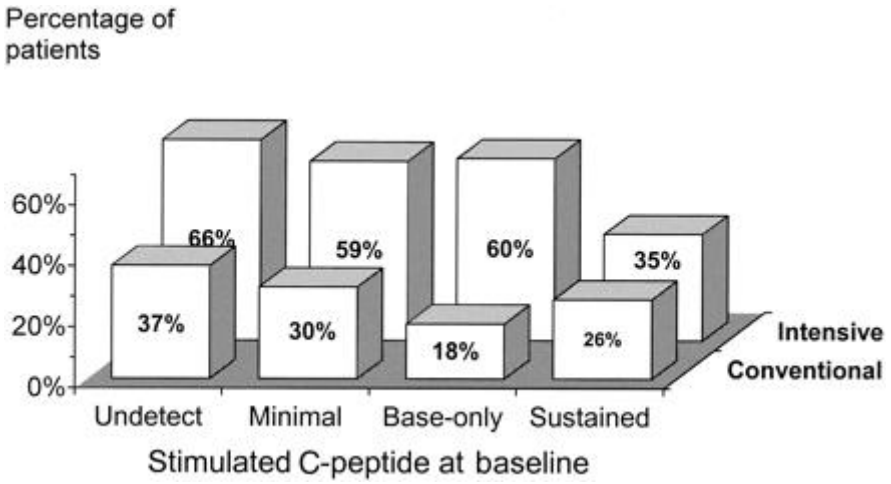
More recent work has demonstrated much greater stability in blood for C-peptide than was previously appreciated (37), with C-peptide levels found to be stable for at least 24 hours at room temperature in both centrifuged and whole blood collected in K^+ EDTA tubes, and stable after 6 freeze-thaw cycles. This has huge potential practical utility for increasing more widespread clinical use of C-peptide measurement.

3.7 C-peptide levels as a clinical marker?

Although Type 1 diabetes is a disease of “absolute insulin deficiency”, the C-peptide level of 200pmol/L has been considered a threshold associated with an increased rate of complications in Type 1 diabetes, in particular hypoglycaemia (38-40). The landmark DCCT study found that in those who were intensively treated (ie tight glycaemic control, HbA1c<7.5%, 58.5mmol/L), the risk of severe hypoglycaemia (seizure or coma) was 17.3 episodes per 100 patient years of follow-up in those with a mixed meal tolerance test stimulated serum C-peptide (sSCP) level <200pmol/L, compared to 6.6 in those with a sSCP >200pmol/L.

Steffes et al (30) further analysed the DCCT data, subdividing patients into those with a baseline sSCP <30pmol/L as “undetectable”, sSCP 40-200pmol/L “minimal”, sSCP >200pmol/L at baseline but <200pmol/L by 1 year follow-up “baseline only”, and sSCP >200pmol/L at baseline and at follow-up “sustained”. They compared at each level of C-peptide the proportion of patients with at least one episode of severe hypoglycaemia in the first 6 months of the study, those treated intensively versus conventionally, Figure 5. Intensively-treated patients had similar rates of severe hypoglycaemia (~65%) at each level of C-peptide, apart from those with “sustained” C-peptide who experienced reduced rates of ~35%, more similar to the rates in seen in all conventionally-treated groups. Of note, the findings in this study remained consistent even when adjusted for duration of diabetes, glycaemic control and other factors.

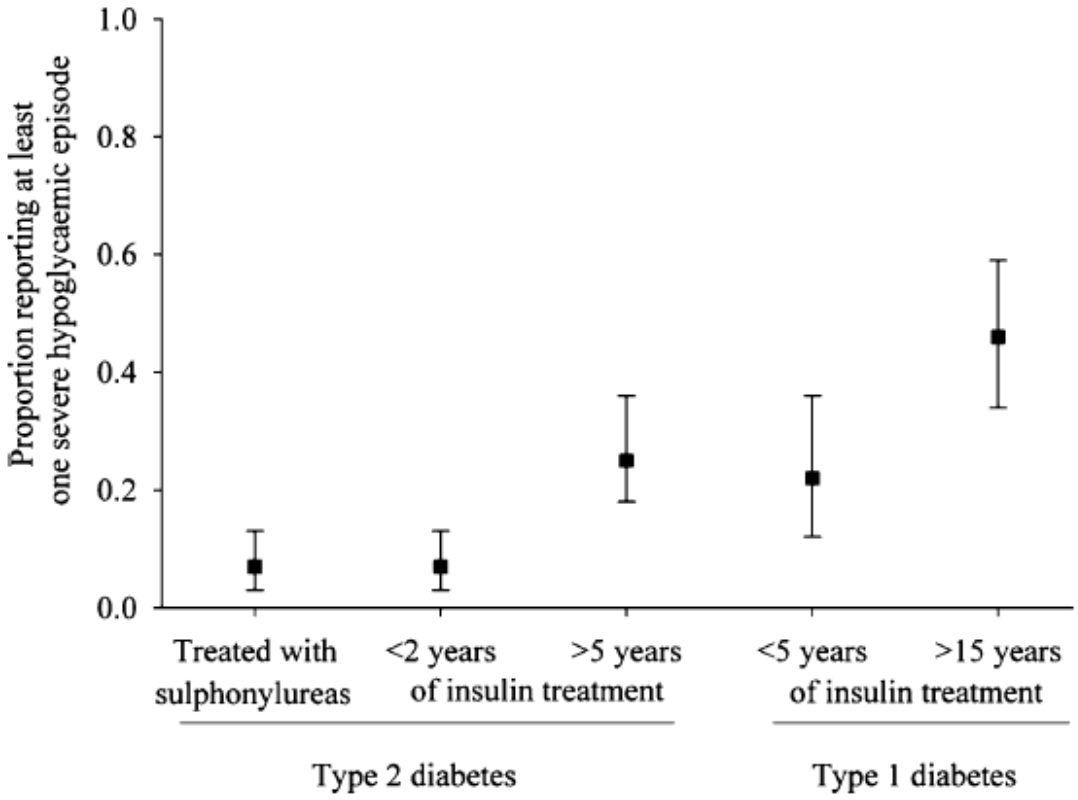
Figure 5: *Percentages of patients who experienced at least one episode of severe hypoglycaemia over the first 6 months of the DCCT (30)*



Modern assays for C-peptide measurement can measure below this range the previous “minimal” range (36, 41), and a relationship between hypoglycaemia and lower levels of C-peptide has been described (42, 43).

A recent analysis of the ACCORD study in Type 2 diabetes suggested that those participants who suffered from severe hypoglycaemia had significantly lower C-peptide levels than those who had similar glycaemic control but who did not experience hypoglycaemia (44). This would be consistent with known progressive insulin deficiency in Type 2 diabetes and the increase in frequency of hypoglycaemia in insulin-treated Type 2 diabetes with increasing duration of diabetes (45), and of insulin treatment (2, 15), Figure 6.

Figure 6: *Proportion of each group experiencing at least one severe self-reported hypoglycaemic episode during 9–12 months of follow-up. Vertical bars, 95% CI (1)*

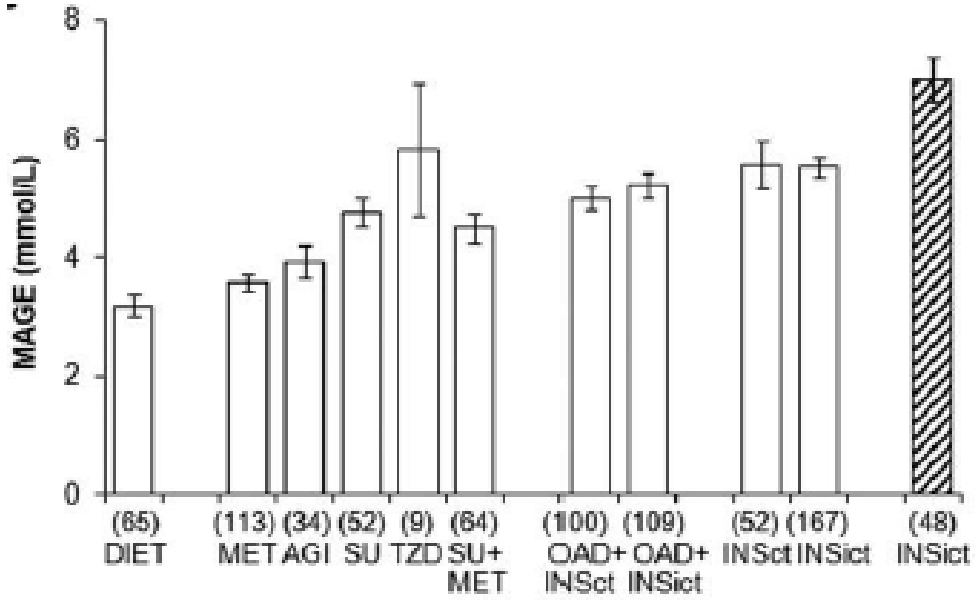


3.8 Glucose variability

Intensification of treatment in Type 2 diabetes is due to progressive insulin deficiency. As well as endogenous insulin levels declining over time in Type 2 diabetes, glucose variability increases with increasing treatment intensity (Figure 7). Glycaemic variability reflects the fact that blood glucose levels fluctuate across the day, and is an important concept, as an equivalent HbA1c does not necessarily reflect the same pattern of glycaemic control, see Figure 8a-c (46, 47). Given the previously described impairments in glucose homeostasis particularly in Type 1 diabetes, it is unsurprising that glycaemic variability is higher in Type 1 than Type 2 diabetes (48). Increased glycaemic

variability has been associated with oxidative stress (49-51), and is proposed to be a predictor for the risk of complications of diabetes (52).

Figure 7: *Glycaemic variability (as measured by mean amplitude of glycaemic excursions) calculated from 72-h continuous glucose monitoring tracings. Between-treatment group differences statistically significant, p < 0.001*



White columns:

patients with T2D treated with
- diet (DIET)
- metformin (MET)
- a-glucosidase inhibitor (AGI)
- sulphonylurea (SU)
- thiazolidinedione (TZD)
- conventional insulin therapy (INSct)
- intensified insulin therapy (INSict)

Hatched column:

patients with T1D

Figures 8a-c: a&b) *These represent the same mean glucose* (46)

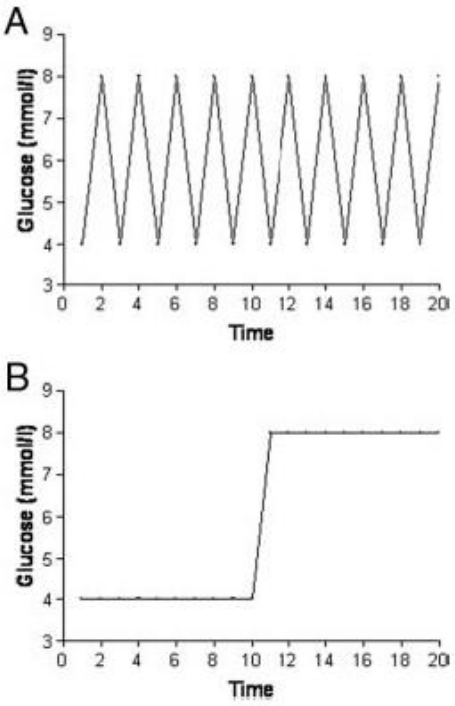
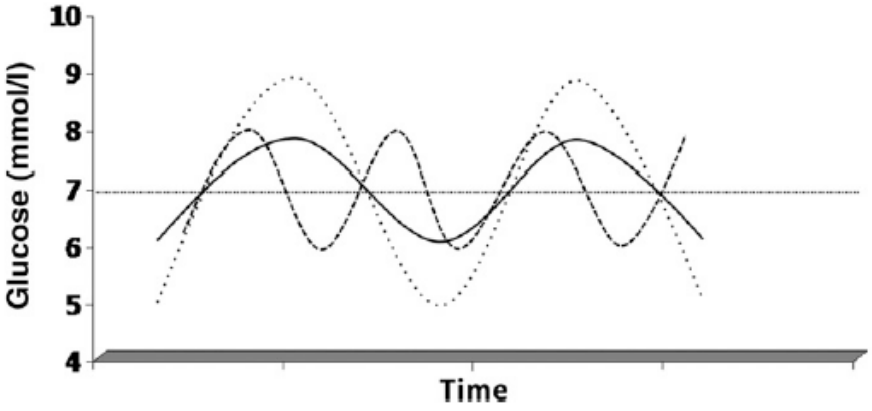


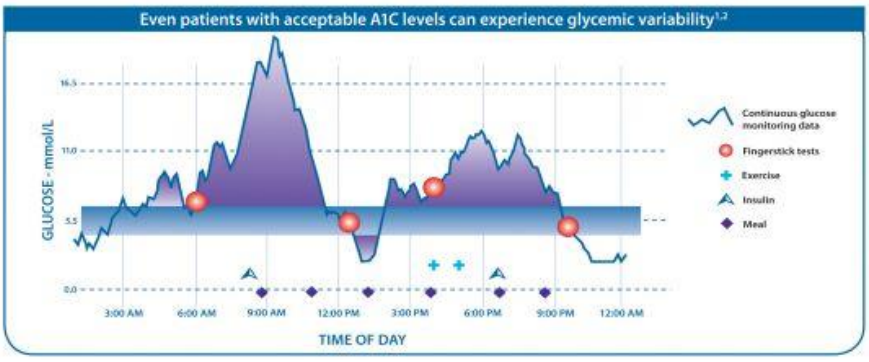
Figure 8c) *different glucose profiles with the same average glucose (and thus HbA1c) but the person with the paler dotted line is at much higher risk of hypoglycaemia* (47)



Glycaemic variability can be measured using continuous glucose monitoring. Appropriate outcome measures have been debated (53-56), and no consensus outcome measure agreed upon. The most commonly reported is the straightforward standard deviation of glucose measurements (48, 57).

By uncovering hidden fluctuations (Figure 9), continuous glucose monitoring also has the ability to expose asymptomatic hypoglycaemia – which may not have previously been revealed eg if blood glucose levels are checked every day at the same time(s). It may be particularly valuable to help detect night-time hypoglycaemia. However until recently continuous glucose monitoring has been prohibitively complex and expensive for widespread use, and as such if there was a way to identify those most at risk of high glucose variability and hypoglycaemia which was more practical (and cheaper) – such as measuring C-peptide - this could be of great clinical utility.

Figure 9: Self-monitoring of blood glucose can miss fluctuations.
http://www.medtronicdiabetes.co.in/treatment-and-products/i-pro-evaluation



3.9 Summary

Hypoglycaemia recognition in the elderly is tricky. Getting the correct diagnosis of type of diabetes is important for subsequent management. People with long-standing Type 2 diabetes have progressive insulin deficiency, and it is not completely clear what clinical consequences this may have when advanced – perhaps increased glycaemic variability, and increased risk of hypoglycaemia, such as in Type 1 diabetes. Measuring endogenous insulin levels by C-peptide measures may have increasing clinical utility, and could have the potential to act as a “biomarker” for high risk of hypoglycaemia.

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CHAPTER 2

Are we missing hypoglycaemia?

Elderly patients with insulin-treated diabetes present to primary care frequently with non-specific symptoms associated with hypoglycaemia

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For submission to a primary care journal

CHAPTER 2

Acknowledgments of co-authors and contributions to paper

The idea originated from an audit that Andrew Hattersley and Phil Taylor had previously done; I developed the study idea from this after discussions with the clinical research team. Willie Hamilton and Sarah were extremely helpful in introducing me to the concept of using GP databases to answer research questions and becoming familiar with the Clinical Practice Research Datalink, which although I did not use specifically in the end, many of the principles were invaluable. Phil Taylor taught me how to use the GP database, and I collected all the data and analysed it. I had help with statistical analysis from Bev Shields and Vasilis Nikolaou. I wrote the manuscript after helpful discussions with the other members of the clinical research team particularly Ali Chakera and Richard Oram, and the other co-authors. Willie Hamilton helped edit the final version.

Are we missing hypoglycaemia? Elderly patients with insulin-treated diabetes present to primary care frequently with non-specific symptoms associated with hypoglycaemia

Abstract

Introduction

Hypoglycaemia is a potentially fatal side-effect of diabetes treatment. Its recognition is difficult in older patients: as with many clinical presentations in this group, the symptoms are non-specific. We explored whether patients at high risk for hypoglycaemia were presenting on other occasions with non-specific symptoms associated with hypoglycaemia, which may represent missed hypoglycaemia.

Methods

Data were collected from the primary care records in a single large practice on people aged ≥65 with and without diabetes. Episodes of hypoglycaemia and consultations for non-specific symptoms - “hypo clues” - were identified (eg unexplained dizziness, confusion, sweating) between 5/2/12 and 4/2/13. Potentially discriminatory symptoms, and their correlation with HbA1c were evaluated.

Results

335 records analysed (79 patients on insulin, 85 on sulphonylureas, 121 on metformin only, 50 without diabetes).

27/79 (34%) insulin-treated patients had ≥1 documented episode of hypoglycaemia, compared to 4/85 (5%) sulphonylurea-treated patients, 2/121 (2%) metformin-only treated patients, and no patients without diabetes, $p<0.001$.

Consultations with “hypo clues” were common in all treatment groups: 1.37 consultations/patient/year in insulin-treated patients, 0.98/patient/year in sulphonylurea-treated patients, 0.97/patient/year in metformin only-treated patients, and 0.78/patient/year in non-diabetic patients, p=0.34. However of insulin-treated patients with a documented episode of hypoglycaemia over the year, 20/27 (74%) attended on another occasion with a “hypo clue” symptom, compared to 21/52 (40%) of those without hypoglycaemia, p=0.008. There was no significant difference in the other treatment groups between the rate of “hypo clue” consultations in those with or without hypoglycaemia.

Nausea, falls and unsteadiness were the most potentially discriminatory symptoms: 7/33 (21%) patients with hypoglycaemia attended on another occasion with nausea compared to 14/302 (5%) without hypoglycaemia, p=0.002; 10/33 (30%) vs 36/302 (12%) presented with falls, p=0.007; and 5/33 (15%) vs 13/302 (4%) presented with unsteadiness, p=0.023.

There was no difference overall in the rate of hypoglycaemia or “hypo clue” consultations across the HbA1c range.

Conclusions

Non-specific symptoms which can represent hypoglycaemia are common in a population aged $\geq$65. However in insulin-treated patients at risk of hypoglycaemia, these “hypo clue” symptoms, in particular nausea, falls and unsteadiness, should serve as a reminder to consider hypoglycaemia and review medication.

INTRODUCTION

Tight glycaemic control in order to prevent long-term complications of diabetes (1, 2) has been associated with an increased prevalence of hypoglycaemia in Type 1 (3) and Type 2 diabetes (4). The increasing prevalence of diabetes coupled with longer life expectancy, and thus longer duration of diabetes, means there is an increasing elderly population on potentially hypoglycaemia-causing medications such as insulin and sulphonylureas (5-7).

Hypoglycaemia is associated with risks such as falls, accidents, hospitalisation, impact on driving (8-11), fear and adverse effects on quality of life (12, 13), arrhythmias (14), and adverse effects on short and long-term cognition (15-19). Recurrent hypoglycaemia can precipitate hypoglycaemia unawareness – reduced awareness of symptoms, which leads into a vicious circle (20, 21).

The recognition that elderly people on hypoglycaemia-causing medications may be particularly vulnerable has led to alterations of guidelines, incorporating more relaxed HbA1c targets for frail elderly, or those with multiple comorbidities (22-24).

Hypoglycaemia symptoms in elderly people are less pronounced than in younger patients (25-27). Hypoglycaemia is under-reported, and under-recognised – by patients, carers and healthcare professionals (28-30). Symptoms also vary much more between episodes in the same person than is often appreciated (31). These factors complicate estimates of the prevalence of hypoglycaemia, almost certainly leading to under-estimation.

As blood sugar levels fall, the autonomic symptoms of sweating, palpitations, and anxiety first occur; these stimulate food intake, in order to restore blood glucose levels (32). However autonomic symptoms become less prominent in older age (25, 33), and glucose levels may thus fall into the “neuroglycopenic” range before self-correction. Symptoms of insufficient cerebral glucose are non-specific, including unsteadiness, light-headedness, tiredness and confusion (34,

35) – symptoms seen commonly in the general population (36-38), and particularly in elderly patients for many other reasons too (39, 40).

The symptoms most associated with hypoglycaemia have been reported (20, 32, 41, 42), including those particularly seen in the elderly (26). However, their non-specific nature, along with multiple alternative explanations, including possible co-morbidities, mean that hypoglycaemia may not be recognised. This study aimed to establish if patients at risk of hypoglycaemia present more to primary care with non-specific symptoms which may represent unrecognised episodes of hypoglycaemia.

METHOD

We performed a cross-sectional survey in one primary care practice (list size: ~11,000, ~3300 ≥65 years old) based in a small market town and with a large rural patient population. The practice's Egton Medical Information Systems (EMIS) database was used to identify all patients aged 65 or over who were treated with insulin (n=79), sulphonylureas (but not insulin) (n=85), or metformin only (n=121), and 50 age-matched non-diabetic patients.

One author (SH: a geriatrician) systematically reviewed patients' consultation notes over a one year period (5/2/12-4/2/13), to identify any episodes of hypoglycaemia (defined below), or any "hypo clue consultations" - consultations with non-specific symptoms known to be associated with hypoglycaemia (see below), where no other obvious explanation or subsequent diagnosis was recorded. The records were reviewed sequentially using the practice's internal computer number for each patient (essentially a random number). Review of the consultation records was performed independently of patient characteristics which were collected on a separate occasion: age, diabetes details, treatment, and glycated haemoglobin (HbA1c) blood test results.

Definition of hypoglycaemia

Hypoglycaemia episodes were defined as episodes having been directly confirmed by a doctor or nurse, paramedic or hospital (although the blood glucose was not always recorded).

Definition of "hypo clue" consultations

A "hypo clue consultation" was defined as one or more of the following symptoms recorded in the primary care records, without an obvious explanation or subsequent diagnosis documented – or documentation that hypoglycaemia had been considered. The symptoms (or synonyms) included were shivering, shaking, sweating, pounding heart/palpitations, lip tingling, dry mouth, apprehension, anxiety, agitation, confusion, odd behaviour, lethargy/fatigue, tiredness, drowsiness, weakness, speech difficulty, light-headedness, dizziness,

unsteadiness, incoordination, falls, feeling unwell, nausea, hunger, headache, double or blurred vision, unexplained waking, depression symptoms, difficulty concentrating, and memory complaints (26).

Analysis

The majority of the data was non-parametric; thus median results and interquartile ranges are presented, and chi^2/Fisher's exact tests used for comparing frequencies across groups and for the binary analyses of "at least one" hypoglycaemia episode or "hypo clue" consultation over the year by treatment group.

Frequency of presentation with individual "hypo clue" symptoms was assessed. Individual symptom frequencies were compared in patients who had, and those who had not had, a recognised episode of hypoglycaemia on another occasion, using chi^2/Fisher's exact tests.

The median HbA1c of those with/without at least one hypoglycaemia or "hypo clue" consultation per treatment group was compared using the Mann Whitney test.

Ethics

The research project was based on an initial audit within the practice, which did not require ethical permission.

RESULTS

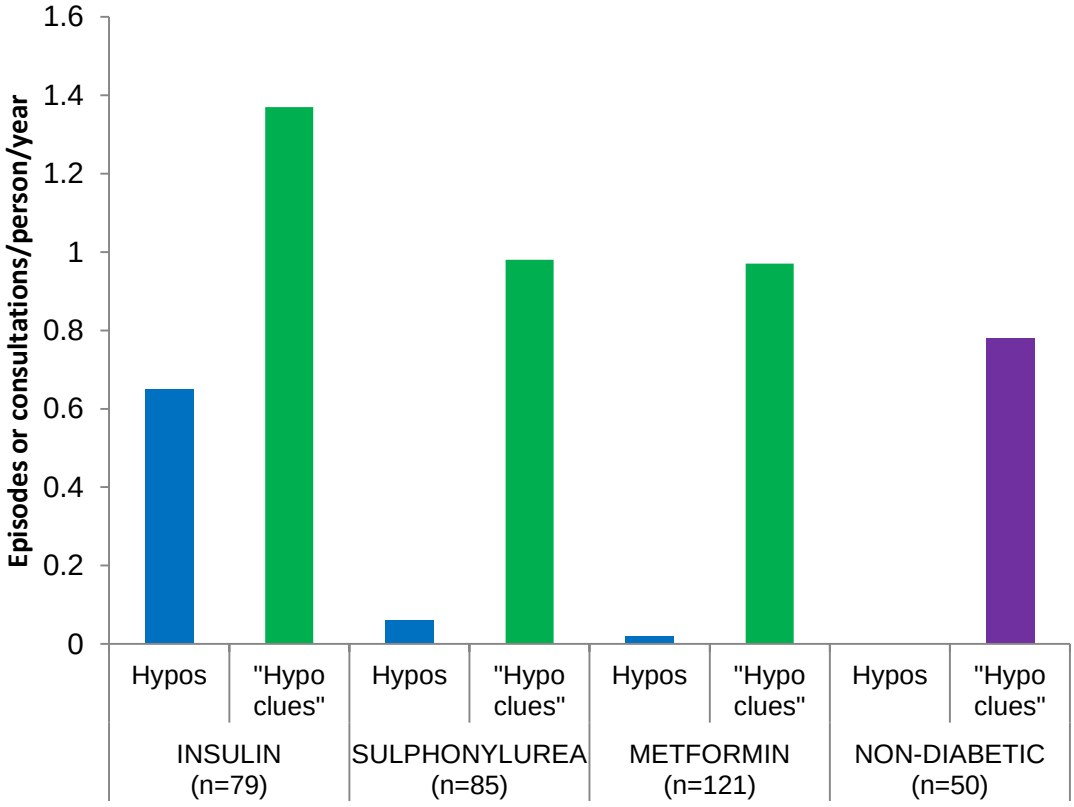
Frequency of hypoglycaemia

At least one episode of hypoglycaemia was recorded for 27/79 (34%) insulin-treated patients, compared to 4/85 (5%) sulphonylurea-treated patients, 2/121 (2%) metformin-only treated patients, and none in patients without diabetes. The total frequency was significantly higher in insulin-treated patients: 51 episodes (0.65 episodes/patient/year), compared to 5 episodes in the 85 patients with sulphonylureas (0.06 episodes/patient/year), and 3 in the 121 (0.02 episodes/patient/year) for the metformin-only treated patients, $p<0.001$, **Figure 1**.

Frequency of "hypo clue" consultations

Even patients without diabetes had frequent consultations with at least one non-specific symptom without other obvious documented explanation (feasibly due to hypoglycaemia in an at-risk patient), 0.78 consultations/patient/year (39 consultations in 50 patients). Rates of hypo-clue consultations were similar for all patients with diabetes, regardless of treatment (insulin 1.37, sulphonylureas 0.98, metformin 0.97 consultations/patient/year; $p=0.34$), **Figure 1**.

Figure 1: *Frequency of documented hypoglycaemia and "hypo clue" consultations (per person per year) according to treatment group, in patients ≥65 years. p<0.001 for a difference in rates of hypoglycaemia across the groups; p=0.34 for a difference in rates of "hypo clue" consultations.*



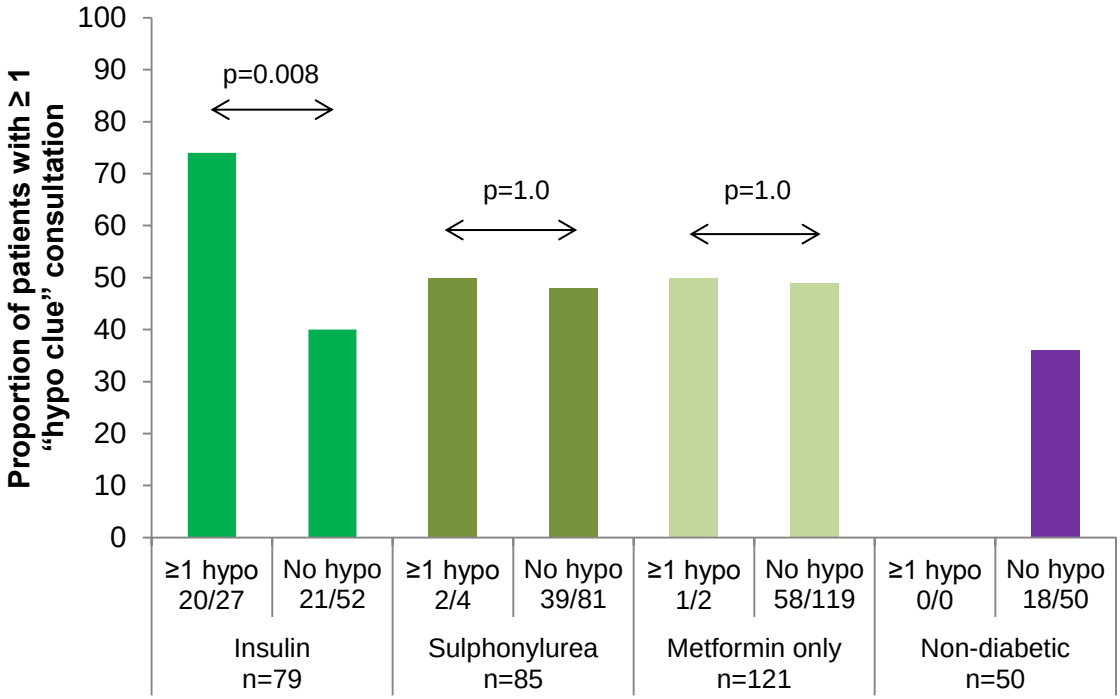
Reported symptoms in "hypo clue" consultations

The most commonly reported non-specific symptoms overall in this study, in decreasing order of frequency, were lethargy/tiredness (47/335, 14%), falls (46/335, 13.7%), feeling unwell (37/335, 11%), dizziness/light-headedness (35/335, 10.5%), depression symptoms (28/335, 8.4%), nausea (21/335, 6.3%), and unsteadiness (18/335, 5.4%).

Consultation with possible "hypo clue" symptoms in those with/without documented hypoglycaemia

In those patients who were insulin-treated and had at least one documented episode of documented hypoglycaemia over the year, 20/27 (74%) had presented on at least one other occasion with a "hypo clue" symptom, **Figure 2**. This was in comparison to 21/52 (40%) of those insulin-treated patients without a documented hypoglycaemia episode, p=0.008. In sulphonylurea and metformin treated patients with at least one document episode of hypoglycaemia over the year, 2/4 (50%) and 1/2 (50%) respectively had also presented at least once with possible "hypo clue" symptoms, with no difference in rates between those with or without documented hypoglycaemia, p=1.0. The odds ratio for insulin-treated patients having a hypoglycaemia episode if they had consulted on another occasion with a possible "hypo clue" symptom, was 4.2, compared to 1.1 in sulphonylurea or metformin only-treated patients.

Figure 2: *Proportion of patients who had at least one documented "hypo clue" consultation" over the year, and whether they had also had a documented episode of hypoglycaemia over the year*



Symptoms in "hypo clue" consultations in those with/without documented hypoglycaemia

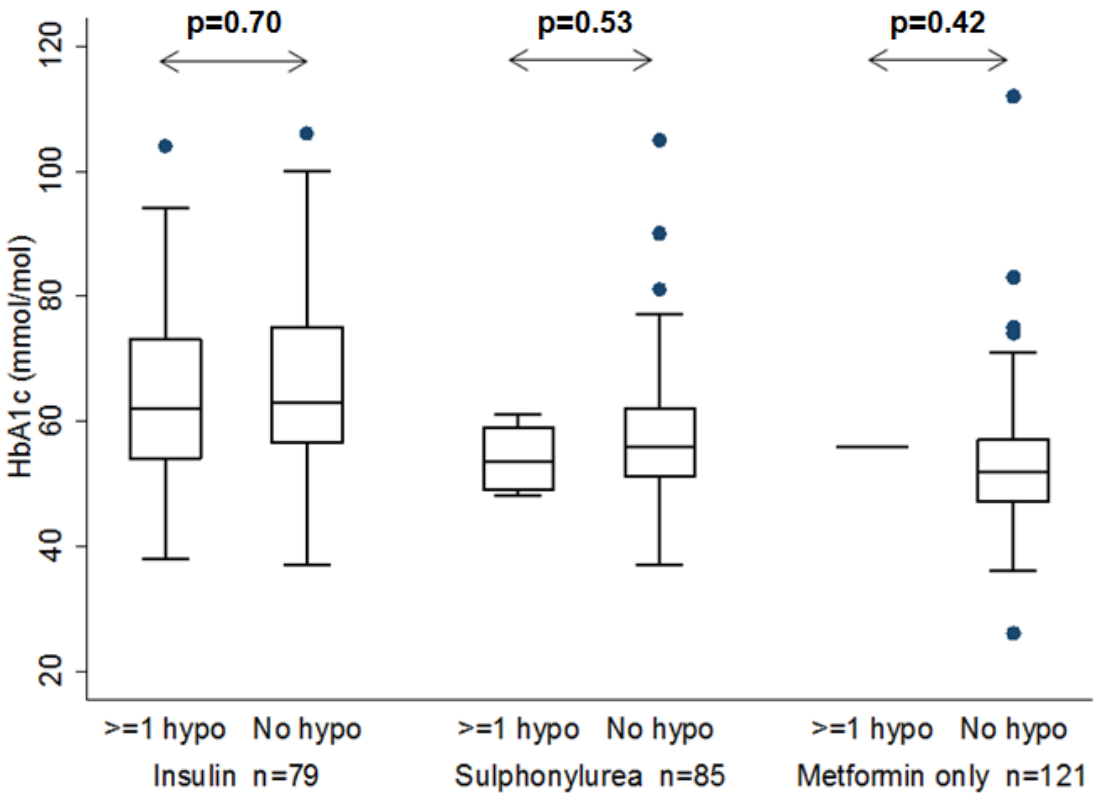
When the rates were compared overall in those with/without at least one episode of documented hypoglycaemia, the symptoms that were significantly more common were nausea (7/33, 21.2% vs 14/302, 4.6%, p=0.002), falls (10/33, 33.3% vs 36/302, 11.9%, p=0.007), unsteadiness (5/33, 15.2% vs 13/302, 4.3%, p=0.02), and depression symptoms (6/33, 18.2% vs 22/302, 7.3%, p=0.044).

The majority of patients (27/33, 81.8%) with at least one documented episode of hypoglycaemia were insulin-treated. Of these, 9/27 (33.3%) had presented on another occasion with a fall, compared to 4/52 (7.7%) insulin-treated patients without a documented episode of hypoglycaemia, p=0.008; and a higher proportion had presented with unsteadiness (5/27, 18.5% vs 2/52, 3.9%), p=0.043. Presentation with nausea was also more frequent in those insulin-treated patients with a recognised/reported episode of hypoglycaemia over the year: 6/27 (22.2%) vs 1/52 (1.9%), p=0.006.

Relationship with HbA1c

Hypoglycaemia was unrelated to HbA1c (p>0.4), **Figure 3**. There was also no relationship with "hypo clue" consultations and HbA1c, p>0.3 for all.

Figure 3: *HbA1c in those patients with or without a documented episode of "definite" hypoglycaemia*



DISCUSSION

Non-specific symptoms are a common presentation to primary care in patients over 65 with and without diabetes. However we have shown that patients at high risk of hypoglycaemia, i.e. patients over the age of 65 who are insulin-treated and have had a recognised episode of hypoglycaemia, present to primary care on other occasions with unexplained non-specific symptoms which may represent unrecognised hypoglycaemia. Falls, unsteadiness and nausea are particularly worth noting.

Strengths and limitations

This study examined a difficult question, namely that of whether additional episodes of hypoglycaemia might be being missed in the older population. The method used was systematic, although reliant on documentation – coding and free text - in primary care consultation records, as well as their interpretation during analysis. The study was performed in just one primary care practice, albeit a reasonably large one. This meant reliance on documentation by a limited number of staff; however this may also have offered more internal consistency for recording and comparing the vague symptoms between patients in this population. The similar rates of “hypo clue” consultations seen in sulphonylurea, metformin and non-diabetic patients is reassuring that the approach for identification of these consultations was reasonably consistent.

The “hypo clue” consultation definition used was deliberately all-embracing – hence high rates were seen in the patients without diabetes. Even so, more “hypo clue” consultations were seen in insulin-treated patients who had also had a documented episode of hypoglycaemia. Conceivably this rate could be artificially elevated as insulin-treated patients may consult more often than the other groups. However, we have tried to address this by presenting the results comparing those with and without episodes of hypoglycaemia within each treatment group.

The definition of hypoglycaemia, in contrast, was taken as a strict definition, i.e. only those episodes documented as having been confirmed in some way by a healthcare professional. This will certainly under-estimate the actual frequency of hypoglycaemia, and it may preferentially identify more “severe” episodes. This approach combined with the small size of the study may have limited the chance of identifying possible associations with those patients experiencing hypoglycaemia. In order to corroborate any relationships seen, a prospective study of possible “hypo clue” symptoms and hypoglycaemia could be undertaken.

Less “severe” episodes of hypoglycaemia (e.g. those which were self-treated and not reported to primary care) are also important: they may potentially pose a risk for development of reduced hypoglycaemia awareness and a subsequent more “severe” event, in addition to as yet under-appreciated possible effects e.g. on long-term cognition. These were not captured in the current analysis, but a study which also directly asked patients about their experience/frequency of hypoglycaemia may prove valuable. This could potentially be combined with a more intense but objective assessment of hypoglycaemia, e.g. using continuous glucose monitoring.

At the other end of the spectrum, further study in a bigger dataset could be revealing: e.g. an “index” event of hypoglycaemia taken and preceding consultations analysed to see if “hypo clue” consultations preceded a recognised event – and thus potentially expose more robust “red flag” symptoms – or corroborate those suggested in the current study. A larger study would also allow more sophisticated analyses to be done, in particular corrections for factors which may have an impact on risk, such as age (11, 43), comorbidities (43, 44), and renal function (11). In addition, insulin-treated patients in the current study comprise a heterogeneous group – i.e. some with long-standing Type 1 diabetes, and others with Type 2 diabetes and more recent initiation of insulin treatment. However although these patients may have different rates of presentation with hypoglycaemia or “hypo clues”, the underlying type of diabetes in clinical care is not always clearly defined, and

thus an all-encompassing “insulin-treated” group was felt to be a more useful analysis in the current study.

Finally, HbA1c analysis was limited as it was based on a single HbA1c level from the year; and therefore does not reflect potential variation (and possible altered risks) over the year. No apparent relationship with hypoglycaemia or “hypo clues” was seen in the current study. This particular practice had been subject to a similar audit previously, and thus it is possible that the frequency of patients with very low HbA1c was lower than average.

Comparison with previous literature

Consistent with published literature, we found that documented hypoglycaemia is more frequent in insulin-treated patients, and the finding that 34% of insulin-treated patients had a “definite” episode of hypoglycaemia confirmed by a healthcare professional over the year is consistent with the 7-46% in insulin-treated patients of different durations in the UK Hypoglycaemia Study (6): as previously mentioned, the insulin-treated patients in the current study comprised a heterogeneous group. 5% of sulphonylurea-treated patients having an episode of hypoglycaemia is also consistent with the 7% seen in the UK Hypoglycaemia Study (6).

18/50 (36%) of patients without diabetes had a “hypo clue” consultation by our definition. As previously discussed, this was an all-embracing definition, which included many non-specific symptoms frequently presenting to primary care. Although not directly comparable, other primary care studies have found 22-48% patients presenting with symptoms which could not be given a same-day diagnosis (36).

Regarding presentation with non-specific symptoms, lethargy/fatigue, feeling “generally unwell”, falls, and light-headedness/dizziness were the most frequently reported, each in over 10% of these patients aged $\geq$65. However falls and unsteadiness, along with nausea, were reported significantly more frequently in those who had also had (on another occasion) a hypoglycaemia episode. Overall 21% of those with at least one episode of documented

hypoglycaemia over the year had attended on another occasion with nausea without a documented diagnosis, in comparison to 5% of those without an episode of hypoglycaemia – and 22% vs 2% in those who were insulin-treated.

It is perhaps not surprising falls were one of the most frequently presenting symptoms, as they are one of the most dramatic. However the difference of 30% vs 12% (or 33% vs 8% of insulin-treated) patients presenting with a fall in those who had/had not also had a documented hypoglycaemic episode on a different occasion is marked. Kachroo et al (45) identified in a study of over 21,000 patients with Type 2 diabetes, those who had experienced a documented episode of hypoglycaemia over a one-year period, had an increased risk of fall-related events compared with an age and gender-matched group of patients with Type 2 diabetes without hypoglycaemia. Those $\geq$75 had an adjusted odds ratio (aOR) for a fall-related event of 1.77 (95% CI 1.48-2.12), and those under 75 an aOR of 2.20 (95% CI 1.77-2.12). The greatest risk was seen within the first 30 days after a fall (aOR 5.86, 95% CI 4.08-8.43) – and increased risk seen in patients with recurrent episodes of hypoglycaemia.

Recognised symptoms associated with hypoglycaemia which could predispose to falls include shakiness, anxiety, confusion, lethargy/fatigue, tiredness, drowsiness, weakness, light-headedness, dizziness, unsteadiness, incoordination, and double or blurred vision (10). The finding that unsteadiness was the other most notable discriminatory symptom may be consistent with this: 15% vs 4% (or 19% vs 4% of insulin-treated) patients with/without hypoglycaemia on another occasion presented with unsteadiness. When originally reviewing the symptoms associated with hypoglycaemia in the elderly in comparison to younger adults, Jaap et al identified that unsteadiness and light-headedness were amongst the most frequently occurring and intense (26). This study was done by asking 102 insulin-treated patients with Type 2 diabetes who had experienced hypoglycaemia in the preceding 2 months their subjective experience of the presence of 22 symptoms of hypoglycaemia during a ‘typical’ hypoglycaemic episode. Falls were not given as an option in this study, and interestingly nausea had a low frequency (6%).

In contrast to the current study, a large meta-analysis showed a 30% increase in severe hypoglycaemia with tight glycaemic control in people with Type 2 diabetes (4). The apparent lack of relationship in the current study may reflect the low rates of “definite” hypoglycaemia documented, and at the other end of the spectrum, the all-embracing definition for “hypo clue” consultations used in this relatively small study may have masked results.

Clinical implications

Patients over the age of 65 and who are insulin-treated are at the highest risk of hypoglycaemia, as will be well-recognised and documented by primary care practitioners. However, hypoglycaemia in older adults is associated with non-specific and less intense symptoms than in younger people (25-27). It is known to be under-reported to healthcare professionals (30, 46), which can be due to a failure to appreciate its significance, or poor recognition (28, 29) perhaps particularly in those with Type 2 diabetes, who may not have had education to go with the increased risk of hypoglycaemia with insulin (6), or with increasing duration of diabetes (20). Symptoms can differ between episodes in the same person, which can make recognition especially challenging (31). Additionally episodes of hypoglycaemia can be poorly recalled by patients (47, 48), which may be exacerbated by cognitive impairment. There may also be a fear of its implications such as relating to driving (46). However, as previously discussed, it carries a high morbidity (35). This means healthcare professionals need to take a more pro-active approach in enquiring about hypoglycaemia.

This study suggests those who have had a recognised episode of hypoglycaemia seem more likely to present on another occasion with a non-specific symptom which could conceivably be due to hypoglycaemia, and nausea, falls and unsteadiness seem to be particularly notable. The likelihood of this is corroborated by other published data, and as such, insulin-treated patients presenting with these symptoms should be reviewed with hypoglycaemia in mind.

More recent guidelines for older adults (22-24) favour a more common-sense approach in actively addressing glycaemic targets, particularly in a more elderly

and frail population, who can ill-afford to be exposed to risk factors for accidents (8-10) and cognitive decline (15-17). As previously discussed the current study did not show a clear relationship between hypoglycaemia or “hypo clue” consultations and HbA1c, but much larger meta-analyses have (49) – and additionally increased all-cause mortality has been observed with HbA1c results below 7.5% (50). On the other hand, avoidance of hypoglycaemia is not as simple as relaxing HbA1c targets – Munshi et al (51) demonstrated using continuous glucose monitoring that 65% of a group of (mainly insulin-treated) elderly patients with HbA1c >8% experienced at least one episode of hypoglycaemia (blood glucose <3.9mmol/L) over 3 days’ monitoring.

At the very least however, clinicians should be alert to the possibility of unrecognised hypoglycaemia in their older insulin-treated patients, and review them with this in mind.

CONCLUSION

Non-specific symptoms which can be symptoms of hypoglycaemia are common in a population over 65. However in insulin-treated patients at risk of hypoglycaemia, these “hypo clue” symptoms, in particular nausea, falls and unsteadiness, may represent episodes of hypoglycaemia not recognised by the patient. Thus GPs should consider a review, including of diabetes medication, when patients report or present with these symptoms.

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CHAPTER 3

Assessment of Practical Classification Guidelines for Diabetes in insulin-treated patients

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CHAPTER 3

Acknowledgments of co-authors and contributions to paper

Andrew Hattersley and Kamlesh Khunti designed the study. Maggie Shepherd and Bea Knight wrote the study protocol and obtained ethical approval. Maggie Shepherd, Bea Knight and myself recruited patients in the Exeter region. Charles Fox co-ordinated recruitment in Northampton, and Kamlesh Khunti in Leicestershire. Bev Shields, Sophie King and I performed statistical analysis on the results, and all authors contributed to interpretation of the results and planning the paper. Sophie Wienand-Barnett, Sophie King and I co-wrote the early version of the manuscript. Bev Shields and I updated the analysis and completed the manuscript, which all co-authors reviewed and contributed to. Post-submission to the British Journal of General Practice I did the final editing to incorporate reviewers' comments, with help from Bev Shields.

Assessment of Practical Classification Guidelines for Diabetes in insulin-treated patients

Abstract

Background

Differentiating between Type 1(T1D) and Type 2 diabetes (T2D) is fundamental for appropriate treatment and management of patients, but can be challenging, especially when patients are insulin-treated. UK Practical Classification Guidelines (using age at diagnosis and time to insulin treatment) were developed, but their accuracy has not been assessed.

Aim

To assess the diagnostic accuracy of the UK guidelines against “gold-standard” definitions of T1D and T2D based on measured C-peptide levels.

Design & Setting

601 adults with insulin-treated diabetes and diabetes duration ≥5years were recruited in Devon, Northamptonshire & Leicestershire.

Method

Baseline information and a home urine sample for urinary C-peptide creatinine ratio (UCPCR, a measure of endogenous insulin production) were collected. “Gold-standard” T1D was defined as continuous insulin treatment within 3 years of diagnosis and absolute insulin deficiency (UCPCR<0.2nmol/mmol ≥5years post-diagnosis); all other patients classed as T2D. Diagnostic performance of the clinical criteria assessed and other criteria explored using ROC curves.

Results

UK guidelines correctly classified 86% of participants.

Most misclassifications occurred in patients classed as T1D who had significant endogenous insulin levels (57/601; 9%); the majority in those diagnosed ≥35y and treated with insulin from diagnosis(37/66;56% misclassified).

Time to insulin and age at diagnosis performed best in predicting long-term endogenous insulin production (ROC AUC=0.904 and 0.871); BMI at diagnosis was a less strong predictor of diabetes type (AUC=0.824).

Conclusion

Current UK guidelines provide a pragmatic clinical approach to classification that reflects long-term endogenous insulin production; caution is needed in older patients commencing insulin from diagnosis, where misclassification rates are increased.

Introduction

Correctly classifying patients with diabetes with Type 1 or 2 is fundamental to ensuring they receive correct management(1-3). In clinical practice this can be challenging, with 7-15% patients misclassified, and large variations in practice (4-7).

Historical lack of clear clinical guidelines for diabetes classification is likely to have contributed to this variation. International guidelines from WHO(8) and ADA(9) base classification on underlying aetiology, with Type 1 described as a destruction of beta cells leading to absolute insulin deficiency. However these guidelines do not provide clear criteria or classification pathways for clinical use (8, 9).

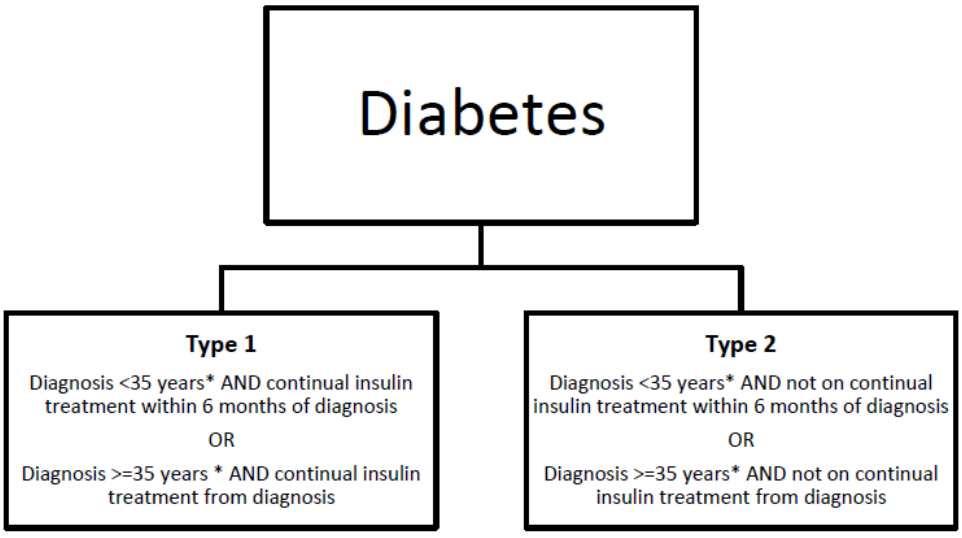
A pragmatic classification algorithm was thus developed in 2010 by key diabetes stakeholders in the UK, and published by the Royal College of General Practitioners (RCGP) and NHS Diabetes in their Coding, Classification and Diagnosis of Diabetes document(4), Figure 1. This uses age at diagnosis and time to commencing insulin treatment from diagnosis as its diagnostic criteria. The efficacy of this algorithm has not yet been tested on a large cohort of patients with diabetes.

The fundamental difference between Type 1 and Type 2 diabetes is the rapid development of absolute insulin deficiency in Type 1, forming the basis of their different treatment and management. Patients with Type 1 require accurate insulin dose replacement(10, 11); patients with Type 2 continue to produce substantial amounts of their own insulin, responding to non-insulin therapy, or if insulin is needed good control can be achieved with non-physiological insulin regimes(12, 13). Measuring endogenous insulin secretion (using C-peptide, a component of the insulin pro-hormone secreted in equimolar amounts to insulin) in longstanding diabetes may be a useful “gold standard” marker of endogenous insulin production, confirming a diagnosis of Type 1 versus Type 2 diabetes. Development of the spot urine test urinary C-peptide creatinine ratio

(UCPCR)(14-17) has enabled practical testing in a community setting. UCPCR is well-correlated with mixed meal tolerance test measures(16, 17), and a UCPCR cut-off of 0.2nmol/mmol gives a sensitivity and specificity of 100% and >95% for detecting severe insulin deficiency(16, 17) as defined by the gold-standard mixed meal test 90-minute C-peptide level of 200pmol/L(18).

We thus aimed to determine the reliability of the 2010 UK Practical Classification Guidelines(4) to correctly classify diabetes in a large cohort of insulin-treated participants against "gold-standard" classification based on measurement of C-peptide, in those with diabetes of ≥5 years' duration. Although UCPCR can be used at any stage in diabetes to confirm endogenous insulin levels, in the current study we chose ≥5 years' duration in order to avoid misclassifying people with early Type 1 who may have been still producing their own insulin.

Figure 1: *UK Practical Classification Guidelines for Diabetes (extract showing classification guidelines for Type 1 and Type 2 diabetes)*



Methods

Subjects

Adults with insulin-treated diabetes in 3 UK centres (Devon, Northamptonshire & Leicestershire) were invited to participate when attending for routine diabetes appointments (in primary and secondary care). 601 white Caucasian and 30 Asian patients with a duration of diabetes ≥5years provided data on age at diagnosis, weight at diagnosis, current age, weight and height, treatment, and time to insulin from diagnosis. BMI at diagnosis and recruitment were calculated where possible; weight at diagnosis for those diagnosed as children converted to the adult equivalent using the UK Child Growth Reference Standards(19).

Participants were asked to collect a urine sample for UCPCR(14) two hours after their largest meal of a day, and return by post for analysis in the Exeter Biochemistry laboratory.

Classification of Diabetes

Participants were classified as having Type 1 or Type 2 diabetes using the UK guidelines(4), Figure 1. We developed "gold-standard" criteria:

- Type 1 diabetes: continuous insulin treatment within the first 3 years of diagnosis and absolute insulin deficiency (UCPCR<0.2 nmol/mmol ≥5 years post-diagnosis)(16)
- Type 2 diabetes: if Type 1 diabetes criteria were not met

Statistical analysis

Proportions of patients correctly classified by the UK guidelines according to the "gold standard" C-peptide-based definition were calculated, and differences in clinical characteristics between those correctly and incorrectly categorised were explored using the Mann-Whitney test.

Diagnostic performance of continuous variables (age at diagnosis, time to insulin, BMI at diagnosis and recruitment) was assessed using receiver

operating characteristic (ROC) curves. Optimal cut-offs for these variables (with maximum specificity and sensitivity for discrimination) were calculated, and we explored whether use of these optimal cut-offs led to improvements in classification over and above the RCGP algorithm using net reclassification improvement(20).

Detailed subgroup analysis could not be carried out on the Asian patients due to small numbers.

Analysis was carried out on Stata version 13.1 and R version 3.1.2.

Results

We compared the UK clinical classification criteria with "gold-standard" C-peptide-based criteria for defining Type 1 and Type 2 diabetes in this cohort of 601 patients (Figures 2&3). Table 1 shows participant characteristics.

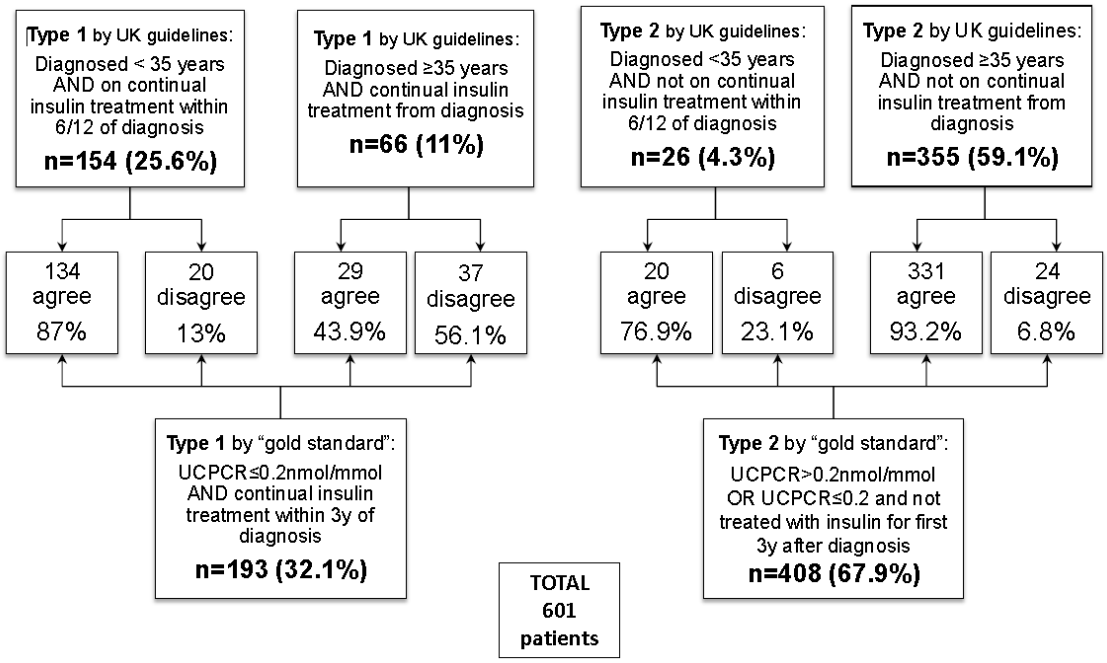
Table 1: *Participant characteristics: median (interquartile range)*

	Overall	Gold standard Type 1	UK guidelines Type 1	Gold standard Type 2	UK guidelines Type 2
Age at recruitment (yrs)	64 (53-73)	54 (41-64)	53 (41-64)	68 (60-74)	68 (61-75)
Gender (% male)	58.2	48.7	52.7	62.8	61.4
Recruitment BMI (kg/m2)	28.7 (25.3-33.3)	26.5 (23.1-29.3)	26.8 23.8-29.7)	29.7 (26.6-34.5)	30 (26.6-34.1)
Age at diagnosis (yrs)	45 (30-56)	24 (12-36)	25 (13-39)	50 (42-59)	50 (43-58)
BMI at diagnosis (kg/m2)	27 (23.9-32)	22 (19.4-26.3)	22.9 (20-27.6)	28.4 (25.6-33.8)	28.3 (25.2-33.6)
Latest HbA1c (%)	8 (7.3-8.8)	8.1 (7.4-8.9)	8 (7.3-8.9)	7.9 (7.2-8.8)	7.9 (7.3-8.8)
Insulin dose/kg/24 hours	0.64 (0.44-0.9)	0.61 (0.5-0.84)	0.61 (0.49-0.88)	0.65 (0.42-0.93)	0.64 (0.43-0.92)
UCPCR (nmmol/mmol)	0.6 (0.03-1.6)	0.019 (0.019-0.03)	0.019 (0.019-0.22)	1.19 (0.59-2.25)	1.1 (0.4-2.1)

UK guidelines correctly classify 86% of insulin-treated patients ≥5 years post-diagnosis

514/601 (86%, 95% confidence interval, CI, 83-88%) of patients overall were correctly classified by the UK guidelines when compared with our "gold-standard" criteria: 163/193 (84%, 95% CI 79-89%) with Type 1, and 351/408 (86%, 95% CI 82-89%) with Type 2 (Figure 2). In the Asian group the criteria (taking note of the age cut-off of 30 years for high risk racial groups) performed less well classifying only 21/30 (70%) correctly (p=0.02 for comparison with white Caucasians).

Figure 2: *Classification of type of diabetes according to UK guidelines' clinical criteria compared to "gold-standard" C-peptide-based criteria*



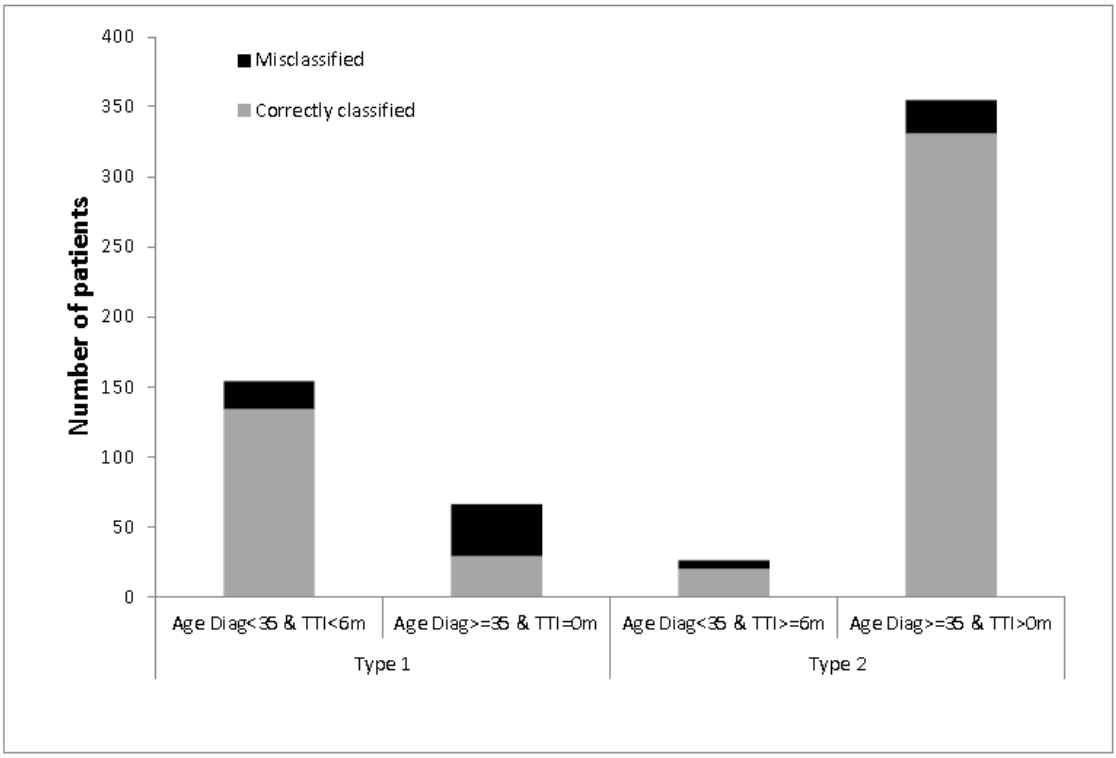
Most misclassifications were in patients classified as having Type 1 diabetes according the UK guidelines

Of patients misclassified by the UK guidelines' clinical criteria in comparison to our C-peptide derived "gold-standard" criteria, the majority, 57/87(66%) were misclassified as having Type 1 diabetes but were still producing substantial endogenous insulin ≥5 years post-diagnosis. 30/87(34%) were misclassified as having Type 2 (but were severely insulin-deficient and had started insulin treatment within 3 years of diagnosis). The majority of misclassifications (8/9) in the Asian group were also cases where the UK guidelines' criteria suggested Type 1 (NB UK guidelines age cut-off 30) but the patients were still producing their own insulin.

The majority of misclassified patients with Type 1 diabetes were diagnosed aged ≥35 years, and went immediately onto insulin

By UK guidelines these 66 patients had Type 1 diabetes, but 37/66(56%) had a UCPCR >0.2nmol/mmol, and thus by "gold-standard" criteria had Type 2 diabetes.

Figure 3: *Proportion of patients classified as Type 1 or Type 2 diabetes according to the UK guidelines (Figures 1 & 2). Grey bars: proportion whose classification is correct according to the C-peptide–derived "gold standard" definition; black bars: proportion misclassified. Age diag - age at diagnosis, TTI - time to insulin treatment from diagnosis*



Those misclassified as having Type 1 had clinical characteristics consistent with Type 2; those misclassified as having Type 2 had clinical characteristics more consistent with Type 1

Those misclassified as having Type 1 diabetes were older than those correctly classified (median age (IQR) 44 (30-59) vs 20 (11-30), p<0.001), and had a higher BMI at diagnosis (26.4kg/m^2 (23-30.3) vs 21.8(18.9-25.4), p=0.002).

In contrast, those who were insulin deficient but were incorrectly classified by the UK guidelines as having Type 2 diabetes, went onto insulin more quickly than those correctly classified as having Type 2 (time to insulin from diagnosis 12 months(2-18) vs 84 months(42-138), p<0.001), had lower BMI (22.5kg/m^2 (21.1-26.3) vs 28.1(25.4-33.3), p<0.001), and were younger at diagnosis (44y(35-56) vs 51(43-59), p=0.014).

Assessment of optimal clinical criteria for differentiating Type 1 and Type 2 diabetes

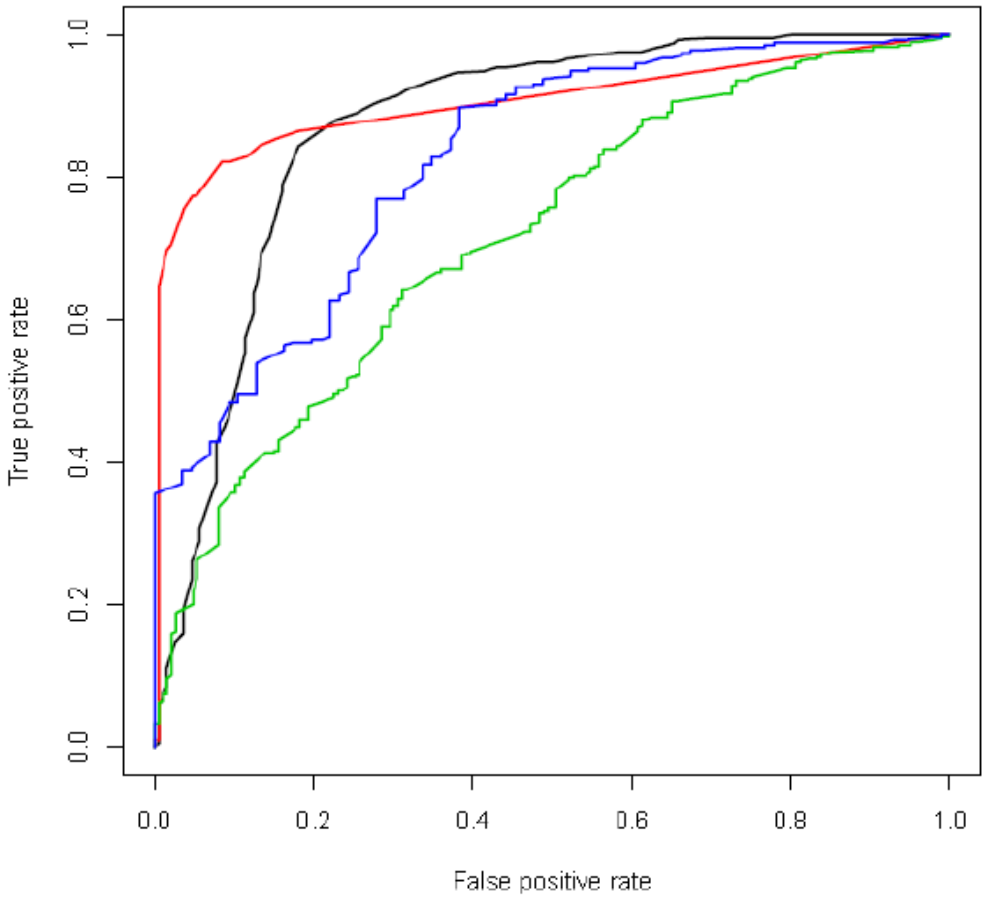
We used ROC curves (Figure 4) to examine the discriminative ability of key clinical criteria (time to insulin, age at diagnosis, BMI at diagnosis, and BMI at recruitment) and to identify the best cut-offs for classification based on the "gold-standard" criteria. An area under the curve (AUC) equal to 1 represents the perfect discrimination between types of diabetes, and an AUC>0.8 is generally deemed clinically useful.

The most discriminatory individual characteristic (Figure 4) was months from diagnosis to insulin treatment (AUC 0.904, 95% CI 0.88-0.93), with the optimal cut-off at 12 months, classifying 91.5% patients correctly as having Type 1 and 82.1% correctly as Type 2.

Age at diagnosis was also a useful discriminator between Type 1 and Type 2 diabetes (AUC 0.871, 95% CI 0.84-0.9), with the optimal cut-off being ≤39y for Type 1. This correctly classified 81.9% of patients with Type 1 and 84.3% of those with Type 2 diabetes.

BMI at diagnosis gave an AUC of 0.824 (95% CI 0.77-0.87; data available in 359/601(59.7%) patients), with the optimal cut-off being $\leq$23.1kg/m^2. However, although this correctly classified 89.4% of those with Type 2 diabetes, it only classified 65.7% patients with Type 1 correctly. BMI at recruitment was less discriminatory again, giving an AUC of 0.715(95% CI 0.67-0.76) and an optimal cut-off of 28.0kg/m$^{2.}$ This correctly classified just 66.8% people with Type 2 diabetes, and 61.8% people with Type 1.

Figure 4: *ROC curve for discriminating between Type 1 and Type 2 diabetes based on the gold standard definition. Red: time to insulin from diagnosis (AUC=0.904); black: age at diagnosis (AUC=0.871); blue: BMI at diagnosis (AUC=0.824); green: BMI at recruitment (AUC=0.715)*



Modifying the UK guidelines' clinical criteria only results in marginal improvements in accuracy

The UK guidelines use age at diagnosis and time to insulin as the classification criteria for differentiating between Type 1 and 2 diabetes, with 84.5% correctly classified with Type 1, and 86% as Type 2, compared to the gold-standard. On the basis of the ROC curve data, we incorporated the optimal cut-offs for time to insulin (12 months), age at diagnosis (39), BMI at diagnosis (23.1kg/m^2) and recruitment (28.0kg/m^2) into modified criteria in various combinations, to see if these improved diagnostic accuracy. Aiming for a sensitivity and specificity of >80% (equivalent to an ROC AUC of >0.8), none were superior to the UK guidelines, as improvements in sensitivity led to greater decreases in specificity and vice versa. The best performing alternative was the combination of age cut-off of 39 and time to insulin of 12 months; this improved correct classification of those with Type 2 diabetes to 94%, but reduced to 78.3% those correctly classified with Type 1 diabetes. In general, adding BMI at diagnosis/recruitment improved the proportion of those with Type 2 correctly classified, but markedly reduced the proportion correctly classified with Type 1 diabetes.

Discussion

The UK guidelines are an accurate method of predicting long-term endogenous insulin production

Our results show the UK guidelines perform well in correctly classifying those with insulin-treated diabetes based on the development of absolute insulin deficiency, with 86% agreeing with a "gold-standard" based on endogenous insulin levels and time to insulin from diagnosis. This supports their use as a useful pragmatic way of classifying patients. When all patients with diabetes are considered, the performance of the UK guidelines will be even better as the vast majority of patients who are not insulin-treated will be correctly classified as having Type 2 diabetes.

Patients diagnosed at an older age (≥35 years) with insulin treatment commenced at diagnosis are at the highest risk of being misclassified when using the UK guidelines

The majority of classification errors occur when using the UK criteria to define Type 1 diabetes in participants diagnosed ≥35 years and on insulin treatment from diagnosis. Clinically, where the subtype of diabetes is unclear, giving insulin from diagnosis is a rational decision to avoid the potential consequences of untreated Type 1 diabetes such as ketoacidosis. This study demonstrates that the majority of these patients are likely to have Type 2 (and therefore may potentially not require insulin), so revisiting the diagnosis following an acute presentation may be worthwhile.

Time to insulin from diagnosis and age at diagnosis are the best predictors of long-term endogenous insulin production

In clinical practice, emphasis is often placed on BMI to help in differentiating between Type 1 and Type 2 diabetes. Our data suggests that amongst insulin-treated patients, time to insulin and age at diagnosis are better predictors of diabetes subtype than BMI, with ROC AUCs of 0.904 and 0.871 respectively, and 0.824 for BMI at diagnosis. Median BMI at diagnosis of those with Type 1 by our "gold-standard" criteria was lower than those with Type 2 diabetes -

21.8kg/m^2 vs 28.1kg/m^2 ($p<0.001$), but the interquartile ranges overlapped (19.8-26.3 and 25.4-32.9kg/m^2). By time of recruitment (ie $\geq$5 years from diagnosis), the difference in BMI between those with Type 1 and 2 was smaller: 26.5kg/m^2 (23.1-29.3) vs 29.7 (26.6-34.5), although still significant ($p<0.001$), and the ROC AUC was low (0.715), highlighting the reduced discriminative ability of this as a clinical marker to differentiate between Type 1 and 2 diabetes once on insulin.

Strengths and limitations

These are the only pragmatic clinical guidelines produced by clinical bodies for the classification of T1D and T2D, and to our knowledge this is the first assessment of them in comparison to a C-peptide based gold-standard, rather than coding errors (4, 6, 21, 22). We studied insulin-treated patients with a duration of $\geq$5 years. If considering all patients with diabetes the misclassification rate of 14% is likely to be significantly lower: patients tablet or diet-treated $\geq$5y from diagnosis are likely to have been correctly diagnosed with Type 2 diabetes. In patients with a diabetes duration of <5y, a few patients with Type 1 may be still producing insulin (the “honeymoon period”) and not yet insulin-treated; however it is rare for patients with Type 1 diabetes to treated without insulin for prolonged periods.

Due to recruitment locations and difficulty in recruiting Asian patients (23), the majority of our recruited patients were white Caucasian, with only 30 Asian patients studied. We thus cannot comment on these criteria for high prevalence populations and further work is needed in these groups.

We had limited data on BMI at diagnosis (available for 60% participants), which could be improved in future prospective study. Age and gender could be considered in more detail in any future (larger) classification studies. It would be interesting to follow up those identified as misclassified, and those diagnosed with Type 2 and still producing insulin beyond 5 years, to find out if some might be able to withdraw successfully from insulin.

We have concentrated on the two main types of diabetes, but recognise there are alternative subgroups such as genetic forms of diabetes (e.g. MODY). These are rare but also part of the UK guidelines(4), and have their own criteria for diagnosis(24). It is important the clinician takes into account other factors that may indicate these. The term latent autoimmune diabetes in adults (LADA) is sometimes proposed for adults with islet autoantibodies who eventually (up to 12 years) become severely insulin-deficient, but do not require insulin for at least the first 6 months (25-28). However LADA is not included in international guidelines for classification or treatment, and given endogenous insulin status determines treatment requirements, we feel it appropriate to classify according to UCPCR status as per our “gold-standard” criteria.

Our gold-standard criteria used a UCPCR cut-off of 0.2nmol/mmol, which has a sensitivity and specificity of 100% and >95% to detect absolute insulin deficiency (16, 29). It is the best “gold-standard” we have in this context, being practical for use in large numbers of community-dwelling adults. Insulin treatment has the potential to suppress endogenous insulin (30-32), but we have shown this rarely affects diabetes classification (32) – and the small possibility of an over-diagnosis of Type 1 diabetes is a safer direction of error than the opposite.

Comparisons with existing literature

Previous reports on “misclassification” of diabetes(4, 6, 21, 22) were mainly based on contraindications in coding rather than on gold standard definitions of insulin deficiency(18, 33, 34).

A recently published systematic review systematically identified diagnostic accuracy studies in the literature which compared clinical criteria with C-peptide cut-offs(7). Age at diagnosis, time to insulin, and BMI are the clinical characteristics most frequently used to classify Type 1 and 2 diabetes, but few studies have addressed clearly which are most strongly associated with long-term C-peptide secretion(7). Where strength of association has been measured, time to insulin and age at diagnosis appear stronger than BMI. Combinations of the former two improve diagnostic accuracy, with BMI adding little(7).

Implications for clinical practice

Correct classification of Type 1 or Type 2 diabetes is important so the appropriate treatment and management guidelines are followed(3, 35), to include treatment, education (eg DAFNE for those with Type 1), and monitoring of complications – all of which are based on the presence or absence of endogenous insulin.

The clinical problem facing GPs and other healthcare professionals is that classification can be tricky at diagnosis – and all guidelines, including these UK classification guidelines, rely on information available further down the line (eg time to insulin). The gold-standard classification using UCPCR at/beyond 5 years from diagnosis by definition cannot completely solve this conundrum: UCPCR>0.2nmol/mol <5 years from diagnosis may represent someone with Type 1 diabetes still in the "honeymoon" phase, or someone with Type 2 diabetes. UCPCR <0.2nmol/mmol within 5 years of diagnosis can diagnose Type 1 diabetes however. Studies designed to improve classification at diagnosis, eg by using islet antibodies, are needed to address this problem.

We have shown that the UK guidelines based on time to insulin and age at diagnosis are accurate and pragmatic for classifying patients with diabetes. "Time to insulin" is subject to many influences - physician or patient factors, or guidelines for treatment in a particular area/patient population – but the high rate of correlation of diagnosis with the gold-standard suggests overall timing of insulin initiation may be reasonably consistent. However it is important to revisit the diabetes diagnosis particularly in those diagnosed >35 years of age, given the high rates of misclassification seen in this category of patients. We suggest if there is diagnostic uncertainty, a review of diagnosis is made, specialist advice sought and further investigations (eg C-peptide and islet autoantibodies) be considered.

We did not find that modification of the criteria used or the cut-offs proposed would improve their diagnostic performance. Our study, like others (7), suggest age of diagnosis is a better clinical predictor of Type 1 diabetes than BMI which

is often used clinically to determine diabetes subtype in intermediate patients - supporting that more emphasis should be placed on age of diagnosis in uncertain cases. This is perhaps particularly relevant in a time when the average population BMI is ever increasing.

Conclusion

Our study demonstrates that the UK Practical Classification Guidelines for Diabetes are an accurate means for determining diabetes subtype, with time to insulin and age at diagnosis being the most discriminatory clinical characteristics. Older patients treated with insulin from diagnosis had the highest rate of misclassification (56% classed incorrectly as having Type 1), and further investigation should be considered in this subgroup.

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CHAPTER 4

Urinary C-peptide creatinine ratio detects absolute insulin deficiency in Type 2 diabetes

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CHAPTER 4

Acknowledgments of co-authors and contributions to paper

The original concept of measuring C-peptide by UCPCR in a large community study was conceived by the team, in particular Tim McDonald and Andrew Hattersley. Maggie Shepherd, Bea Knight, Angus Jones and Rachel Besser wrote the study protocols and obtained ethical approvals. Maggie Shepherd, Bea Knight, Emily Goodchild and myself recruited patients for the UCPCR screening part of the study, and I recruited further for the mixed meal test part of the study. Emily Goodchild and I performed the mixed meal tests. All co-authors contributed to discussions regarding results and planning and reviewing the final manuscript. Please note a preliminary version of this work contributed to the final thesis for my MSc in Diabetes in 2011, from Queen Margaret University, Edinburgh.

Urinary C-peptide creatinine ratio detects absolute insulin deficiency in Type 2 diabetes

Abstract

Aims

It is unclear whether progressive beta-cell failure in type 2 diabetes (T2DM) can result in absolute insulin deficiency, which would cause increased risk of hypoglycaemia and ketoacidosis, as in T1D. We aimed to determine the prevalence and clinical characteristics of absolute insulin deficiency in longstanding T2DM, using a strategy based on home urinary C-peptide creatinine ratio (UCPCR) measurement.

Methods

2-hour post-home meal UCPCR was assessed in 191 insulin-treated participants with T2DM (diagnosis age ≥45, no insulin in the first year). Where initial UCPCR was≤0.2nmol/mmol (representing absolute insulin deficiency) it was repeated. A standardized mixed-meal tolerance test (MMTT) with 90-minute stimulated serum C-peptide (sSCP) measurement was performed in 9 subjects with UCPCR≤0.2nmol/mmol (and 9 controls with UCPCR>0.2nmol/mmol) to confirm absolute insulin deficiency.

Results

2.7% of participants had absolute insulin deficiency confirmed by MMTT. They were identified initially using UCPCR: 11/191(5.8%) had two consistent UCPCRs≤0.2nmol/mmol; 9/11 completed a MMTT and had a median sSCP of 0.18nmol/L. 5/9 had sSCP<0.2nmol/L. 9/9 participants with UCPCR>0.2 had confirmed endogenous insulin secretion in MMTT.

Compared to participants with UCPCR>0.2, those with confirmed absolute insulin deficiency had shorter time to insulin (median 2.5v6years,p=0.005) and lower BMIs (25.1v29.1kg/m^2, p=0.04). 2/5 were GAD autoantibody positive.

Conclusions

Absolute insulin deficiency may occur in long-standing T2DM, and cannot be reliably predicted by clinical features or autoantibodies. Its recognition should help guide treatment, education and management. UCPCR is a practical non-invasive method to aid detection of absolute insulin deficiency, with UCPCR>0.2nmol/mmol being a reliable indicator of retained endogenous insulin secretion.

Introduction

Most older patients with diabetes have type 2 diabetes (T2DM), which is typically a disease where endogenous insulin persists. Progressive beta-cell dysfunction occurs in T2DM(1-4), but it is unclear if this leads to absolute insulin deficiency. In contrast, in type 1 diabetes (T1D) absolute insulin deficiency is usual outside the initial “honeymoon period” (ie the period soon after diagnosis when some residual beta-cell function may persist) (5).

Some patients may present later in life clinically as having T2DM, but have the autoimmune destructive process as seen in T1D. These patients can be recognised by pancreatic autoantibodies, known as latent autoimmune diabetes in adults (LADA)(6). People with LADA may develop absolute insulin deficiency (7-10). However in practice, autoantibody levels are rarely measured in patients presenting with adult-onset diabetes: a clinical diagnosis of T2DM is usually made, and seldom revisited. Hence later subsequent development of absolute insulin deficiency is rarely suspected or tested for.

Absolute insulin deficiency in patients with T2DM is likely to carry similar risks to those associated with T1D, such as fluctuant blood glucose levels, high hypoglycaemia risk and diabetic ketoacidosis(11). The patient with T2DM however is unlikely to be offered a similar level of education to deal adequately with these, such as the Dose Adjustment For Normal Eating programme (DAFNE)(12). Frail older people in particular may be ill-equipped to cope with such complications, with less functional reserve both physically and cognitively, and in terms of their social support. The development of absolute insulin deficiency in T2DM will alter treatment: oral hypoglycaemic agents (especially sulphonylureas) will not be effective, the newer agents e.g. GLP-1 receptor analogues and DPP4 inhibitors are not suitable, and the most appropriate insulin regimen may be for example basal-bolus rather than background long-acting insulin. With an estimated 870,000 people with insulin-treated T2DM in the UK, development of absolute insulin deficiency in even a small proportion could have significant impact on both individuals and society.

Endogenous insulin levels are rarely measured in routine clinical practice - even in secondary care - due to practical limitations including the need for rapid laboratory analysis of blood tests. The majority of patients with T2DM are cared for in primary care where this is even less practical. Recently a simple urine test - Urinary C-Peptide Creatinine Ratio (UCPCR)(13) has been shown both in T1D and T2DM, to be excellently correlated with the gold-standard measure of endogenous insulin secretion, the formal mixed meal tolerance test (MMTT) - and a sensitive and specific test for absolute insulin deficiency(5, 14). The UCPCR test has the advantages of being widely available, and stable at room temperature for 3 days so offering the potential for widespread non-invasive testing which may be particularly useful for a more frail older population. Our study aimed to use UCPCR to test for absolute insulin deficiency in older people with insulin-treated T2DM.

Methods

Subjects

191 insulin-treated participants with type 2 diabetes (clinical diagnosis of type 2 diabetes, diagnosis > 45 years of age, no insulin within 1 year of diagnosis) were recruited from primary care at the time of their routine retinal screening appointment, and written consent obtained for participation in the study. Baseline data collected included duration of diabetes, current treatment, BMI and most recent HbA1c.

Urine collection and analysis

Participants were asked to provide an initial urine sample, collected at home, 2 hours after their largest meal of the day. The urine sample was collected in a standard mid-stream urine boric acid-containing specimen pot, and returned by post to the routine pathology labs for UCPCR analysis. UCPCR <0.2nmol/mmol is equivalent to a stimulated serum C-peptide of 0.2nmol/L in a mixed meal tolerance test (MMTT) (15, 16), representing an absence of clinically significant insulin secretion (11). This level is associated with unstable glycaemia, increased risk of hypoglycaemia and microvascular complications (as well as absolute insulin requirement) in T1D (11, 16).

All patients identified as insulin-deficient were asked to provide a repeat sample to confirm their initial result, as were a random group of those with a UCPCR >0.2nmol/mmol.

Mixed Meal Tolerance Test

In those patients with consistent UCPCR results <0.2nmol/mmol, we performed a formal mixed meal tolerance test (MMTT) with their insulin excluded, to confirm the absolute insulin deficiency(5). A comparison group of age-matched participants with UCPCR >0.2nmol/mmol also underwent the standardised MMTT(17). In brief, subjects fasted from midnight, and omitted their morning medications including insulin. Fasting serum and urine samples were taken before participants consumed 6ml/kg Ensure Plus HP (Abbott). A blood sample

for stimulated serum C-peptide (sSCP) was taken 90 minutes later, and a urine sample for UCPCR at 2 hours. As above, an sSCP of <0.2mmol/L was used to represent absolute insulin deficiency (5, 18).

Sample Analysis

Urine and serum samples were analysed for C-peptide using electrochemiluminescence immunoassay (intraassay CV <3.3%; interassay CV <4.5%) on a Roche Diagnostics E170 analyzer (Mannheim, Germany) by the Biochemistry department at the Royal Devon & Exeter Hospital. Urine creatinine was analyzed on the Roche P800 platform using creatinine Jaffé reagent (standardized against ID-MS) to obtain a urinary C-peptide creatinineratio (nmol/mmol). Blood samples for all patients completing the MMTT were analysed for GAD65 and IA2 autoantibodies, using the Biokit automated Elisa System (BEST 2000, Biokit, Barcelona) following manufacturers' instructions. Cut-offs used were those based on the 99th centile for 500 non-diabetic individuals; for GAD65 the reference positive value was >64 units/ml, for IA-2 the reference positive value >15 units/ml.

Data analysis

The data were not normally distributed, and so are presented as medians and interquartile ranges. Results were analysed primarily in terms of clinical characteristics between those with confirmed absolute insulin deficiency on MMTT, versus those with endogenous insulin secretion, using Mann-Whitney U and chi-squared tests (using Predictive Analytic Software PASW 17.0). The full group of 167 participants with initial home UCPCR >0.2nmol/mmol was used to represent those with significant insulin secretion, given the consistency of repeat UCPCR and MMTT results in subgroups drawn from these (see Results and Figure 1).

Ethics approval was obtained from the Southwest Research Ethics Committee.

Results

191 participants (median age 73.5 years, interquartile range (IQR) 67-78, 37% women) provided an initial urine sample for UCPCR measurement. They had a median age at diagnosis of 58 years (IQR 50-65), duration of diabetes of 13.5 years (9-19), and BMI at recruitment of 29kg/m2 (25.9-33.54). Their median time to insulin from diagnosis was 6 years (3.5-11).

UCPCR detected subjects with low endogenous insulin levels

Figure 1 summarises flow of patients through the study. Of the 191 participants screened, 24 (12.5%) had UCPCR<0.2nmol/mmol. Of these, 21 provided a repeat sample, and 11/188 (6% of the whole cohort) had two consistent UCPCR results of <0.2nmol/mmol.

Figure 1: *Flow of participants through the study*

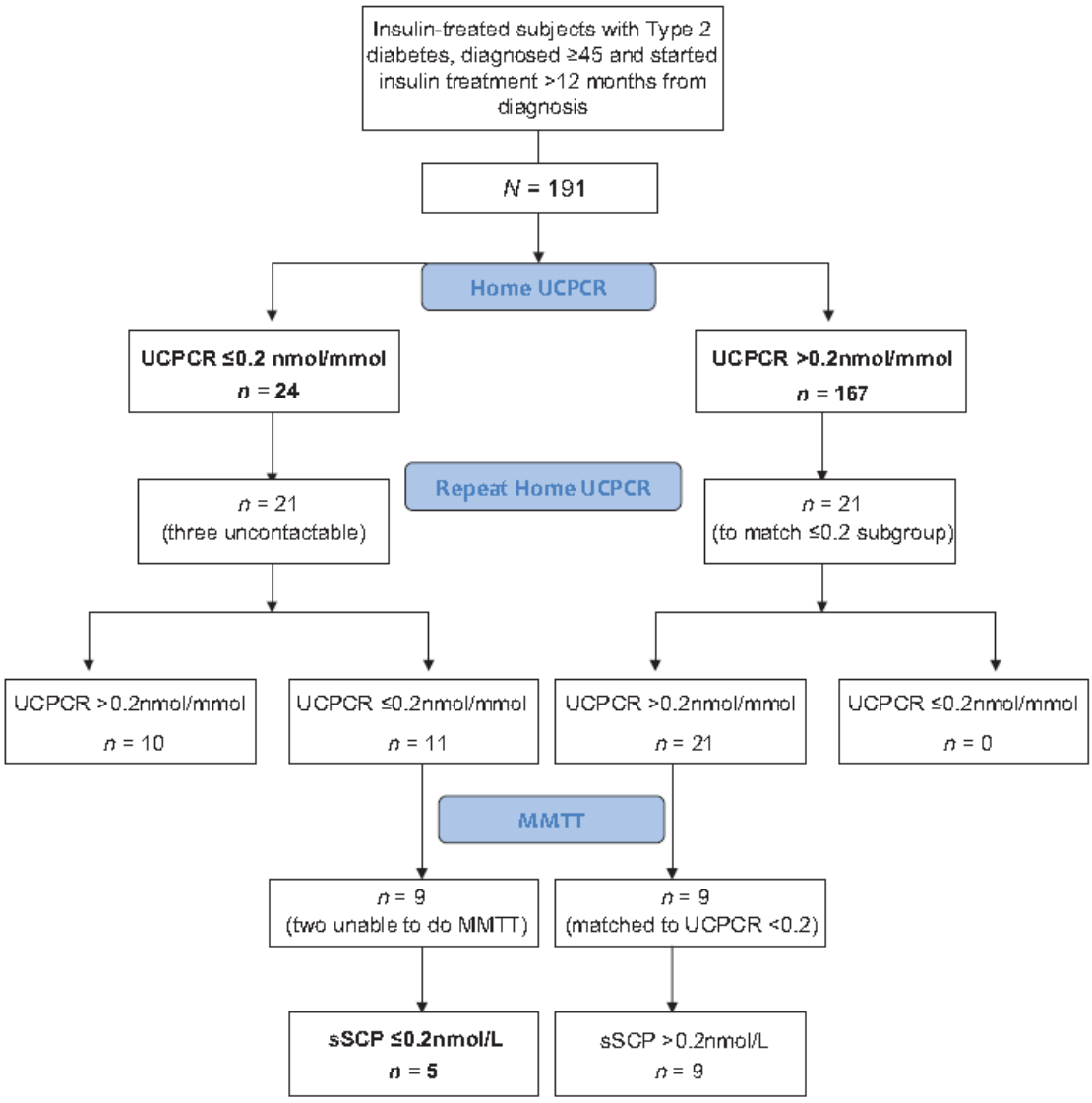

Table 1: *UCPCR and serum C-peptide values in 9 subjects with two home UCPCRs of ≤0.2nmol/mmol, compared to 9 matched subjects with two home UCPCRs of >0.2nmol/mmol. Data shown as median values and interquartile ranges. fSCP: fasting serum C-peptide; sSCP: stimulated serum C-peptide; MMTT: mixed meal tolerance test.*

	UCPCR ≤0.2	**UCPCR >0.2**	**P value**
UCPCR (home) (nmol/mmol)	<0.02 (<0.02-0.2)	1.7 (0.8-7.1)	<0.001
UCPCR (MMTT) (nmol/mmol)	0.07 (<0.02-0.7)	2.6 (1.9-5.6)	0.001
fSCP (nmol/L)	0.13 (0.08-0.35)	0.59 (0.45-0.88)	0.003
sSCP (nmol/L)	**0.18 (0.08-0.64)**	**2.0 (1.53-2.52)**	**0.002**

Patients with clinically diagnosed T2DM show absolute insulin deficiency with mixed meal tolerance testing

Table 1 summarises the MMTT results of the two groups selected on the basis of their UCPCR. These two groups were similar in age, duration of diabetes, time to insulin from diagnosis, and BMI. As expected the stimulated serum C-peptide (sSCP) was lower in those with a low UCPCR compared to those with a high UCPCR (median 0.18 nmol/L v 2.0 nmol/L, p= 0.002). 5 of the 9 patients with a low UCPCR had a stimulated C-peptide <0.2nmo/L representing absolute insulin deficiency(18) in contrast to none with a high UCPCR. This suggests the minimum prevalence of absolute insulin deficiency in insulin-treated T2DM is 3% (5/186, excluding the 5 who were unable to provide repeat urine samples or participate in the MMTT, see Figure 1).

Of note, the UCPCR results obtained in both groups were substantially higher after the MMTT than the home meal. For those four patients with two home UCPCRs <0.2nmol/mmol but an sSCP>0.2nmol/L, the post-MMTT UCPCR

results were also >0.2nmol/mmol. This suggests the MMTT provided more beta-cell stimulation than the meals consumed at home.

Patients with absolute insulin deficiency went onto insulin sooner and are slimmer

The 5 patients with confirmed absolute deficiency on MMTT were slimmer (BMI 25.1 kg/m^2 versus 29.1, p=0.04), and commenced insulin more rapidly after diagnosis (2.5 years versus 6, p=0.005), although there was substantial overlap for both these measures between those with (n=5) and without (n=167) absolute insulin deficiency. There was no difference in age of diagnosis, duration of diabetes, glycaemic control or insulin dose (Table 2).

Two of the five participants with absolute insulin deficiency were GAD-positive (titre in both >2000 units/ml); one of these was also IA-2 positive (titre 74.9 units/ml). In addition, one patient who had two low UCPCR measurements from home but a stimulated C-peptide of 0.37nmol/L,was GAD-positive (titre >2000 units/ml). None of the 9 participants from the comparison MMTT group, ie with home UCPCR demonstrating residual endogenous insulin secretion and confirmed on MMTT, were positive for GAD or IA-2 antibodies.

Of note only 2 of the 5 participants with absolute insulin deficiency were on a basal-bolus regimen, and two were treated with oral agents in combination with insulin.

Table 2: *Clinical characteristics of those with absolute insulin deficiency as confirmed by MMTT, versus those with endogenous insulin secretion (UCPCR >0.2nmol/mmol). Data shown as medians (interquartile range). *OHA: oral hypoglycaemic agent(s); **Basal bolus regime: 4 or 5 injections of insulin a day. $^{\$}$Chi-square tests; all others Mann-Whitney U*

	Absolute insulin deficiency	**Endogenous insulin secretion**	**P**
N	5	167	
Age at diagnosis (years)	63 (54-72)	58 (50-66)	0.28
Duration of diabetes (years)	12 (9.5-19.5)	13 (9-17)	0.87
BMI (kg/m^2)	25.1 (22.8-28.8)	29.1 (26.3-33.6)	**0.04**
HbA1c (mmol/mol) **HbA1c (%)**	72 (57-85) 8.7 (7.4-9.9)	62 (55-69) 7.8 (7.2-8.5)	0.24
Time to insulin (years)	2.5 (1.5-3)	6 (3-10.75)	**0.005**
Insulin/kg/24hrs (units/kg/24h)	0.72 (0.54-0.88)	0.51 (0.31-0.84)	0.26
On OHA (in add^n to insulin)*$^{\$}$	2/5 (40%)	115/167 (69%)	0.17
On basal bolus regime$^{\$}$**	2/5 (40%)	19/167 (11%)	**0.05**

Discussion

2.7% of insulin-treated patients with a clinical diagnosis of T2DM in this study have been shown to have absolute insulin deficiency. Patients who may have had absolute insulin deficiency were detected using the simple non-invasive testing method, Urinary C-Peptide Creatinine Ratio (UCPCR), and the MMTT was used to confirm findings. These patients cannot be solely identified on the basis of clinical characteristics, or by testing of GAD antibodies.

Prevalence & aetiology of absolute insulin deficiency in T2DM

Our prevalence of absolute insulin deficiency of 2.7% (5/186) in an insulin-treated group of patients with a clinical diagnosis of T2DM is similar to the 2.3% (3/133) found at 10 years from diagnosis in an observational study by Niskanen et al (7). This looked at adult patients over the age of 45 with new-onset non-insulin-dependent diabetes, and measured sSCP and GAD titres at 0, 5 and 10 years. By including only insulin-treated patients in our study, one might have expected a more insulin-deficient group and hence a comparatively higher proportion of patients with absolute insulin deficiency than in Niskanen's study. The aim for tighter glycaemic control (and hence earlier initiation of insulin) in the post-DCCT/UKPDS era may provide explanation for why this was not seen. Additionally, the 2.7% prevalence in our study population is a minimum: there were 5 additional participants with initial UCPCR suggestive of absolute insulin deficiency who were either uncontactable or unable to undergo a MMTT (Figure 1), if all these had confirmed sSCP<0.2nmol/L the prevalence would have risen to 5.2% (10/191).

In patients with high titres of GAD antibodies, reasonably long duration (10-12 years) prospective longitudinal studies have shown many (but not all)develop absolute insulin deficiency (7, 9). When combined with the clinical features of adult-onset diabetes not immediately requiring insulin treatment, the presence of pancreatic autoantibodies has been called "latent autoimmune diabetes in adults" (LADA) (7, 9, 10). Two of the 5 participants with absolute insulin deficiency in our study fit these criteria, having high GAD-titres (>2000units/ml,

reference value >64units/ml). However, with 3 participants with confirmed absolute insulin deficiency not exhibiting GAD antibodies, it suggests that the presence of these antibodies is not a sensitive test for detecting the development of absolute insulin deficiency in those with longstanding diabetes.

Our study has hence identified 3 people with apparent non-autoimmune T2DM and confirmed absolute insulin deficiency. Of the three patients developing absolute insulin deficiency in Niskanen et al's study [7], one was GAD-antibody negative. This was the only other case we found in the literature of absolute insulin deficiency confirmed using stimulated serum c-peptide, in non-autoimmune T2DM(7). It is possible that the cross-sectional measurement of pancreatic autoantibodies in our study may have missed some patients who were antibody-positive at an earlier stage but lost this positivity over time. However numerous studies have found high GAD-titres persist (7, 9, 19, 20).The cross-sectional design of this study meant we were able to look a wide range of durations of diabetes, longer than that looked at before in T2DM, and this may help explain why we have detected absolute insulin deficiency where others have not. No previous studies we have found were designed to look for absolute insulin deficiency in T2DM; the majority have looked at the significance of GAD antibodies on the deterioration in beta-cell function over time.

UCPCR testing

UCPCR was used in this study as a practical test in a large number of individuals, and was able to detect patients at risk of absolute insulin deficiency. The gold-standard MMTT was used to confirm findings. Those with evidence of endogenous insulin secretion on an initial UCPCR test had consistent results both on repeat UCPCR and MMTT. As would be expected by regression to the mean when selecting a low cut-off, those with initial low UCPCR suggesting absolute insulin deficiency had a tendency to higher results upon repeat testing, taking them above the designated 0.2nmol/mmol threshold. In addition, some practical issues were identified which may have led to erroneously low UCPCR results upon initial testing: these included patients tipping out the boric acid preservative from the sample pots, and postal delays. Additionally in those with low endogenous insulin levels, variation in meal stimulus may have contributed

to a low UCPCR on one occasion versus a UCPCR over the 0.2nmol/mmol threshold on another occasion. This is supported by the finding that in four patients, despite two home UCPCR results suggestive of absolute insulin deficiency, a higher UCPCR and measurable sSCP (though low) levels were seen under controlled MMTT conditions. This suggests the MMTT was more stimulating than the home meals of these patients and they were still able to mount an insulin response when maximally stimulated. However insulin secretion with their normal diet may be clinically more relevant.

The screening method did identify individuals with genuine absolute insulin deficiency. With clear instructions on how to optimally take a sample for UCPCR testing, and advice to repeat a low UCPCR in the first instance, it is a very easy and practical test which has the advantage of being widely available, avoids the need for venepuncture, and can be done at home and posted in. Since the completion of this study, it has been shown that the previously widely perceived practical limitations in measurement of c-peptide in blood may be to some extent overcome by using EDTA sample tubes: these can improve the stability of C-peptide concentrations to over 24 hours at room temperature(21). This would also make measurement of C-peptide in blood a viable test in the outpatient/primary care setting.

In the increasingly complex climate of diabetes management options, confirmation (or not) of insulin deficiency should help guide treatment, education and management decisions, which will be valuable in optimising care for any patient, but perhaps particularly the more frail older patient. We would suggest a measure of c-peptide, such as UCPCR, may have an important role when clinical features like marked variation in blood glucose values suggest absolute insulin deficiency.

Clinical characteristics

Those with confirmed absolute insulin deficiency had started insulin sooner after diagnosis than those with retained endogenous insulin (2.5 years versus 6), and had lower BMIs (25 versus 29). In terms of other easily available and

measurable baseline patient characteristics, there was little else to distinguish them.

Although 2 of the 5 patients with confirmed absolute insulin deficiency were on basal bolus regimens, the three others, and several of those with low endogenous insulin levels, were on unusual regimens more suited to patients with endogenous insulin secretion. 2 of the 5 were still on oral hypoglycaemic agents, and none had had any training such as DAFNE(12) to help them understand and manage their diabetes better.

Theoretically despite a clinical diagnosis of T2D, the patients with absolute insulin deficiency may be at risk of complications as seen in T1D. This was reflected in all of the patients with absolute insulin deficiency – and those with low endogenous insulin levels - reporting difficulty in managing their blood glucose levels due to seemingly unpredictable fluctuations in blood glucose levels, and one patient having had an episode of diabetic ketoacidosis (DKA).

Implications for clinical practice

Identification of absolute insulin deficiency in patients with a clinical diagnosis of T2DM may enable optimisation of their treatment such as basal bolus regimens, management and education such as DAFNE(12) courses, and recognition of potential complications such as higher risks of hypoglycaemia or DKA. All these have not been traditional considerations in many patients with T2DM, and recognition should help improve the quality of life of these individuals.

UCPCR is a practical and useful test to detect absolute insulin deficiency in T2DM and should be used in “T2DM” individuals developing DKA, severe hypoglycaemia or having large fluctuation in blood glucose values, to help inform optimal diagnosis and/or management. A UCPCR suggestive of endogenous insulin production is reliable, and in this clinical context may suggest other explanations for the clinical features (such as compliance). A low UCPCR suggestive of insulin deficiency should be repeated in the first instance, but may help guide management and education as described above.

Conclusion

We have shown that absolute insulin deficiency is present in 3% of insulin-treated patients with T2DM and may be detected using Urinary C-peptide creatinine ratio, UCPCR. Clinical features such as GAD antibodies, starting insulin sooner after diagnosis, and having a lower BMI are pointers to help recognise those at risk, but are not diagnostic. Those with absolute insulin deficiency are at risk of more fluctuant blood glucose levels, hypoglycaemia and diabetic ketoacidosis, which may adversely affect quality of life as well as potentially having more severe consequences especially in the older population. Recognition of absolute insulin deficiency is thus important as it will aid optimal management of these individuals, and UCPCR is a useful test that can be used in general practice or outpatients to confirm a clinical suspicion of insulin deficiency.

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CHAPTER 5

Random non-fasting C-peptide: bringing robust assessment of endogenous insulin secretion to the clinic

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CHAPTER 5

Acknowledgments of co-authors and contributions to paper

Angus Jones, Bea Knight and I designed the study and wrote the study protocol. Bea Knight and I obtained ethical approval. I recruited the patients and performed the study, with help from Bea Knight. Tim McDonald co-ordinated analysis of the samples, and all co-authors contributed to discussions regarding results and reviewing the final manuscript.

Random non-fasting C-peptide: bringing robust assessment of endogenous insulin secretion to the clinic

Abstract

Background

Measuring endogenous insulin secretion using C-peptide can assist diabetes management, but standard stimulation tests are impractical for clinical use. Random non-fasting C-peptide assessment would allow testing when a patient is seen in clinic.

Methods

We compared C-peptide at 90 minutes in the mixed meal tolerance test (sCP) with random non-fasting blood C-peptide (rCP) and random non-fasting urine C-peptide creatinine ratio (rUCPCR) in 41 participants with insulin-treated diabetes. We assessed sensitivity and specificity for previously reported optimal mixed meal test thresholds for insulin deficiency (<200pmol/L) and Type 1 diabetes/inability to withdraw insulin (<600pmol/L), and assessed impact of concurrent glucose.

Results

rCP and sCP levels were similar (median 546pmol/L and 487pmol/L, p=0.92). rCP was highly correlated with sCP, r=0.91, p<0.0001, improving to r=0.96 when excluding samples with concurrent glucose <8mmol/L.

An rCP cut-off of 200pmol/L gave sensitivity of 100% and specificity of 93% for detecting severe insulin deficiency (sCP<200pmol/L), with area under the ROC curve 0.99. An rCP <600pmol/L gave sensitivity of 87% and specificity of 83% to detect sCP<600pmol/L, with specificity improving to 100% when excluding samples with concurrent glucose <8mmol/L.

rUCPCR (0.52nmol/mmol) was also well-correlated with sCP, r=0.82, p<0.0001. An rUCPCR cut-off of <0.2nmol/mmol gave sensitivity and specificity of 83% and 93% to detect severe insulin deficiency, with ROC AUC 0.98.

Conclusions

Random non-fasting C-peptide measures are strongly correlated with mixed meal C-peptide, and have high sensitivity and specificity for identifying clinically relevant thresholds. These tests allow assessment of C-peptide at the point patients are seen for clinical care.

INTRODUCTION

Assessment of endogenous insulin secretion using C-peptide is useful in clinical practice to assist classification and treatment of diabetes (1). Assessment of a stimulated blood C-peptide level following a standardised stimulus such as a mixed meal (mixed meal tolerance test, MMTT) provides a gold-standard measure of endogenous insulin secretion, but is impractical for clinical use (2). Other C-peptide measures such as fasting blood C-peptide (3), or a post-home meal urinary C-peptide creatinine ratio (UCPCR)(4-6), give a reasonable approximation to the gold-standard, and high sensitivity and specificity in classifying diabetes (7-10). However, for routine clinical care, the most practical test would be a spot “random” non-fasting sample, sent when a patient is seen in an outpatient or primary care clinic.

Random non-fasting blood C-peptide (rCP) has been shown to have superior performance to both post-glucagon and fasting blood C-peptide assessment in differentiating clinically well-defined Type 1 and Type 2 diabetes (7, 8), and to have clinical utility in detecting MODY (11, 12). However rCP has never been formally validated against a gold-standard MMTT C-peptide assessment. While UCPCR changes little from 2 to 4 hours post-meal in those with Type 2 diabetes (McDonald, unpublished), utility of a random non-fasting UCPCR sample has never been assessed.

We aimed to compare non-fasting random blood C-peptide and UCPCR with ‘gold-standard’ blood C-peptide assessment at 90 minutes in the MMTT.

METHODS

Subjects

41 participants with insulin-treated diabetes were recruited to the GREAT study (https://clinicaltrials.gov, NCT02506296). To ensure a range of C-peptide values, participants were selected on the basis of prior C-peptide assessment to include participants with and without severe insulin deficiency (under/over 200pmol/L post-MMTT blood C-peptide or equivalent (1)). All participants had a clinical diagnosis of Type 2 diabetes, and an estimated glomerular filtration rate (eGFR) >30ml/min/1.73m^2. Ethical approval was obtained from the NRES Committee South West, and all participants gave written informed consent.

Mixed meal tolerance test

Participants fasted from 10pm, then attended the following day prior to 11am having not taken their morning medication prior to arrival. Baseline bloods for glucose and C-peptide were taken, morning insulin given (13), and 160ml of Fortisip Compact (Nutricia, Trowbridge, UK) drunk within 10 minutes (content/100ml: carbohydrate 29.7g, protein 9.6g, fat 9.3g). Bloods for C-peptide and glucose analysis were repeated every 30 minutes, up to and including 180 minutes post-mixed meal. Samples were immediately centrifuged after collection and stored at -80^{0}C, for later batched analysis.

Non-fasting tests

On a separate occasion (within 8 days of the MMTT), blood was taken between 9am and 5pm, within 5 hours of a meal, and without restriction on snacks or other intake. Whole blood samples collected in potassium-EDTA (C-peptide) and fluoride oxalate (concurrent glucose) tubes were sent at room temperature to be processed routinely at the Royal Devon & Exeter Hospital Blood Sciences department. Participants were also asked to provide a spot urine sample. This was frozen at -80^{0}C before later batch analysis.

Sample analysis

C-peptide was analysed using the automated Roche diagnostics (Manheim, Germany) E170 immuno-analyser (limit of detection 3.3pmol/L, inter- and intra-assay coefficients of variations <4.5% and <3.3% respectively). Urinary creatinine was analysed on the Roche P800 platform to obtain a urine C-peptide creatinine ratio (UCPCR, nmol/mmol).

Analysis

We compared the median random non-fasting blood C-peptide (rCP) with the median blood C-peptide at 90 minutes in the mixed meal tolerance test (sCP) using Wilcoxon's signed rank test, and correlation coefficient between both rCP and random non-fasting UCPCR (rUCPCR) with sCP using Spearman's rank correlation.

We then assessed the utility of rCP and rUCPCR in correctly classifying participants in relation to previously described clinically relevant MMTT C-peptide thresholds using receiver operating characteristic (ROC) curves, with corresponding specificities and sensitivities:

1. MMTT sCP <200pmol/L: absolute insulin deficiency (1, 14)
2. MMTT sCP <600pmol/L: Type 1 diabetes/inability to withdraw insulin (1)

Finally, we assessed the influence of concurrent glucose repeating the above analyses excluding hypoglycemia (concurrent glucose <4mmol/L), and a previously suggested cut-off of <8mmol/L (1, 8, 15).

RESULTS

Participant characteristics

28/41 (68%) of participants were men. Participants had a median age of 73 (interquartile range, IQR 68-78), diabetes duration 21 (14-31) years, BMI 26.8 (25-29.9) kg/m^2, and HbA1c 68 (58-75) mmol/mol/8.4% (7.5-9.0%).

12/41 (29%) participants had severe insulin deficiency (sCP<200pmol/L). C-peptide was detectable (>2.9pmol/L) at all time-points - fasting and stimulated - in 40/41 participants.

C-peptide was stable 1-3 hours after meal stimulation

There was little change in the C-peptide from 60 mins to 3 hours post-MMTT: median C-peptide ranged from 487 to 622pmol/L across these five time points, Figure 1a. Mean individual coefficient of variation over the 1-3 hour-post MMTT period was 14.3%.

Random non-fasting blood C-peptide level is strongly correlated with the gold-standard 90-minute mixed meal test C-peptide

Median rCP of 546 pmol/L (IQR 76-943) was similar to sCP at 90 minutes, 487 pmol/L(75-985), p=0.92, Figure 1a.

rCP was strongly correlated with sCP: Spearman's rho correlation coefficient=0.913, p<0.0001, Figure 1b. When only participants who had a concurrent lab glucose value of $\geq$8mmol/L were included (66% participants), the correlation coefficient increased to 0.96.

To be expected, results showed more variation in the higher C-peptide range, Figure 2.

Figure 1 (a) *Blood C-peptide levels on random sampling and in the mixed meal test. rCP: random non-fasting; time points reflect minutes post mixed meal ingestion, 0m: fasting sample.*

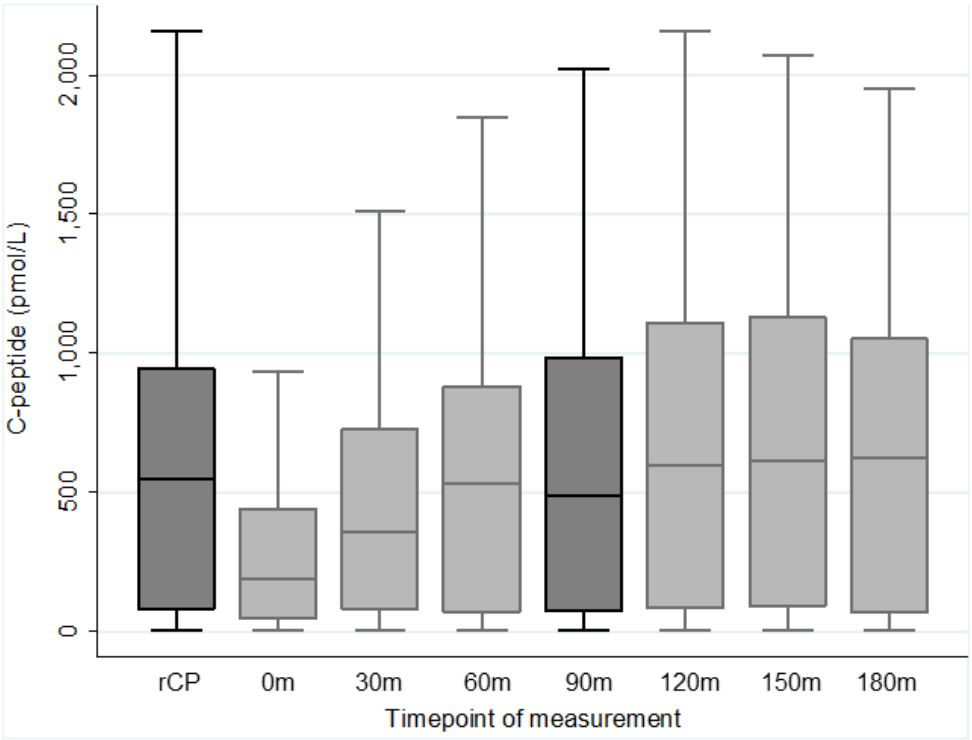


Figure 1 (b) *Random non-fasting C-peptide versus 90 minute C-peptide in the mixed meal tolerance test. Blue diamonds: concurrent blood glucose ≥8mmol/L; green circles: concurrent blood glucose ≥4 to 8mmol/L; red triangles: concurrent blood glucose <4mmol/L.*

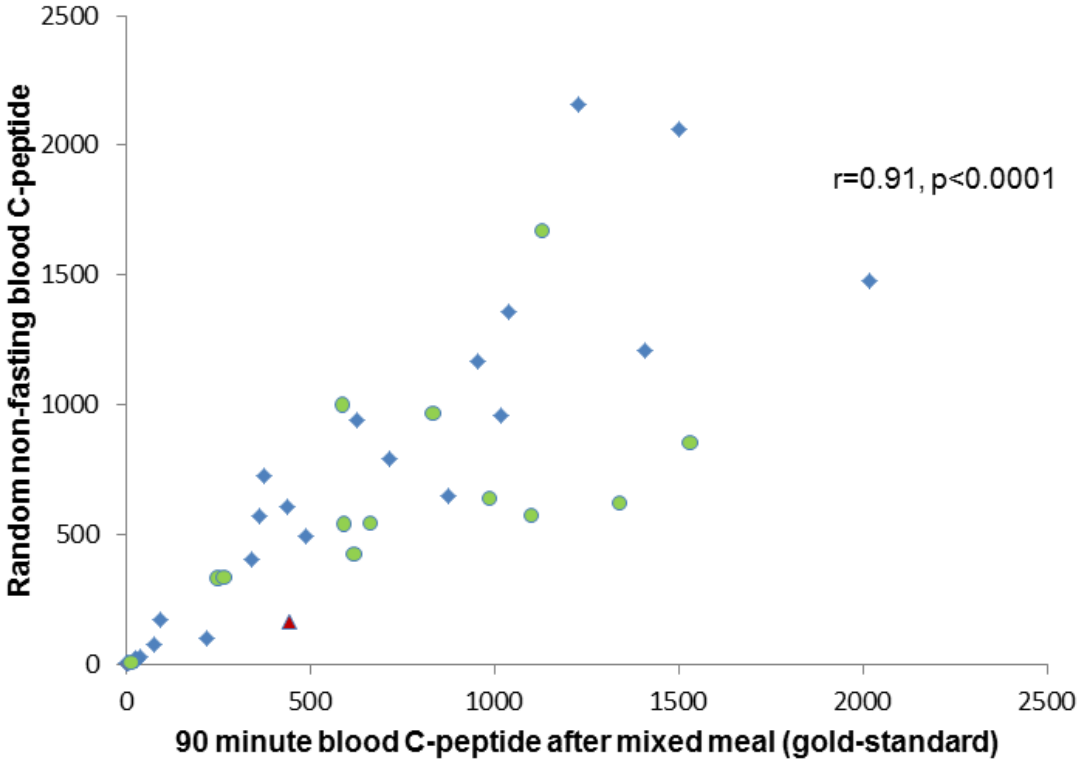
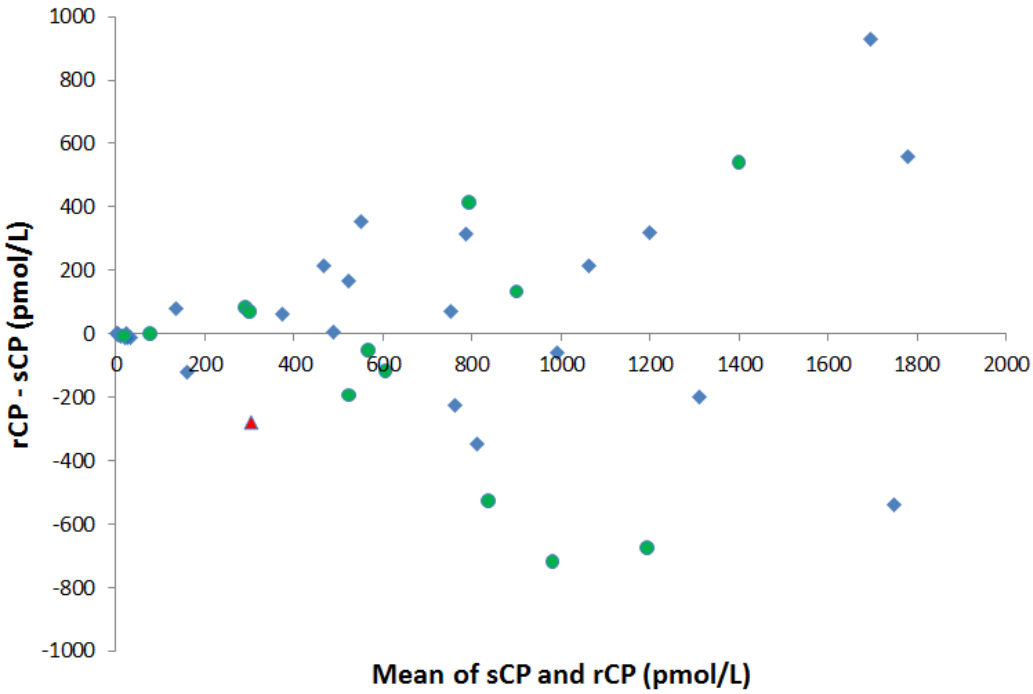


Figure 2: *Bland-Altman plot showing the difference between 90-minute C-peptide (sCP) and random non-fasting C-peptide (rCP). Level of blood glucose measured concurrently with rCP shown by blue diamonds ≥8mmol/L; green circles ≥4 to 8 mmol/L; red triangles: <4 mmol/L*



Random non-fasting blood C-peptide is a highly sensitive and specific test for severe insulin deficiency

rCP was a highly sensitive and specific test for severe insulin deficiency (sCP<200pmol/L), with area under the ROC curve (AUC ROC) of 0.99 (Table 1). An rCP cut-off of <200pmol/L gave a sensitivity of 100% and specificity of 93% for severe insulin deficiency, with 95% of participants correctly classified. This did not alter significantly with concurrent glucose (Table 1).

rCP was also able to identify participants with sCP <600pmol/L (Type 1 diabetes/inability to withdraw insulin): AUC ROC 0.94 (95% CI 0.84-0.99). An rCP value <600pmol/L gave a sensitivity of 87% and specificity of 83% to detect sCP<600pmol/L - with 85% correctly classified. Excluding concurrent glucose values <8mmol/L improved specificity to 100% without altering sensitivity, Table 1.

Random non-fasting UCPCR is also strongly correlated with the gold-standard blood C-peptide measure and a sensitive and specific test for severe insulin deficiency

rUCPCR (median 0.52nmol/mmol (IQR 0.095-1.57nmol/mmol), was well-correlated with sCP, r=0.82, p<0.0001 (n=40). rUCPCR was also a sensitive and specific test for detecting the clinically relevant thresholds of sCP <200pmol/L and <600pmol/L: ROC AUC 0.98 and 0.90 respectively, Table 1. For identifying severe insulin deficiency (sCP<200pmol/L), an rUCPCR cut-off of <0.2nmol/mmol gave a sensitivity and specificity of 83% and 93%, with 90% participants being correctly classified. An rUCPCR cut-off of <0.6nmol/mmol had a sensitivity and specificity of 82% and 83% for detecting sCP<600pmol/L.

Table 1: *Ability of random non-fasting blood C-peptide (rCP) and UCPCR (rUCPCR) to define absolute insulin deficiency (90-minute mixed meal tolerance test C-peptide (sCP) <200pmol/L) and type 1 diabetes/insulin dependence (sCP <600pmol/L) using equivalent thresholds, with and without exclusion based on concurrent glucose (blood C-peptide only). Sensitivity, specificity and % correct classification are given for numerically equivalent thresholds (rCP 200 and 600pmol/L, UCPCR 0.2 and 0.6nmol/mol) as these were close to optimal on ROC analysis.*

Mixed meal test C-peptide threshold	Concurrent glucose cut- off (mmol/L)	n	AUC	AUC 95% confidence intervals	**Specificity** 95% CI (%)	**Sensitivity** 95% CI (%)	Correctly classified (%)
Random non-fasting blood C-peptide							
<200pmol/L	**All**	**41**	**0.99**	**0.91 – 1.0**	**93** 77-99	**100** 74-100	**95** 83-99
	≥4	39	1.0	0.91 – 1.0	96 82-100	100 72-100	97 87-100
	≥8	27	0.99	0.87 – 1.0	94 73-100	100 66-100	96 81-100
<600pmol/L	**All**	**41**	**0.94**	**0.84 – 0.99**	**83** 59-96	**87** 66-97	**85** 71-94
	≥4	39	0.94	0.79 – 0.98	83 59-96	86 64-97	85 69-94
	≥8	27	0.99	0.87 – 1.0	100 74-100	87 60-98	93 76-99
Random non-fasting UCPCR							
<200pmol/L	All	40	0.98	0.87 – 1.0	93 76-99	83 52-98	90 76-97
<600pmol/L	All	40	0.90	0.76 – 0.97	83 59-96	82 60-95	83 67-93

DISCUSSION

Our results show that random non-fasting blood C-peptide and UCPCR measurements taken when a patient attends clinic are highly correlated with the gold-standard mixed meal test assessment of endogenous insulin secretion, and are sensitive and specific tests for clinically relevant thresholds. These tests, combined with the demonstration of stability at room temperature of blood C-peptide for >24 hours (in EDTA (16)) and UCPCR for >72 hours (in boric acid (17)), offer a practical way of assessing endogenous insulin excretion when contact is made for clinical care.

Our findings are consistent with previous research demonstrating that a random non-fasting blood C-peptide offers similar performance to C-peptide in a formal glucagon stimulation test when classifying clinically well-defined Type 1 and 2 diabetes (8), is superior to fasting C-peptide when identifying autoimmune diabetes (7) and has high clinical utility for detecting patients with undiagnosed monogenic diabetes (11). This is the first study to formally evaluate use of a random non-fasting C-peptide sample against a gold-standard in a mixed meal test. The use of a random non-fasting UCPCR has not been previously assessed.

Limitations of our study include that our modest sample size limits our ability to assess the impact of concurrent glucose on rCP testing. In addition our population may not be representative of the patients where C-peptide testing has most utility (difficult to classify diabetes) in that they are elderly and have been selected on the basis of a clinical diagnosis of Type 2 diabetes with or without discordant C-peptide.

Our results suggest that a random non-fasting blood C-peptide or UCPCR could be used to assess endogenous insulin secretion in clinical practice. This would have major practical advantages in that the test can be conducted when a patient is seen for clinical care. While our sample size is too small to robustly assess the impact of concurrent glucose our results suggest this has only

modest impact. While a high value in the presence of any glucose is likely to be robust it may be prudent to treat rCP values below a clinical threshold where concurrent glucose is <8mmol/L with caution, and consider a repeat sample.

CONCLUSION

We have shown that random non-fasting blood and urine C-peptide measures are strongly correlated with the gold-standard C-peptide test and have high sensitivity and specificity in identifying clinically relevant C-peptide thresholds. These tests allow assessment of C-peptide at the point patients are seen for clinical care.

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CHAPTER 6

A clinically collected random non-fasting C-peptide sample may be used as a risk assessment tool for hypoglycaemia frequency and awareness in insulin-treated patients

Hope SV, Hill A, Knight BA, Shields BM, Jones AG, Hattersley AT, McDonald TJ

For submission

CHAPTER 6

Acknowledgments of co-authors and contributions to paper

Tim McDonald and his lab team set up the method for testing C-peptide on consenting participants, after Gill Baker, Anita Hill, Bea Knight and I had obtained an amendment for the DARE diabetes ethics agreement. The project idea was mine, developed from ideas from my MSc and discussions in team meetings, and enthusiastically supported in particular by Andrew Hattersley, Tim McDonald and Angus Jones. Andrew vitally also provided the funding from a pre-existing grant in order to enable it to get going. Anita Hill and Rob Bolt have co-ordinated receipt of C-peptide results and amalgamating in the DARE database, and Anita worked tirelessly to keep the database up-to-date and patiently providing information, help and advice when needed! Tina Libretto sent out all the Hypoglycaemia Questionnaires, the receipt of which was co-ordinated by her, Dionne McGill and Rob Bolt. The CRF nurses also administered the questionnaires to new recruits to DARE. Bethan Knight, Rachel Kelland, Aylish Tregarthen and I entered/second-entered the Hypoglycaemia Questionnaire results into the DARE database, and I cleaned the database. Richard Oram and Suzie Hammersley kindly increased the number of participants undergoing a MMTT results to 50. All co-authors contributed to discussions regarding results and reviewing the final manuscript.

A clinically collected random non-fasting C-peptide sample may be used as a risk assessment tool for hypoglycaemia frequency and awareness in insulin-treated patients

Abstract

Background and aims

There is considerable variation in the degree of endogenous insulin secretion in insulin-treated diabetes. Recently we have shown that C-peptide is stable >6 hours at room temperature. We aimed to assess whether random non-fasting C-peptide (rCP) correlates well with the gold-standard C-peptide measure (90 minute stimulated sample (sCP) in mixed meal test (MMTT), and whether rCP analysis could be done on routinely collected diabetes blood samples from primary care, in order to provide a model for integration with research and clinical care.

We then aimed to assess the relationship between rCP and hypoglycaemia frequency and hypoglycaemia awareness in insulin-treated patients (Type 1 or 2 diabetes).

Methods

50 participants underwent a standardised MMTT for comparison of rCP and sCP. 480 insulin-treated patients, median age 66 (IQR 54-74), diabetes duration 19 (13-29) years, and HbA1c 65mmol/mol (57-74) provided rCP samples and completed Clarke's Hypoglycaemia questionnaire. Results were analysed across C-peptide deciles, and non-parametric analysis performed.

Results

rCP was strongly correlated with sCP: rho correlation coefficient=0.93, $p<0.0001$. Median rCP was 415pmol/L (IQR 19-789) compared to sCP, 368pmol/L (28-954), $p=0.67$.

Increased frequency of recognised hypoglycaemia episodes with blood glucose <3.5mmol/L was associated with lower C-peptide deciles, p=0.0001, regardless of the clinical diagnosis of Type 1 or Type 2. HbA1c levels were similar across all C-peptide deciles, p=0.44.

37/429 (8.6%) patients had impaired hypoglycaemia awareness. Median C-peptide was lower than in those with awareness: 12pmol/L (2.9-977) vs 370(12-910), p=0.044. Other than duration of diabetes (31(16-43) vs 18(13-27)years, p=0.0015), clinical characteristics were similar including age, gender, BMI, HbA1c, and type of diabetes.

Conclusion

We have demonstrated a clear link between patient-reported hypoglycaemia frequency and awareness, and C-peptide level in all insulin-treated patients, regardless of clinical diagnosis. We propose measuring rCP could be a useful clinical tool in assessment and management of insulin-treated patients and their risks of hypoglycaemia.

INTRODUCTION

C-peptide and hypoglycaemia risk

C-peptide is used for assessing endogenous insulin secretion in diabetes, for assisting in classification and treatment (1-7), and as a marker for intervention (8) - eg success of islet transplantation (9). Patients with Type 1 diabetes have a higher risk of hypoglycaemia than those with Type 2 diabetes (10). The DCCT demonstrated that there was a correlation between C-peptide levels (in its broadly stratified groups of stimulated C-peptide level in people with Type 1 diabetes) and risk of hypoglycaemia (11, 12), with lower levels correlated with increasing risk.

With increasing sensitivity of C-peptide assays in recent years, it has been demonstrated that the vast majority of people with Type 1 diabetes continue to produce small amounts of endogenous insulin – termed “microsecretors” (13-15), and even at these very low levels of endogenous insulin, there appears to be a continuous correlation with diabetes complications, including hypoglycaemia (16, 17).

Frequency of hypoglycaemia in insulin-treated Type 2 diabetes is correlated with duration of diabetes (18), and of insulin treatment (19, 20). A recent analysis of ACCORD suggested that those participants (with Type 2 diabetes) who suffered from severe hypoglycaemia had significantly lower C-peptide levels than those who had similar glycaemic control but who did not experience hypoglycaemia (21).

Measuring C-peptide

C-peptide is secreted in equimolar amounts with endogenous insulin following cleavage of proinsulin, and has the advantage (over measuring insulin) that it can be used to measure endogenous insulin levels in those receiving subcutaneous insulin treatment (8, 22).

Previously perceived instability of C-peptide in blood samples and need to collect on ice, centrifuge and get to the laboratory rapidly, has limited its use to mainly research. However it has now been shown that C-peptide is stable for at least 24 hours in EDTA tubes at room temperature (23) – thus markedly increasing its potential use.

Historically fasting levels of C-peptide, glucagon-stimulated levels, or those stimulated in a standardised mixed meal tolerance test, have most often been used. Fasting levels can under-represent insulin secretory capacity. Stimulated levels in formal glucagon or mixed meal tolerance tests are not practical for routine clinical use – especially where the majority of patients are looked after in outpatients or primary care.

There have been increasing calls recently for more routine use of C-peptide in clinical care (24). The practical feasability of this has been increased by the demonstration of stability of C-peptide in routine blood tubes (23), and if a random non-fasting measure of C-peptide could be shown to correlate well with the gold-standard 90 minute stimulated C-peptide in a mixed meal tolerance test (25)), it could mean a spot “random” non-fasting sample could be sent when a patient is seen in an outpatient or primary care clinic.

More widespread C-peptide analysis in the population with diabetes may enable further assessment of its relationship with complications such as hypoglycaemia – and raises the possibility of random non-fasting C-peptide as part of a risk assessment tool for hypoglycaemia.

Linking routine C-peptide analysis with clinical evaluation of hypoglycaemia

We thus aimed to establish a method by which random non-fasting C-peptide analysis could be done on routinely collected diabetes blood samples from primary care, to provide a model for integration with research and clinical care.

We aimed to assess the relationship between C-peptide and hypoglycaemia frequency and hypoglycaemia awareness in insulin-treated patients (with a clinical diagnosis of Type 1 or Type 2 diabetes), by integrating random non-fasting C-peptide results with results from the standardised Clarke & Gold Hypoglycaemia questionnaire (26).

METHODS

Participants

All insulin-treated patients on the Diabetes Alliance for Research England (DARE) database in Exeter (27) were invited to participate. DARE is a national UK study aiming to explore environmental and genetic influences in diabetes and its associated complications, by acquiring information from as many patients with diabetes as possible.

Establishing reflex testing for C-peptide

Potential participants were asked for permission to test C-peptide on residual blood from their routine HbA1c tests sent in from primary care. Ethical approval was obtained from the NRES Committee South West. Consenting participants were flagged on the Blood Sciences database at the Royal Devon & Exeter Hospital, and any HbA1c sample received for these patients automatically also got tested for C-peptide. Results from 1.6.14 to 30.10.15 were included. These results were reported back to primary care physicians along with HbA1c results, and generic guidelines developed for reference.

C-peptide was analysed using the automated Roche diagnostics (Manheim, Germany) E170 immuno-analyser (limit of detection 3.3pmol/L, inter- and intra-assay coefficients of variations <4.5% and <3.3% respectively).

Demonstration of random non-fasting C-peptide as a robust and pragmatic measure of beta cell function

Spearmans' correlation coefficient between random non-fasting C-peptide levels and gold-standard 90 minute C-peptide values were assessed in a subgroup of 50 participants who had undergone a standardised mixed meal tolerance test (MMTT).

All participants in this evaluation were insulin-treated; 9/50 had a clinical diagnosis of Type 1 diabetes, and 41/50 Type 2 diabetes. To ensure a range of C-peptide values, participants were selected on the basis of prior C-peptide

assessment to include participants with and without severe insulin deficiency (under/over 200pmol/L post-MMTT blood C-peptide or equivalent (22)), and thus 19/50 (38%) had C-peptide <200pmol/L. All had an estimated glomerular filtration rate (eGFR) >30ml/min/1.72m^2.

In brief, for the 50 patients completing a MMTT, participants attended before 11am having fasted from 10pm the evening before, without taking morning medication prior to arrival. Baseline bloods for glucose and C-peptide were taken, morning insulin given (28), and 160ml of Fortisip Compact (Nutricia, Trowbridge, UK) drunk within 10 minutes (content/100ml: carbohydrate 29.7g, protein 9.6g, fat 9.3g). Bloods for C-peptide were taken at 90 minutes, immediately centrifuged and stored at -80^{0}C, for later batched analysis.

Hypoglycaemia questionnaires

All insulin-treated patients in DARE were posted the Clarke & Gold hypoglycaemia questionnaire in May/June 2014 (26), and provided with a stamped addressed envelope for returning it. New DARE recruits were asked to complete the questionnaire on recruitment throughout the study time period.

Hypoglycaemia frequency and awareness were evaluated from the questionnaire, including calculation of the Clarke score (26).

Statistical analysis

The majority of clinical characteristics reported on were distributed in non-parametric fashion, so medians and interquartile ranges are reported throughout. C-peptide results were also non-parametric, not corrected by logging results. As such, the random non-fasting C-peptide results were split into deciles for the purpose of analysis.

Hypoglycaemia frequency was assessed by the Clarke Hypoglycaemia questions 3-6, asking respectively about frequency of experience of “moderate” or “severe” hypoglycaemia, and episodes where blood glucose was <3.5mmol/L with/without symptoms. Questions 5 and 6 categorise frequency of episodes into groups, thus for statistical analysis we assigned an estimated frequency per

answer for an approximation of numbers of episodes in the last month: 1-3 times became 2, 1 time/week became 1x4=4, 2-3 times/week became 2.5x4=10, 4-5 times/week became 4.5x4=18, and almost daily was estimated at 25 episodes. We evaluated the frequency of episodes with symptoms (Q5) and without symptoms (Q6) both added together for a total estimate of the number of episodes of blood glucose <3.5mmol/L in the last month, and separately.

Hypoglycaemia awareness was assessed by calculating the Clarke score (26): questions are allocated a score of “aware” (scoring 0) or “reduced awareness” (scoring 1), and added together. A score of 4 or more is classed as “reduced awareness”, and a score of 2 or fewer “aware”.

Non-parametric analysis was used for assessing the frequency of hypoglycaemia episodes and hypoglycaemia awareness in relation to C-peptide deciles and clinical diagnosis. Chi2 or Fisher’s exact tests were used for comparing proportions.

RESULTS

Participant characteristics

480 participants had both a random non-fasting C-peptide blood sample processed in the study period and had completed the hypoglycaemia questionnaire. Random non-fasting C-peptide levels reported here thus were measured a maximum of 1 year after questionnaire completion.

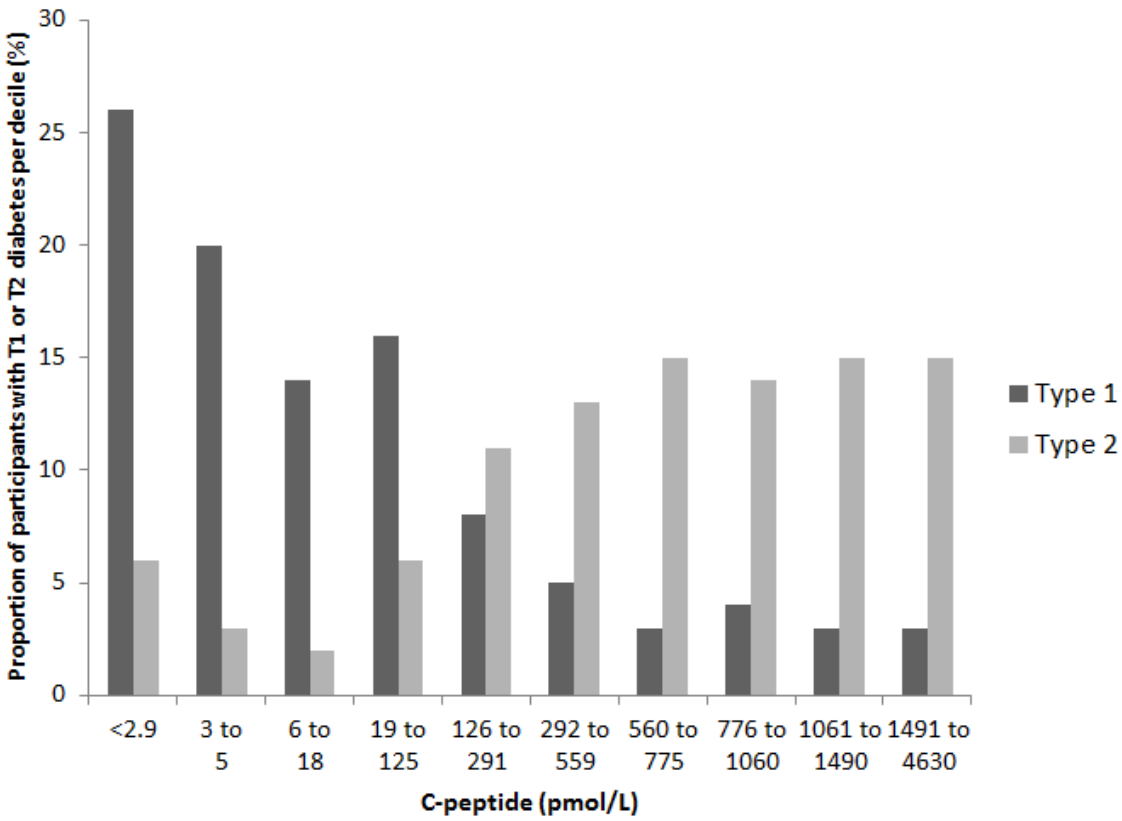
Clinical characteristics are summarised in **Table 1**. Participants were classified as having Type 1 or Type 2 diabetes by the RCGP classification guidelines (29): 40.3% had Type 1 diabetes, and 59.7% Type 2 diabetes.

Table 1: *Clinical characteristics (overall, Type 1 and Type 2 by RCGP guidelines). Median and interquartile range shown.*

	Overall	Type 1	Type 2
Number	480	194	286
Age now (yrs)	66 54-74	54 44-66	70 64-78
Male gender (n, %)	261 54%	93 48%	168 59%
BMI (kg/m^2)	28.6 25.3-32.7	26.8 23.4-30.1	29.9 26.9-34.7
Diabetes duration (yrs)	19 13-29	24 12-39	18 13-25
Time to insulin (months)	4 0-60	0 0-0	60 18-120
On any OHAs (n, %)	219 46%	22 11%	197 69%
On metformin (n, %)	203 42%	20 10%	183 64%
HbA1c (mmol/mol)	65 57-74	64 57-76	65 57-74
C-peptide (pmol/L)	291 6-920	8 2.9-125	697 250-1180

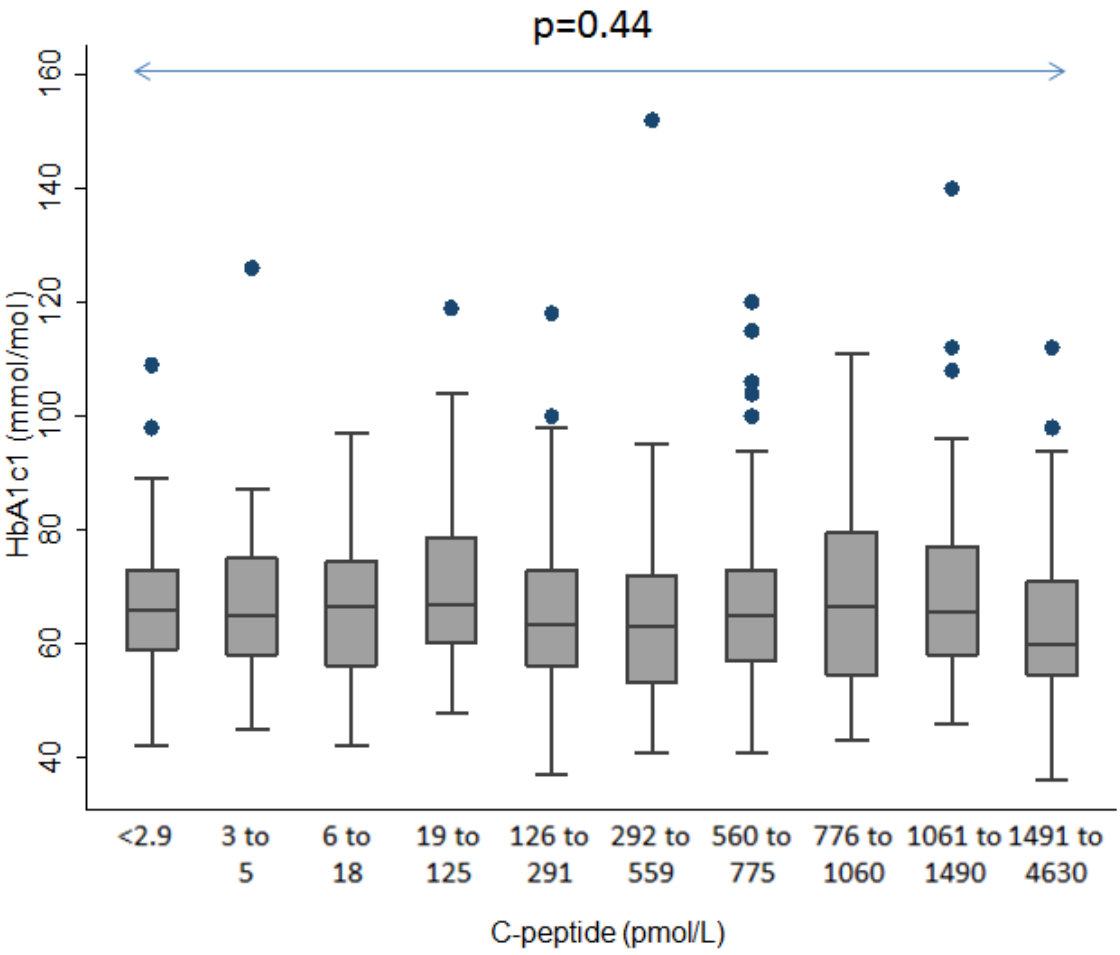
Median random non-fasting C-peptide was 291pmol/L (interquartile range 6-920pmol/L), and was significantly different between those with Type 1 and Type 2 diabetes: 8 (2.9-125) versus 697pmol/L (250-1180) respectively, p<0.0001. Results were analysed by C-peptide deciles for the whole group (approximately 48 participants in each group). **Figure 1** shows the distribution (proportion) of those with Type 1 or Type 2 in each C-peptide decile.

Figure 1: *Proportion of participants with Type 1 or Type 2 diabetes per C-peptide decile*



Of note, the distribution of HbA1c results across the C-peptide deciles did not significantly differ, p=0.44. The median HbA1c was 65mmol/mol (57-74), and the distribution across C-peptide deciles shown in **Figure 2**.

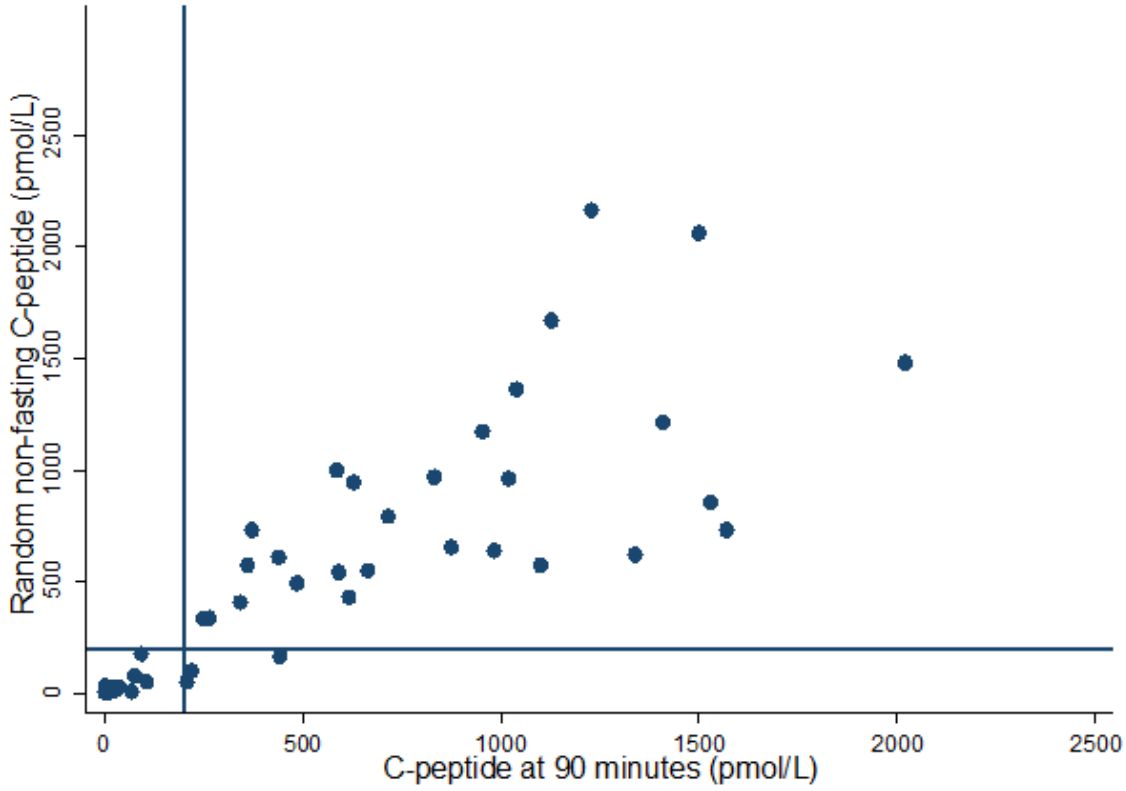
Figure 2: *Distribution of HbA1c values per C-peptide decile*



Random non-fasting blood C-peptide is strongly correlated with gold-standard 90 minute blood C-peptide in mixed meal tolerance test

Random non-fasting blood C-peptide (rCP) was strongly correlated with the 90 minute blood stimulated C-peptide (sCP): Spearman's rho correlation coefficient=0.93, p<0.0001, **Figure 3**. Median rCP of 415 pmol/L (IQR 19-789) was similar to sCP at 90 minutes, 368pmol/L (28-954), p=0.67.

Figure 3: *Correlation between random non-fasting blood C-peptide (rCP) and 90 minute blood stimulated C-peptide (sCP) in the mixed meal tolerance text for 50 patients: Spearman's rho correlation coefficient=0.93, p<0.0001. Reference lines at 200pmol/L.*



Lower C-peptide levels are associated with more frequent hypoglycaemia, regardless of clinical diagnosis

The total number of self-reported estimated episodes of blood glucose <3.5mmol/L in the last month (Q5 + Q6 on the Clarke questionnaire (26)), was significantly different across the C-peptide deciles, p=0.0001: the more episodes the lower the C-peptide decile, **Figure 4**. The pattern of hypoglycaemia frequency with C-peptide remained the same regardless of clinical diagnosis: the median number of episodes of blood glucose <3.5mmol/L in the last month decreased according to C-peptide decile, **Figure 5**.

Figure 4: *Total self-estimated number of episodes of blood glucose <3.5mmol/L in the last month, by C-peptide decile*

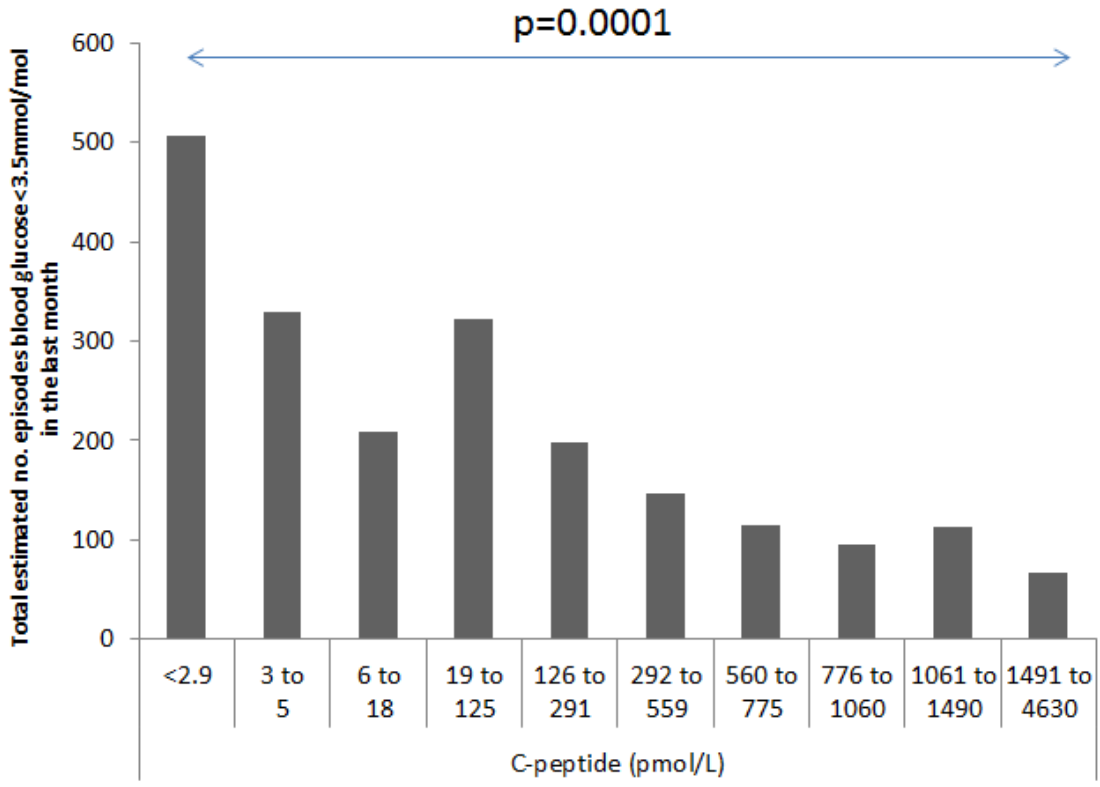
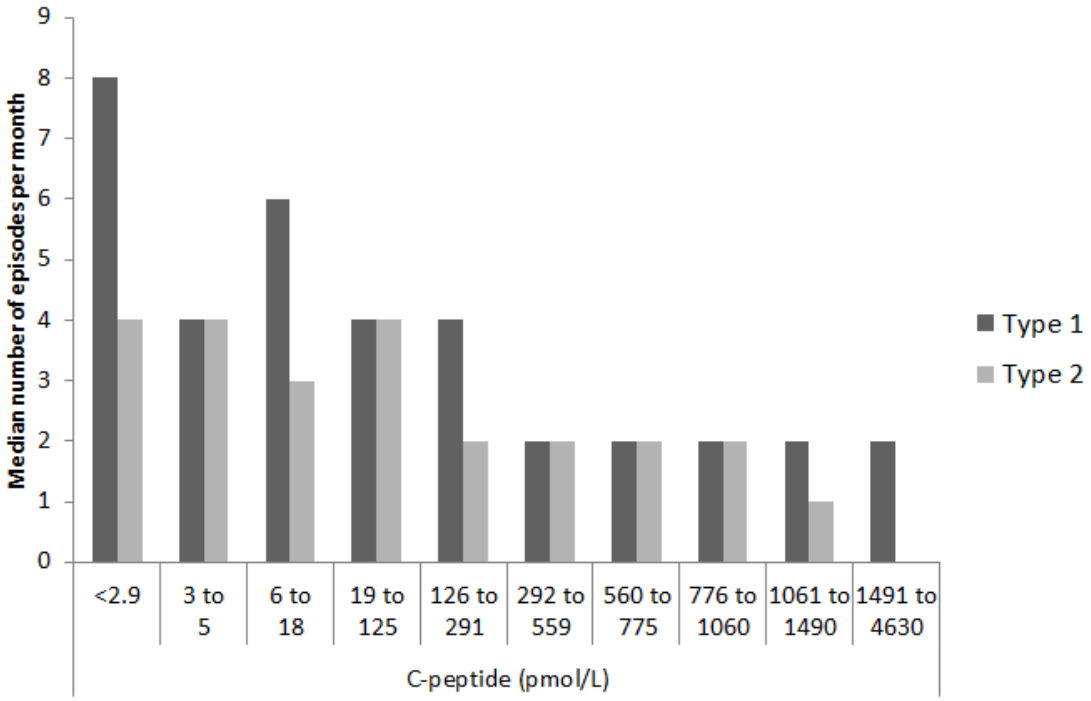
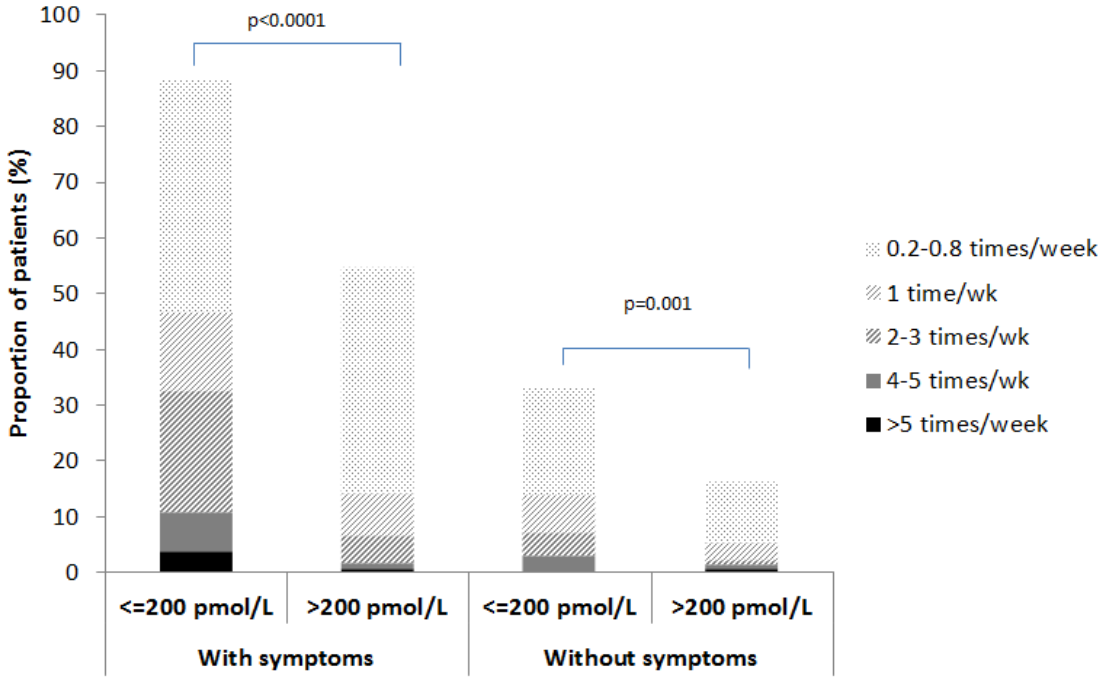


Figure 5: *Median estimated number of episodes of blood glucose <3.5mmol/L in the last month, by C-peptide decile and clinical diabetes diagnosis*



When looking at the accepted “traditional” level of C-peptide for severe insulin deficiency, 200pmol/L (22, 30), both self-reported episodes <3.5mmol/L with (Q5) and without (Q6) symptoms separately were significantly higher in those with a random non-fasting C-peptide less than 200pmol/L: p<0.0001 and p=0.001 respectively, **Figure 6**.

Figure 6: *Self-reporting of blood glucose levels <3.5mmol/L in the last month with and without symptoms, in those with a random non-fasting C-peptide above or below 200pmol/L*



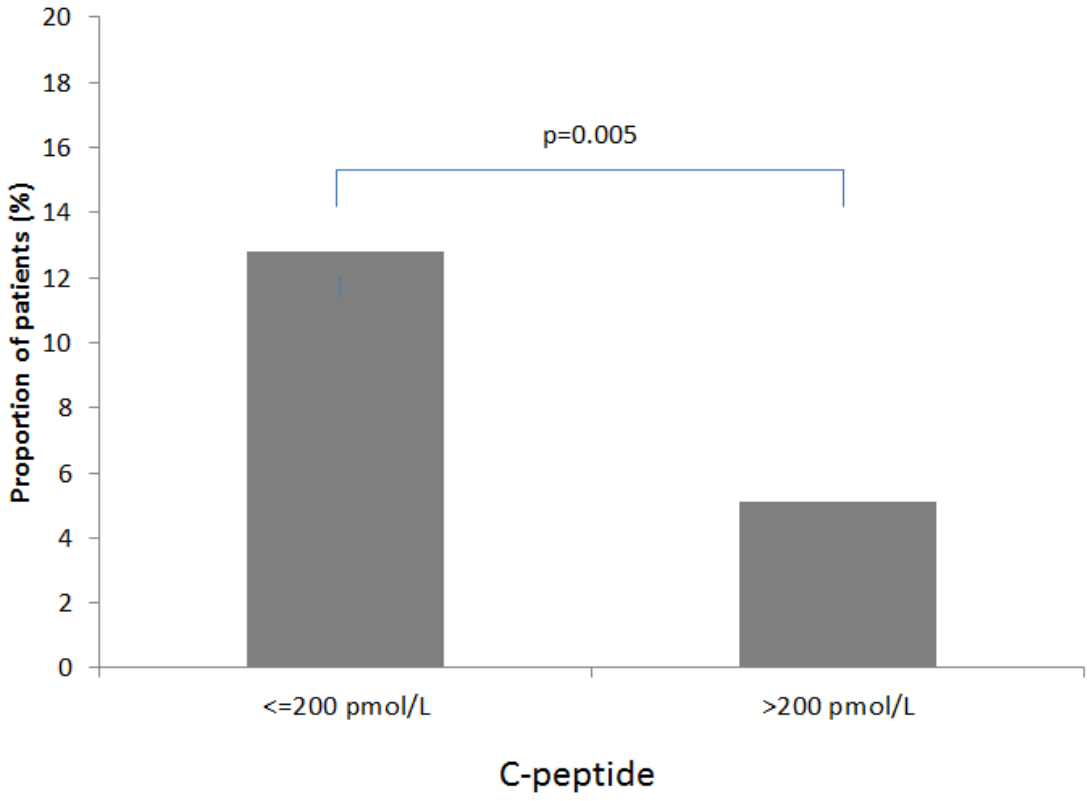
More severe episodes of hypoglycaemia – Q3 (“in the past 6 months how often have you had moderate hypoglycaemia episodes where you might feel confused, disorientated or lethargic and were unable to treat yourself?”), or Q4 (“in the past year how often have you had severe hypoglycaemic episodes where you were unconscious or had a seizure and needed glucagon or intravenous glucose?”), were not seen to increase significantly in frequency with lower C-peptide deciles (p=0.38 and p=0.53 respectively). The median C-

peptide seen in the 9.9% of people who reported at least one “severe” episode of hypoglycaemia in the past year (Q4), was 52 (IQR 5-785), compared to 319 (7-916) in those who had not reported an episode of severe hypoglycaemia, although this did not reach statistical significance, p=0.06.

Low C-peptide levels are associated with hypoglycaemia unawareness

Overall, 8.6% of participants had reduced hypoglycaemia awareness by the Clarke method, ie a score of ≥4 out of a possible 7. Those with a random non-fasting C-peptide below 200pmol/L had a higher rate of reduced awareness than those with a C-peptide above 200pmol, 12.8% vs 5.2%, p=0.005, **Figure 7**. This was more discriminatory than separating by clinical diagnosis, where 11.8% of those with Type 1 had reduced awareness compared to 6.6% of those with Type 2, p=0.06.

Figure 7: *Proportion of patients with C-peptide under or over 200pmol/L with reduced hypoglycaemia awareness (Clarke method)*



Comparing those with reduced hypoglycaemia awareness by the Clarke method, clinical features were similar in terms of age, gender, BMI, HbA1c, whether they were on oral agents in addition to insulin, and even type of diabetes, **Table 2**. Those with reduced awareness did have lower C-peptide with median C-peptide of 12 (2.9-977) compared to 370 (12-910), p=0.044, and had had diabetes for significantly longer 31 (16-43) vs 18 (13-27) years, p=0.0015.

Table 2: *Clinical characteristics for those with reduced awareness by the Clarke method, compared to those without reduced awareness*

	Aware	**Reduced awareness**	**P score for difference**
Number (%)	392 (91.4%)	37 (8.6%)	
Age (years)	66 (54-74)	67 (52-75)	0.6
Gender (% male)	204 (52%)	25 (68%)	0.07
BMI (kg/m^2)	28.7 (25.2-32.7)	28.2 (25.6-31.6)	0.7
Clinical diagnosis of Type 1 (%)	150 (38%)	20 (55%)	0.06
Duration of diabetes (years)	18 (13-27)	31 (16-43)	**0.0015**
Time to insulin (months)	10 (0-72)	0 (0-36)	0.068
Currently on OHAs (%)	46%	33%	0.14
HbA1c (mmol/mol)	65 (57-75)	66 (54-71)	0.35
C-peptide (pmol/L)	370 (12-910)	12 (2.9-977)	**0.044**

DISCUSSION

Summary

We have set up a simple automated system whereby random non-fasting C-peptide blood levels can easily be measured on routine HbA1c samples sent to the hospital laboratory, and have demonstrated good correlation between random non-fasting C-peptide levels and the gold-standard stimulated measure in a mixed meal tolerance test. The ability to monitor C-peptides longitudinally in patients is proving an invaluable source of information for further research, and will allow prospective studies to be done.

We have demonstrated a clear link between patient-reported hypoglycaemia frequency and C-peptide level in all insulin-treated patients, regardless of clinical diagnosis and glycaemic control. We have also reported an association between C-peptide levels and hypoglycaemia awareness.

Lower C-peptide levels are associated with more frequent hypoglycaemia, regardless of clinical diagnosis

Our findings of a correlation between decreasing C-peptide levels and frequency of self-reported hypoglycaemia frequency is consistent with the findings of the DCCT (11, 12, 17), and more recent findings at lower levels of C-peptide (16, 17). Although our C-peptide results cover a range of levels lower than that seen in the DCCT, it is interesting that in the current study the "traditional" 200pmol/L threshold appears to remain a significant one for increased frequency of hypoglycaemia seen. Recent modelling analysis based on the DCCT results concluded that there was a continuous relationship between C-peptide levels and the 200pmol/L cut-off may be too simple a definition for clinically significant residual insulin secretion (17); future additional data and statistical modelling in our expanding cohort may help explore this further.

The direct demonstration of a relationship between C-peptide levels and hypoglycaemia frequency in those with a clinical diagnosis of Type 2 diabetes is

less widely recognised. Certainly it is known that the frequency of hypoglycaemia in Type 2 diabetes is correlated with the duration of diabetes (18), and those with long-standing insulin-treated Type 2 diabetes can have similar rates of hypoglycaemia to those with Type 1 diabetes (10). It has been demonstrated that those with a clinical diagnosis of Type 2 diabetes can develop levels on insulin deficiency comparable to that of those with Type 1 (31, 32).

We have concentrated on reporting the results where participants reported episodes of blood glucose <3.5 with or without symptoms, as these had a wider range of responses. The prevalence of “severe” or “moderate” (requiring help) episodes were much lower, and our study is underpowered to detect a significant difference in the low rates of severe hypoglycaemia reported according to C-peptide levels, although a trend was seen. This is consistent with the recent analysis of ACCORD which suggested those participants with Type 2 diabetes who were unable to achieve the study’s treatment target of <6.0% (42mmol/mol) due to severe hypoglycaemia had significantly lower C-peptide levels than those who had similar glycaemic control but who did not experience hypoglycaemia (21) – with an adjusted odds ratio of 23.2 [95% CI 9.0, 59.5], p<0.0001.

Overall the finding that there is a clear relationship between C-peptide levels and hypoglycaemia frequency in those with a clinical diagnosis of Type 2 diabetes - is of significance. Combined with the increasing evidence of random non-fasting C-peptide as a practical routine test which can be done in routine clinical care, it may help support the argument that knowledge of C-peptide levels in insulin-treated diabetes patients may greatly enhance clinical care, and perhaps contribute to a risk assessment tool in identifying patients at high risk of hypoglycaemia.

Low C-peptide levels are associated with hypoglycaemia unawareness

We found that those with a C-peptide $\leq$200pmol/L had significantly higher rates of reduced awareness than those with a C-peptide over 200pmol/L. Clinical characteristics between those with reduced awareness and those without

reduced awareness did not differ significantly, apart from duration of diabetes, and C-peptide. This included clinical diagnosis of type of diabetes, age, and HbA1c. Impaired awareness being associated with duration of diabetes is consistent with previous findings in Type 1 diabetes (33), though not consistently in insulin-treated Type 2 diabetes (34).

The overall rates of hypoglycaemia awareness in the current study (11.8% of those with Type 1, and 6.6% with Type 2) were lower than those sometimes reported in the literature – eg estimated prevalence of 19-25% in Type 1 (33, 35), and 8-10% in Type 2 (18, 34). However these studies used the less discriminatory Gold score. If applied to the current study, 19.6% of those with Type 1 and 22.4% with Type 2 fitted the Gold criteria for having reduced hypoglycaemia awareness.

Strengths and weaknesses

This is a cross-sectional study performed in community-dwelling insulin-treated adults with diabetes, which simply required the participants to complete and post back in a stamped addressed envelope a standard hypoglycaemia questionnaire. The C-peptide samples were analysed on routine blood samples sent into the blood sciences laboratory. As such, this study includes a good cross-section of participants, including a lot of older adults who are often excluded from research studies.

The standardised hypoglycaemia questionnaire is not the most user-friendly, and some patients reported difficulty or frustration in completing it. However given its wide use in clinical and research settings it seemed appropriate to use. The random non-fasting C-peptide measures were taken as close to completion of the questionnaires as possible. The possible lag between the two means it is conceivable there may have been the occasional participant whose C-peptide levels were rapidly falling and as such there may have been a discrepancy between their results. However this is unlikely to have been a major problem: rapidly changing C-peptide levels are most likely to occur in those with recently diagnosed Type 1 diabetes, and during the course of the study new recruits to

DARE were completing the questionnaire at recruitment, and C-peptide levels would have been taken at the same time.

Random non-fasting C-peptide is well-correlated with the gold-standard 90 minute C-peptide in a mixed meal tolerance test, although can be suppressed in the setting of a low ambient glucose (22). It has the advantage over studies done with fasting C-peptide that it is a stimulated sample (16). Endogenous insulin levels (and thus C-peptide levels) have the potential to be suppressed by exogenous insulin treatment (28, 36, 37). It is possible that those with hypoglycaemia as a result of over-treatment with exogenous insulin, may have lower C-peptide levels on random non-fasting measurement than they are capable of. Taken in a clinical context however, a review of anyone with apparent low C-peptide or frequent hypos would be relevant, and thus a low C-peptide level could serve as an alert for review.

Clinical implications

Of particular importance in the current study, are the findings that there are a high proportion of people with a clinical diagnosis of Type 2 diabetes who have a C-peptide less than the traditional threshold of 200pmol/L, and that regardless of diagnosis type, lower C-peptide levels are associated with increasing frequency of hypoglycaemia.

Given the heterogeneity of the population of those with Type 2 diabetes, which is ever-expanding, a clinical tool which can help determine risk of one of the most feared (38-40) complications of diabetes, and thus help with choices regarding next-line therapy, monitoring and education needed, must be beneficial. C-peptide is cheap and practical for widespread outpatient clinical use, and could contribute hugely to this field.

CONCLUSION

We have demonstrated a clear link between patient-reported hypoglycaemia frequency and C-peptide level in all insulin-treated patients, regardless of clinical diagnosis. We have also reported an association between C-peptide levels and hypoglycaemia awareness. We propose measuring random non-fasting C-peptide could be a useful clinical tool in assessment and management of insulin-treated patients and their risks of hypoglycaemia.

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CHAPTER 7

Low c-peptide is associated with high glycaemic variability and hypoglycaemia in insulin-treated patients with Type 2 diabetes

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For submission

CHAPTER 7

Acknowledgments of co-authors and contributions to paper

Angus Jones and I came up with the project concept, and had useful discussions with David Strain as well as the other co-authors. Pratik Choudhary contributed invaluable advice re practicalities of continuous glucose monitoring. Angus Jones, Bea Knight and I wrote the project protocol, and Bea Knight and I obtained ethics approval. I recruited the patients and performed the study, with help from Bea Knight, and data support from Anita Hill and administrative support from Tina Libretto. I analysed the continuous glucose monitoring data for the study, with help and advice from Pratik Choudhary and Kai Tan Horng. Kash Patel has also been incredibly helpful in helping interpret the monitoring results to feed back to patients and clinicians. I wrote the manuscript with help from Angus Jones, and all co-authors contributed to discussions regarding results and reviewing the final manuscript.

Low c-peptide is associated with high glycaemic variability and hypoglycaemia in insulin-treated patients with type 2 diabetes

Abstract

Aims

We aimed to determine whether random c-peptide could be used as a biomarker for hypoglycaemia risk in insulin-treated Type 2 diabetes.

Methods

We assessed hypoglycaemia risk using continuous glucose monitoring (CGM, mean duration 4.1 days/person) and Clarke's hypoglycaemia questionnaire, in 34 insulin-treated participants with type 2 diabetes (diagnosed >35 years old, >2 years before starting insulin). 17 participants with severe insulin deficiency (random non-fasting serum c-peptide (rCP) <200pmol/L) were matched with 17 controls with rCP >500pmol/L, for HbA1c and gender.

Results

Glucose variability was greater in the low c-peptide group: standard deviation of glucose measurements 4.15 vs 3.01mmol/L, p=0.0005. This was despite similar mean glucose in the low vs high group: 10.2vs9.9mmol/L (p=0.50), HbA1c 72 vs 72mmol/mol (p=0.88), age 72.8 vs 71.8 (p=0.71), and BMI 26.6 vs 27.9kg/m^2 (p=0.19).

The number of episodes of hypoglycaemia on CGM (>20mins <4mmol/L) was higher in the low c-peptide group: median (interquartile range) 5.3(1.7-7.7) vs 0(0-2.3) episodes/person/week, p=0.003.

Participants in the low c-peptide group reported a median of 4 episodes <3.5mmol/L in the last month, compared to a median of 0 in controls with preserved c-peptide, p=0.002; the majority were asymptomatic.

Summary

In insulin-treated participants with type 2 diabetes matched for clinical characteristics and glycaemia, low endogenous insulin is associated with markedly increased hypoglycaemia risk compared to those with preserved insulin secretion. Assessment of a non-fasting C-peptide can identify patients with insulin-treated type 2 diabetes at high risk of hypoglycaemia.

INTRODUCTION

Hypoglycaemia is a serious complication of insulin-treated diabetes, and a limiting factor in obtaining good glycaemic control (1, 2). Detecting hypoglycaemia is not straightforward, particularly in the older population as symptoms are non-specific, and autonomic symptoms are less intense than in younger patients (2-5).

In patients with Type 1 diabetes, measured C-peptide has been shown to be strongly associated with hypoglycaemia risk (6-8) and high glucose variability (9). In the DCCT study intensively treated participants who developed mixed meal test stimulated C-peptide <200pmol/L (the vast majority of patients with Type 1 diabetes) had 3x as many severe hypos as those with preserved C-peptide above this level, despite higher HbA1c (7, 8). The threshold of 200pmol/L is commonly described as identifying absolute insulin deficiency, although modern assays can measure below this range (10, 11) and a relationship between hypoglycaemia and lower levels of C-peptide has been described (12, 13). For this reason clinical guidelines for Type 1 diabetes incorporate intensive strategies to minimise hypoglycaemia such as multiple daily injections, carbohydrate counting and insulin pumps.

Absolute insulin deficiency can occur in people meeting clinical criteria for long-standing type 2 diabetes, but the clinical consequences of this have not been examined (14). People with Type 2 diabetes and increasing duration of diabetes have higher glycaemic variability and higher risk of hypoglycaemia (15-18). A recent analysis of the ACCORD study suggested that those participants (with Type 2 diabetes) who suffered from severe hypoglycaemia had significantly lower C-peptide levels than those who had similar glycaemic control without hypoglycaemia (19). It is likely that patients with severe insulin deficiency will have high glycaemic variability and high hypoglycaemia risk whatever the underlying diabetes aetiology or classification, however the utility of C-peptide as a biomarker for glycaemic variability and hypoglycaemia has not been investigated outside of type 1 diabetes populations.

We aimed to determine whether random c-peptide could be used as a biomarker for hypoglycaemia risk in insulin-treated Type 2 diabetes.

METHODS

Participants

We recruited 34 participants with a clinical diagnosis of Type 2 diabetes receiving insulin therapy. All participants were diagnosed with diabetes ≥35 years old, started insulin ≥2 years after diagnosis, and had an estimated glomerular filtration rate (eGFR) >30ml/min/1.73m^2. Participants were recruited based on known C-peptide status (random non-fasted serum sample at recruitment visit) and clinical characteristics in the Diabetes Alliance for Research in England (DARE) database. 17 participants known to have severe insulin deficiency (previous testing demonstrating a non-fasting C-peptide <200pmol/L or equivalent (20)), were individually matched on gender and HbA1c (+/-10mmol/L) with a control participant who had preserved endogenous insulin secretion (previous testing demonstrating a non-fasting C-peptide >500pmol/L).

Ethics approval was obtained from the NRES Committee South West.

Baseline visit

Participants attended non-fasting within 5 hours of a meal. Following informed consent, baseline characteristics were collected, and blood taken to confirm C-peptide status and HbA1c. Clarke's Hypoglycaemia Questionnaire (21) was completed by all participants.

Continuous glucose monitoring

Following the baseline visit participants commenced at least three consecutive days' continuous glucose monitoring (CGM, iPro2 Professional, Medtronic, Watford, UK). For calibration purposes participants were asked to keep a diary of 3-4 self-monitoring blood glucose tests per day over the CGMS period, and these readings were entered into the iPro2 online software on downloading the data (23).

The following criteria were required for CGMS data to be included in analysis (22): at least three self-monitoring blood glucose (SMBG) calibrations per 24 hours, no more than 24 hours between SMBG readings, no missing data points, correlation between SMBG and iPro2 readings >0.77 in 24 hours, mean variation for each 24 hours (MAD%) <28 %; and a minimum of 24 hours meeting these criteria.

CGM analysis

The mean glucose, standard deviation of glucose measurements and mean amplitude of glycaemic excursions (MAGE) were analysed for individual patients using online software, EasyGV (23, 24).

An episode of hypoglycaemia was defined as >20 minutes at or below the interstitial glucose level of 4, 3 or 2.2 (LIG4, LIG3 and LIG2.2 respectively), and only complete once readings had been above the threshold for >20 mins. The proportion of participants in each group who had at least one episode of hypoglycaemia over the period recorded were compared using a chi2 test. Results were converted for each person to median number of episodes per week and duration of episodes per week, and by day (8am – midnight) and night (midnight – 8am).

Clarke's hypoglycaemia questionnaire

This is scored out of seven, with four or more suggesting reduced awareness (21).

Mixed Meal Tolerance Test

Approximately one week after the recruitment visit participants who were able attended fasted for a standardised mixed meal tolerance test (25), in which a stimulated blood C-peptide test was taken 90 minutes following ingestion of 160ml of Fortisip Compact (Nutricia, Trowbridge, UK). Samples were immediately centrifuged after collection and stored at -80C, for later batched analysis.

Laboratory analysis

Samples were processed by the Blood Sciences department, Royal Devon & Exeter Hospital. C-peptide was analysed using the automated Roche diagnostics (Manheim, Germany) E170 immuno-analyser (limit of detection 3.3pmol/L, inter- and intra-assay coefficients of variations <4.5% and <3.3% respectively).

GAD65 and IA2 autoantibodies were assessed using the Biokit automated Elisa System (BEST 2000, Biokit, Barcelona) following manufacturers' instructions. Cut-offs used were those based on the 99th centile for 500 non-diabetic individuals; for GAD65 the reference positive value was >54 units/ml, for IA-2 the reference positive value >15 units/ml.

Statistical analysis

Differences in continuous measures of glucose variability, hypoglycaemia and baseline characteristics between low and high C-peptide pairs were assessed using paired T tests and where T test assumptions were not met, the Wilcoxon signed rank test. Chi2 tests were used for comparing proportions.

RESULTS

Participant characteristics and data quality

Participant characteristics are shown in **Table 1.** HbA1c, age, duration of diabetes and BMI did not differ by C-peptide status, however participants with severe insulin deficiency had progressed more rapidly to insulin treatment, received higher insulin dose and were more likely to receive basal bolus insulin therapy.

Mean duration of CGM recording meeting inclusion criteria for analysis was 4.1 days (range 1 to 6.2 days), and this was similar between the two groups: 4.3 (range 1-6.2) days in the low C-peptide group compared to 3.9 (1.3-6) days in the high C-peptide group, p=0.34. The mean glucose across the period of CGM monitoring in the low vs high group was similar: 10.2 vs 9.9mmol/L, p=0.5.

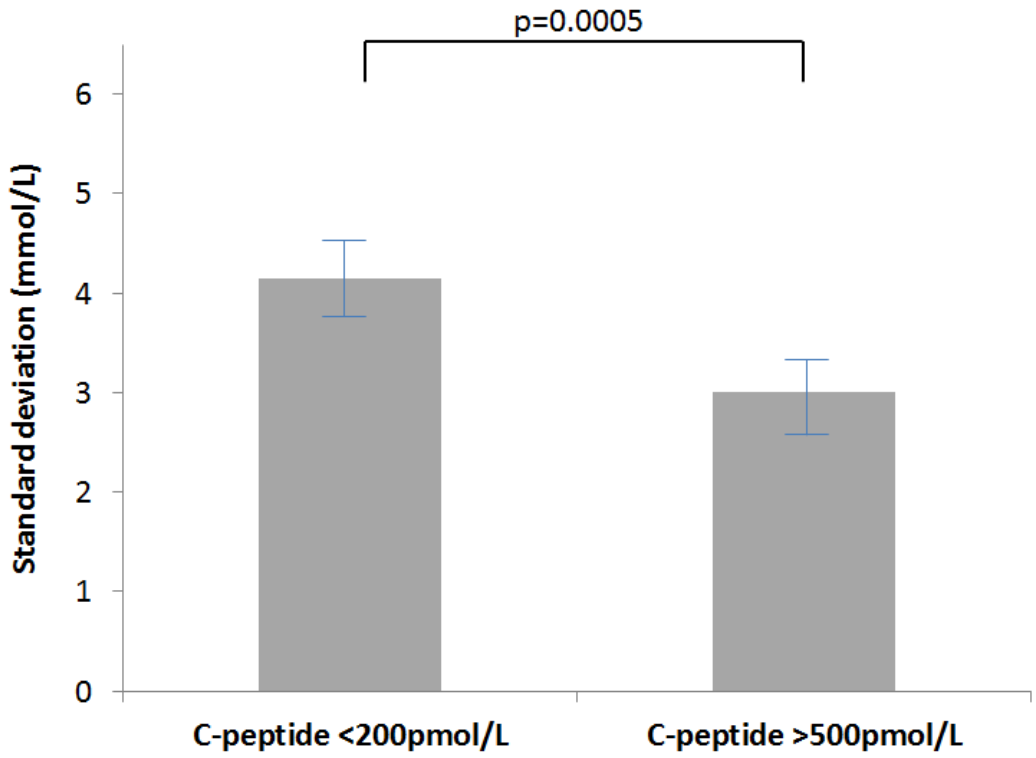
Table 1: *Participant characteristics. Median (interquartile range) shown, and p value for comparison between groups.*

	C-peptide <200	C-peptide >500	p
Number of subjects	17	17	-
C-peptide (pmol/L)	**15 (5-26)**	**1000 (789-1480)**	**<0.0001**
HbA1c (mmol/mol)	74 (63-81)	70 (66-80)	0.88
Age (yrs)	74 (71-80)	70 (67-77)	0.71
Diabetes duration (yrs)	25 (14-34)	21 (15-24)	0.13
BMI (kg/m^2)	26.4 (24.1-28)	27.7 (26.1-29.9)	0.19
Time to insulin (months)	48 (24-90)	108 (60-132)	0.03
Total dose insulin (units/kg/24h)	0.77 (0.54-0.88)	0.49 (0.33-0.66)	0.007
Proportion on basal bolus (%)	77%	6%	<0.0001
Proportion with ≥1 autoantibody (%)	59%	6%	0.0006

Standard deviation of glucose readings on CGM was higher in the low C-peptide group

Glucose variability was greater in the low C-peptide group: standard deviation of glucose measurements 4.15 vs 3.01mmol/L, p=0.0004, **Figure 1**. Mean amplitude of glycaemic excursions (MAGE) did not differ (7.05 (2) vs 6.03 (2.39), p=0.1.

Figure 1: *Mean (and 95% confidence interval) of standard deviation of glucose values on continuous glucose monitoring, by C-peptide group.*

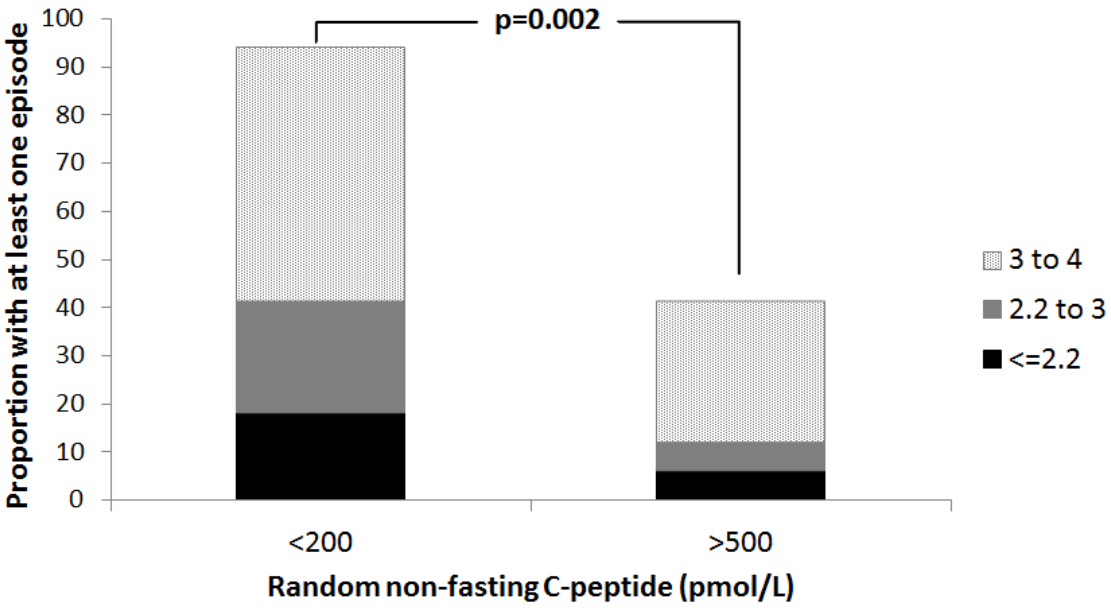


Hypoglycaemia is markedly more frequent in those with low C-peptide

94% of participants in the low C-peptide group experienced at least one episode of hypoglycaemia on CGM (≥20mins ≤4mmol/L), compared to 41% of those in the high C-peptide group, p=0.002, **Figure 2**.

The number of episodes of hypoglycaemia/person/week (≥20mins ≤4mmol/L) on CGM was also markedly higher in the low c-peptide group: median 5.3 (interquartile range (IQR) 1.7-7.7) episodes/person/week compared to 0 (0-2.3), p=0.0031. The total duration of hypoglycaemia was also higher in the low c-peptide group: median 407 (196-988) minutes/person/week, compared to 0 (0-305), p=0.0027.

Figure 2: *Proportion of patients by C-peptide category, with at least 1 episode of hypoglycaemia during continuous glucose monitoring. Shading denotes level of lowest recorded glucose*



88% of participants in the low C-peptide group experienced at least one episode of hypoglycaemia ≤4mmol/L during the daytime, compared to 24% of those in the higher c-peptide group, p<0.001. 71% vs 29% of participants experienced at least one episode overnight, p=0.038.

The frequency and total duration of episodes of hypoglycaemia less than 3mmol/L were also higher in the low C-peptide group (0 (0-3.7) episodes <3 per week vs 0 (0-0), p=0.04; 0 (0-386) hours <3 per week vs 0 (0-0), p=0.039). However differences in severe hypoglycaemia <2.2 were not significant (p=0.25). All hypoglycaemia episodes below 3mmol/L in the high C-peptide group occurred at night.

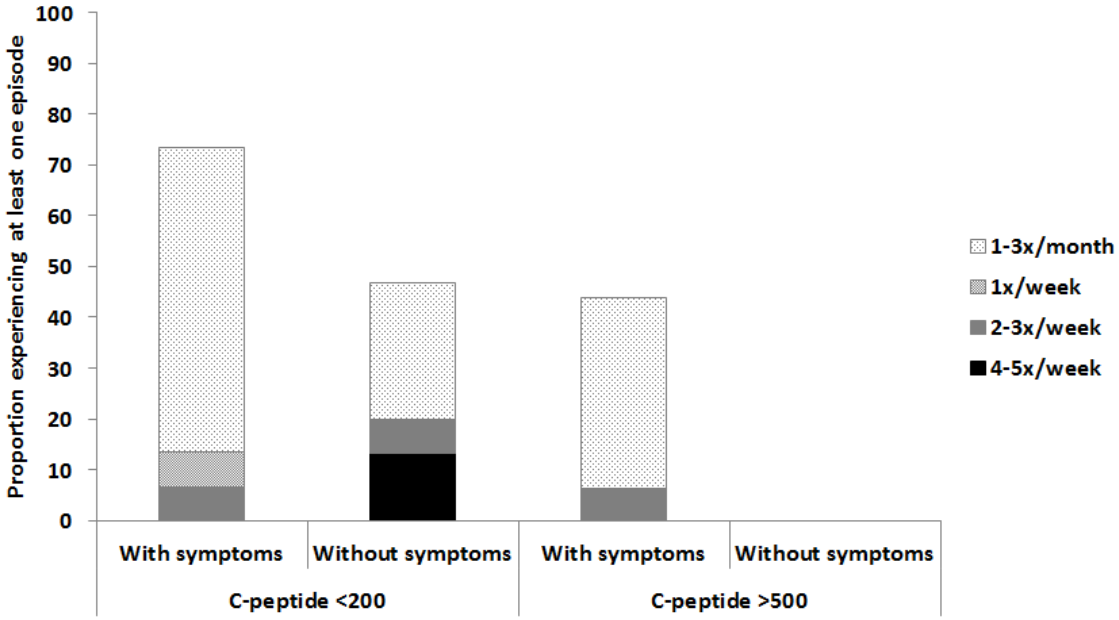
Participants in the low C-peptide group self-reported more hypoglycaemia

Participants in the low C-peptide group reported more episodes of hypoglycaemia (blood glucose level <3.5mmol/L) in the last month than those in the high C-peptide group, **Figure 3.** 47% of participants in the low C-peptide group reported at least one episode without symptoms, compared to none in the high C-peptide group, p=0.003.

The number of participants reporting severe hypoglycaemia episodes (questions 3 & 4 on the Clarke Hypoglycaemia questionnaire (21)) in the last year was low and not statistically different between groups.

“Hypoglycaemia awareness” as measured by a formal Clarke score of ≥4 did not differ between the C-peptide groups: 2/17 (12%) versus 0/17 (0%), p=0.49.

Figure 3: *Self-estimated frequency of episodes of blood glucose <3.5 with or without symptoms in the past month, by C-peptide group (Clarke hypoglycaemia questionnaire questions 5 & 6)*



DISCUSSION

Our results demonstrate that insulin-treated patients with a clinical diagnosis of Type 2 diabetes but low C-peptide levels have markedly higher rates and duration of hypoglycaemia in comparison to those patients with preserved endogenous insulin secretion. Participants with low C-peptide had a median of 5 hypoglycaemic episodes on continuous glucose monitoring per week compared to 0 in those with preserved C-peptide, and both self-reported hypoglycaemic unawareness and more severe daytime hypoglycaemia were entirely confined to participants with low C-peptide. These differences occurred despite similar glycaemic control, mean glucose, and clinical characteristics in those with and without preserved endogenous insulin secretion.

We have also demonstrated higher glucose variability in those in those with low C-peptide when assessed by standard deviation of blood glucose measured by continuous glucose monitoring, the most commonly used measure of glycaemic variability (26-28), and that which has been viewed as the most practical measure of quantifying glycaemic variability (18). A second marker of glycaemic variability, MAGE, showed a similar pattern but this did not reach statistical significance, which may reflect our small sample size and the higher sensitivity of standard deviation in detecting isolated swings in glucose.

Strengths & limitations

This is to our knowledge the first study specifically looking at whether C-peptide testing can help identify people with Type 2 diabetes at higher hypoglycaemia risk. However our findings are consistent with previous studies showing a strong relationship between C-peptide and hypoglycaemia in Type 1 diabetes (7, 8) and lower hypoglycaemia in those with preserved C-peptide in a Type 2 diabetes interventional trial (19).

Strengths of our study include that the two groups were well-matched by clinical characteristics, and importantly by HbA1c, and had similar mean glucose across the time of CGM. The mean age of participants was high, at 72.3, which

represents an age group at high risk of adverse consequences of hypoglycaemia where it can be especially difficult to optimise treatment and management.

A weakness of our study is that the sample size was small, although power was increased by matching of participants. A larger sample size may have been able to detect differences in other measures of glucose variability, such as MAGE, or episodes of severe hypoglycaemia. With the sample size achieved, at 80% power and at a significance level of 0.05, we were powered to detect only a 1.9 mmol/L mean difference in MAGE between groups therefore negative findings from this cohort should be treated with caution.

Random non-fasting C-peptide (rCP) was used as a practical measure in this study to categorise participants into the two groups, this is the most easily available C-petpide measure in clinical practice as the sample can be taken at the point of clinical contact. rCP has been shown to be well-correlated (r=0.91) with the gold-standard measure of C-peptide, 90 minute stimulated C-peptide in a mixed meal tolerance test (29). Of the 29/34 participants who underwent a mixed meal test 27/29 (93%) remained consistently in the same category on 90 minute post mixed meal C-peptide; the other two had stimulated C-peptide levels of 221 and 443 pmol/L respectively. The latter had an (asymptomatic) blood glucose of 2mmol/mol when the random non-fasting C-peptide sample was taken, confirming the recommendation of avoiding concurrent hypoglycaemia for accurate assessment of C-peptide levels (20), Hope et al, submitted).

Clinical implications

We have shown that insulin-treated patients with a clinical diagnosis of Type 2 diabetes who are similar in their clinical characteristics and HbA1c, but differ in their endogenous insulin levels as indicated by a random non-fasting C-peptide level, have substantially different rates of glycaemic variability and hypoglycaemia risk. This study demonstrates that a random non-fasting C-peptide sample taken when a patient with Type 2 diabetes is seen in clinic can

help identify those patients who - despite a clinical diagnosis of Type 2 diabetes, may have high glucose variability and hypoglycaemia risk.

Although many participants with low C-peptide in this study had positive islet autoantibodies, suggesting autoimmune aetiology, importantly these patients could not be identified by their clinical features and autoantibody status are rarely measured in patients with these characteristics in clinical care. Clinically it is not practical to do continuous glucose monitoring on all patients, and a random non-fasting blood C-peptide test could offer a useful additional tool in identifying those at most risk of hypoglycaemia and difficult glucose control.

This may be particularly pertinent in an older population where hypoglycaemia is often not recognised and consequences more severe. Individualising treatment in this population has been advocated (30-34), but there is little guidance on how to do this. Just one study to date specifically attempted to set individualised treatment targets (35), but found clinicians reluctant to deviate from traditional glycaemic targets even in the frail elderly. It is possible that random non-fasting C-peptide may offer a tool to give more confidence in helping to stratify risk of hypoglycaemia, and decide whether tight glycaemic control is appropriate.

An additional area where a robust biomarker for hypoglycaemia risk would be clinically useful, is in stratification of hypoglycaemia risk in relation to driving. Our results show clearly the markedly increased risk in those with low C-peptide and that this is independent of clinical features. In conjunction with the extensive evidence in Type 1 populations this data supports use of C-peptide testing as a biomarker for hypoglycaemia risk which is additive to knowledge of a person’s clinical features and diabetes subtype.

CONCLUSION

Random non-fasting C-peptide testing can identify patients with insulin-treated type 2 diabetes at markedly higher risk of hypoglycaemia, which may help risk stratification, decision making, and management in routine clinical practice.

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CHAPTER 8

DISCUSSION

CHAPTER 8: DISCUSSION

This thesis addresses several facets of managing diabetes in older patients, ranging from recognition of symptoms of hypoglycaemia, to accurate diagnosis of diabetes, and examining heterogeneity within “Type 2” diabetes – particularly in recognition of the fact that severe insulin deficiency can develop, and this may have significant impact on the risk of hypoglycaemia. Measurement of endogenous insulin levels using random non-fasting C-peptide in routine blood tests is proposed as a useful clinical measure, and two studies using this to demonstrate a relationship with the risk of hypoglycaemia are presented.

This chapter gives an overview of the main findings of the thesis – each chapter is briefly summarised, its implications and limitations discussed, and potential areas for further research suggested.

Complexity sums up physiological, psychological and social aspects of older people, and the complex and far-reaching implications of diabetes combined with this makes for fascinating yet challenging clinical scenarios. This thesis touches the tip of the iceberg, but hopefully has presented a potentially useful clinical tool that has been shown to be relevant, easy to understand and use, in order to help recognise those at high risk of hypoglycaemia, and thus help improve patient care.

Chapter 2

Are we missing hypoglycaemia?

Elderly patients with insulin-treated diabetes present to primary care frequently with non-specific symptoms associated with hypoglycaemia

Summary

Hypoglycaemia symptoms are non-specific, and in the elderly can be difficult to recognise, as may be hard to distinguish from non-specific symptoms caused by other factors associated with older age. This chapter looks in patients over the age of 65 in one general practice, at the frequency of hypoglycaemia, and the frequency of consultations with non-specific symptoms known to be associated with hypoglycaemia, in patients treated with insulin, sulphonylureas, just metformin, or who did not have diabetes.

Insulin-treated patients had a much higher documented rate of hypoglycaemia episodes/patient/year (1.1 vs 0.2 and 0.07 in the sulphonylurea-treated and metformin only-treated patients respectively), $p<0.0001$. In insulin-treated patients, 74% of those with a documented episode of hypoglycaemia over the year attended on another occasion with a non-specific “hypo clue” symptom, compared to 40% of those without a recorded episode of hypoglycaemia, $p=0.008$. Nausea, falls and unsteadiness were the most potentially discriminatory symptoms: 7/33 (21%) patients with hypoglycaemia attended on another occasion with nausea compared to 14/302 (5%) without hypoglycaemia, $p=0.002$; 10/33 (30%) vs 36/302 (12%) presented with falls, $p=0.007$; and 5/33 (15%) vs 13/302 (4%) presented with unsteadiness, $p=0.023$.

Limitations

One should be wary of drawing too many conclusions from this study; it was a small observational study done in one GP practice, and by design was attempting to tackle some of the most “woolly” corners of primary care consultations – ie the symptoms which do not consistently get reported by patients or carers, or asked about or documented by healthcare professionals. They are the sorts of symptoms which are filtered or “interpreted” at every level – ie by patients/carers, by healthcare

professionals - and probably by researchers. The recording is heavily dependent on the style of documentation in consultation notes, and hence on the individuals seeing the patients. They are also not routinely coded, hence manually going through every consultation record for every patient over a one year-period. Due to the likely low rate of recording of these symptoms, and the likelihood that if a healthcare professional was likely to record one from one patient they might be likely to record several, a rather “catch-all” definition of a “hypo clue” consultation was used, ie if one or more symptom was mentioned. Inevitably this led to high rates of “hypo clue” consultations seen, even amongst those without diabetes (0.76 episodes/patient/year) – but the rates did seem to differ when looked at in those most at risk of hypoglycaemia.

A systematic approach was used, although due to the labour intensity involved in looking manually at the free text of every consultation over a one year period for every patient, the recording in this study was done by one person. A sample of patients was repeated on a separate occasion and found to be consistently recorded, but the study could have higher validity if the consultations had been reviewed and recorded by another researcher as well.

The rates are presented as episodes per patient per year. For hypoglycaemia this was defined this as episodes directly confirmed by a doctor or nurse, paramedic or hospital (although the blood glucose was not always recorded). Data on hypoglycaemia episodes reported by patients (eg at their annual diabetes review) was also available, but it was felt to be more robust to concentrate on the confirmed episodes, given the variability in this additional data (being dependent on the patient’s awareness of hypoglycaemia and self-monitoring of blood glucose, and on the healthcare professional doing the annual review (usually practice nurse) to specifically ask and record consistently about the patients’ hypoglycaemia experience – ie not just ask those who are known to have had hypoglycaemia before, or those assumed to be at high risk).

The data collection here did not include a count of the total number of consultations had by each patient, ie it was not possible to calculate a total rate of consultations

per patient per year. It seems likely that insulin-treated patients may consult more often, and hence despite an apparently higher rate of “hypo clue” consultations, this may merely represent a higher rate of consultations per se. Presentation of the data as “at least one episode” attempted to minimise the possible effect this may have had. Additionally, the difference in falls seen in those with and without hypoglycaemia suggests that the findings in this study may be genuine, but would be more robust with this additional evidence.

The original pilot audit which led onto this study was done a few years earlier in the same practice, and thus the results obtained from this practice conceivably are not representative of other primary care practices. A primary care survey done of nearly 25,000 patients over the age of 70 found that of those who were on insulin or sulphonylureas, nearly 30% had an HbA1c of <7% (53mmol/mol), and 12% had an HbA1c <6.5% (48mmol/mol) (1). This is compared to 28% under 7% (53mmol/mol) and 8.5% under 6.5% (48mmol/mol) in this group of patients over the age of 65.

Future work

This was a cross-sectional study looking at any episodes of hypoglycaemia or “hypo clue” consultations over a year. Acknowledging the limitations of this sort of study, it would be of great interest to do a similar study in other practices, for the reasons mentioned above.

Despite the limitations in the current study, there do seem to be differences in the rates of “hypo clue” symptoms that may be teasing out something more than just an increased rate of consultation in those at risk of hypoglycaemia. To explore more clearly the possible link, and determine whether presentations of hypoglycaemia as non-specific symptoms are being missed, an examination of possible “hypo clue” consultations in the run-up to an index event of hypoglycaemia, could be much stronger.

The low rates of definite hypoglycaemia seen in one GP practice mean that in order to assess this properly, this sort of study could be done in a much larger dataset, eg the Clinical Practice Research Datalink (CPRD, previously General Practice Research Database, GPRD). The disadvantage of this is that again the non-specific

symptoms may be less well-coded. However a review of the possible association of more specific symptoms, eg of falls in those insulin-treated patients with hypoglycaemia versus not, could be examined.

As mentioned previously, knowing the rates of all consultations would help in determining whether the apparent increased rates of consultations with non-specific symptoms in those insulin-treated patients with a documented episode of hypoglycaemia over the year was a clinically relevant finding, and any future work on a similar theme should include this. It would require a clear definition of a "consultation", as documented "interactions" in primary care come in many guises.

Additionally, doing a larger scale study with similar design could also examine in more detail the possible relationship of HbA1c and hypoglycaemia/non-specific "hypo clue" consultations. The larger dataset would also allow more exploration/stratification particularly in terms of type of diabetes or duration – in the current study "insulin-treated" patients are reported as one group, but there will be differences in risks and frequencies according to these. Additionally other potential risk factors could be considered, eg comorbidities.

Any simple clinical message that has potential widespread utility in improving management of patients in primary care – including self-management – is worth exploring, even if not straightforward to do so! This study emphasises once again the widely-recognised side effect of hypoglycaemia in insulin-treated patients, but also suggests there may be certain symptoms, such as falls and unsteadiness, and nausea, which when occurring in insulin-treated patients, should serve as extra alerts to review carefully for possible unrecognized hypoglycaemia episodes, and to alter management accordingly.

Chapter 3

Assessment of Practical Classification Guidelines for Diabetes in insulin-treated patients

Summary

This study examines the accuracy of the UK Practical Classification Guidelines for diabetes (using age at diagnosis and time to insulin treatment), which were developed by the Royal College of General Practitioners and NHS Diabetes (2). It uses a "gold-standard" definition of Type 1 and Type 2 diabetes, based on endogenous insulin levels (measured by urinary C-peptide creatinine ratio, UCPCR), and time to insulin, and looked at patients who were insulin-treated, and who had had diabetes for at least five years. These guidelines are important as classification guidelines for diabetes are rare, and the correct diagnosis is vital for patients having the right treatment and education.

Compared to the gold-standard definitions of Type 1 and Type 2 diabetes, the UK Practical Classification Guidelines correctly classified 86% of insulin-treated patients at least 5 years after diagnosis. The group which were most frequently misclassified were those who developed diabetes over the age of 35, but by virtue of being put immediately on insulin were classed as having Type 1 diabetes by the guidelines. In fact 56% of these patients were still producing significant amounts of endogenous insulin after 5 years.

Time to insulin and age of diagnosis were found to be stronger predictors of diabetes type than BMI, and no improvement to the guidelines was found by altering the clinical criteria or cut-offs used – and thus it was concluded that the UK Classification Guidelines were useful in their current form.

Limitations

Only insulin-treated patients with a diabetes duration of >5 years were studied, in order to avoid the honeymoon period where some with Type 1 diabetes may still have been producing insulin. If considering all patients with diabetes the guidelines

would perform even better – ie patients tablet or diet-treated >5y from diagnosis are likely to have been correctly diagnosed with Type 2 diabetes.

The tool which would be of most use to healthcare professionals would be one which could correctly classify people at diagnosis. However at present there is insufficient data to do this too accurately. The UK Classification Guidelines (2) have time to insulin as one of their criteria. UCPCR can correctly identify people with severe insulin deficiency early on (ie Type 1), but as above cannot rule out a diagnosis of Type 1 diabetes in someone who was producing reasonable endogenous levels of insulin early on, as they may still be in the honeymoon period.

The gold-standard criteria used a UCPCR cut-off of 0.2nmol/mmol, which has a sensitivity and specificity of 100% and >95% to detect absolute insulin deficiency. In a large scale study of community-dwelling adults, this is the best – and most practical - “gold-standard” available. UCPCR levels have been shown to be 1.48-fold higher in women than men, due to the lower rates of creatinine excretion as a result of lower muscle mass (3). This could mean that slightly altered cut-offs should be used for the gold-standard definition, which was not done in this study. Insulin treatment has the potential to suppress endogenous insulin, but this rarely affects diabetes classification (4) – and the small possibility of an over-diagnosis of Type 1 diabetes is a safer direction of error than the opposite.

Due to recruitment locations and difficulty in recruiting Asian patients, the majority of recruited patients in this study were white Caucasian, with only 30 Asian patients studied. Comment cannot therefore be made on the accuracy of the UK Classification Guidelines in high prevalence populations, and further work is needed in these groups.

Limited data on BMI at diagnosis was available only for 60% participants; this could be improved in future prospective study.

The two main types of diabetes were addressed in this study, but there are alternative subgroups such as genetic forms of diabetes (e.g. MODY). These are rare but also part of the UK guidelines, and have their own criteria for diagnosis. It is

important the clinician takes into account other factors that may indicate these. The term latent autoimmune diabetes in adults (LADA) is sometimes proposed for adults with islet autoantibodies who eventually (up to 12 years) become severely insulin-deficient, but do not require insulin for at least the first 6 months. However LADA is not included in international guidelines for classification or treatment, and given endogenous insulin status determines treatment requirements, it was felt to be appropriate to classify according to UCPCR status as per these “gold-standard” criteria.

Future work

The most useful clinical tool would be one that could be used close to diagnosis of diabetes, in order to help classify patients accurately. Further large-scale prospective study closer to diagnosis, taking into account clinical factors including age and gender, BMI at diagnosis, time to insulin, and measures such as C-peptide and pancreatic antibodies would be of great value - and to follow results over time – eg regular C-peptide follow up in order to confirm the future course of insulin deficiency. It would also be very important to do these studies in other ethnic groups, and in a paediatric population.

On a smaller scale, it could be interesting to follow up those identified in the current study as misclassified, and those diagnosed with Type 2 and still producing insulin beyond 5 years, eg to find out if some might be able to withdraw successfully from insulin.

Chapter 4

Urinary C-peptide creatinine ratio detects absolute insulin deficiency in Type 2 diabetes

Summary

This study looked at 191 insulin-treated patients with a clinical diagnosis of Type 2 diabetes, and found 6% had two UCPCR tests suggesting severe insulin deficiency (<0.2nmol/mmol). These patients undertook a mixed meal tolerance test, to assess C-peptide levels by the “gold-standard” C-peptide test of 90 minute stimulated serum C-peptide (sCP). This confirmed that overall 3% insulin-treated patients with a clinical diagnosis of Type 2 diabetes had severe insulin deficiency (sCP <0.2nmol/L (200pmol/L)).

Only a third of those with absolute insulin deficiency were treated with (arguably) the optimal treatment regimen (basal bolus insulin regimen), suggesting their severe insulin deficiency had not been recognised by healthcare professionals. This was perhaps not surprising given the only notable clinical differences were that those with confirmed absolute insulin deficiency had shorter time to insulin than those with UCPCR>0.2nmol/mmol (median 2.5 v 6 years, p=0.005), and slightly lower BMIs (25.1 v 29.1kg/m2, p=0.04).

Overall this study therefore showed that absolute insulin deficiency may occur in long-standing T2D, and cannot be reliably predicted by clinical features or autoantibodies. Its recognition should help guide treatment, education and management. UCPCR is a practical non-invasive method to aid detection of absolute insulin deficiency, with UCPCR>0.2nmol/mmol being a reliable indicator of retained endogenous insulin secretion.

Limitations

This was the first time UCPCR had been used in a reasonably large scale community study, and it was possible as a result to iron out some practical methodological issues after reviewing the reasonably high rate of low UCPCR results upon repeat becoming “positive”, and/or MMTT sCP being “positive” despite two low

UCPCR results. The study was done around the time of some postal strikes, and thus some samples returned beyond the 5 days that UCPCR testing had been validated for. Other points were noting that some patients had been tipping out the boric acid preservative from the urine specimen pots. It was also worth considering checking for a concurrent urine infection in the presence of a surprising low UCPCR result. Variability in meal stimulus may have also played a role, supported by the finding that in four patients despite two home UCPCR results suggestive of absolute insulin deficiency, a higher UCPCR and measurable sCP (though low) levels were seen under controlled MMTT conditions. This suggests the MMTT was more stimulating than the home meals of these patients as they were still able to mount an insulin response when maximally stimulated. Finally practically, although every precaution was taken to process (centrifuge) and freeze samples immediately, there is a chance that samples acquired from those MMTTs done by two different investigators in patients' own homes may have encountered some variation in consistency.

Future work

As demonstrated in this thesis, given the potential importance of identifying those people with severe insulin deficiency, and having ironed out the above practical issues, it would be highly valuable to do another study assessing the prevalence of severe insulin deficiency in a population with a clinical diagnosis of "Type 2 diabetes" – and as per the discussion around the previous chapter, look further as to whether there are any clinical features particularly associated with it. In other studies UCPCR has been found to be particularly practical for measuring endogenous insulin levels in children, but in adults with insulin-treated diabetes the relative frequency of blood tests means that random non-fasting blood C-peptide may prove more practical (as well as accurate) for any such future studies.

Chapter 5

Random non-fasting C-peptide:
bringing robust assessment of endogenous insulin secretion to the clinic

Summary

Traditionally it was thought that C-peptide degraded rapidly and thus needed to be sent on ice to the lab for rapid centrifuging and analysis. As such it has only really been measured regularly in the context of research studies – and hence the development of a clinical speciality which does not routinely measure the hormone which it is trying to respond to. UCPCR was hence a turning point. However this study came about because of the realisation that C-peptide is substantially more stable in blood than previously thought (5), and thus samples could be sent in at room temperature from outpatients or primary care without degrading. If measuring random non-fasting C-peptide was accurate, this could potentially revolutionise clinical measurement of C-peptide.

This study therefore compared the "gold-standard" measure of C-peptide at 90 minutes in a MMTT (sCP) with random non-fasting blood C-peptide (rCP) and random non-fasting urine C-peptide creatinine ratio (rUCPCR) in 41 participants with insulin-treated diabetes. The impact of concurrent glucose when taking the random samples was also evaluated.

rCP and sCP levels were similar: median 546pmol/L and 487pmol/L, p=0.92. rCP and sCP were also highly correlated, which improved even further when excluding samples with concurrent glucose <8mmol/L. For detection of severe insulin deficiency (<200pmol/L), rCP was highly sensitive (100%) and specific (93%); for looking at a cut-off often taken to define insulin "requirement" (<600pmol/L), rCP was less discriminatory (sensitivity 87% and specificity 83%). rUCPCR was also well-correlated with sCP, and an rUCPCR cut-off of <0.2nmol/mmol gave a sensitivity and specificity of 83% and 93% to detect severe insulin deficiency.

Random non-fasting C-peptide measures may thus give the potential of assessing C-peptide levels at the point patients are seen for clinical care, which will certainly increase their utility.

Limitations

The main limitation of this study was the very modest sample size, and this meant that the confidence intervals for the results were wide. The sample size also limited our ability to assess the impact of concurrent glucose on rCP testing, which seems to have some impact.

Additionally the population in this study may not be wholly representative of the patients where C-peptide testing may have most utility (difficult to classify diabetes) in that they are older patients (median age 73), nearly all white Caucasian, and were selected on the basis of a clinical diagnosis of Type 2 diabetes with or without discordant C-peptide.

Future work

Further validation of the random non-fasting C-peptide measure in a larger and more heterogeneous insulin-treated group would allow more confidence in its accuracy in comparison to the “gold-standard” 90 minute C-peptide in the MMTT, and in particular the impact of concurrent glucose.

Obtaining understanding of the variability of the random non-fasting C-peptide measure in the same individual on different occasions over a short time period would also be highly valuable for interpretation purposes.

It would be fascinating to see if people who had “undetectable” rCP measures on some occasions had measurable rCP levels on other occasions. Overall the opportunity to easily prospectively assess C-peptide levels over time is immensely exciting.

Chapter 6

A clinically collected random non-fasting C-peptide sample may be used as a risk assessment tool for hypoglycaemia frequency and awareness in insulin-treated patients

Summary

Further to the previous study, we set up in this study an automated system for measuring random non-fasting C-peptide on routine blood samples sent in for HbA1c testing from 480 consenting insulin-treated patients (Type 1 and Type 2). We asked those participating to complete a questionnaire regarding their experience of hypoglycaemia frequency and awareness.

An increased frequency of recognised hypoglycaemia episodes with blood glucose <3.5mmol/L was associated with lower C-peptide deciles, p=0.0001, regardless of the clinical diagnosis of Type 1 or Type 2. This was despite similar HbA1c levels across all C-peptide deciles, p=0.44.

Median C-peptide was lower in those with hypoglycaemia unawareness: 12pmol/L (2.9-977) vs 370(12-910), p=0.044. Other than duration of diabetes (31(16-43) vs 18(13-27) years, p=0.0015), clinical characteristics were similar including age, gender, BMI, HbA1c, and type of diabetes.

This clear link between patient-reported hypoglycaemia frequency and awareness, and C-peptide level in all insulin-treated patients, regardless of clinical diagnosis, is exciting. We propose measuring rCP could be a useful clinical tool in assessment and management of insulin-treated patients and their risks of hypoglycaemia.

Limitations

Although given its wide use in clinical and research settings it seemed appropriate to use the standardised hypoglycaemia questionnaire, it is not the most user-friendly, and some patients reported difficulty or frustration in completing it. There were instances where answers for some individuals were slightly inconsistent between questions, but we did not over-interpret, and the hope is that the reasonably large

number of participants means any inconsistencies will not affect the overall results. For reasons of consistency we chose to use the more “robust” Clarke method for assessing hypoglycaemia unawareness, which takes results from several questions. This may identify a slightly different (smaller) group of patients who meet the criteria for having unawareness than might be detected in an outpatient clinic (where they might be screened with the one-off Gold question regarding their awareness).

The random non-fasting C-peptide measures were taken as close to completion of the questionnaires as possible, and within one year. The possible lag between the two means it is conceivable there may have been the occasional participant whose C-peptide levels were rapidly falling and as such there may have been a discrepancy between their results. However this is unlikely to have been a major problem: rapidly changing C-peptide levels are most likely to occur in those with recently diagnosed Type 1 diabetes; during the course of the study new recruits to DARE were completing the questionnaire at recruitment, and C-peptide levels were taken at the same time.

Random non-fasting C-peptide as previously discussed is well-correlated with the gold-standard MMTT measure of C-peptide. Endogenous insulin levels (and thus C-peptide levels) have the potential to be suppressed by exogenous insulin treatment (4, 6), and hence it is possible that those with hypoglycaemia as a result of over-treatment with exogenous insulin may have lower C-peptide levels on random non-fasting measurement than they are capable of. Unfortunately we did not have concurrent glucose levels available. Taken in a clinical context however, a review of anyone with apparent low C-peptide or frequent hypos would be relevant, and thus a low C-peptide level could serve as an alert for review.

Future work

Expansion of this simple study in terms of size, and in terms of following up these participants over time would be extremely valuable, and now the precedent of setting up automated rCP analysis has been set, should be relatively easy to do. With a bigger sample size it may also be possible to explore further whether endogenous insulin levels and self-reported hypoglycaemia frequency is indeed a continuous relationship, or whether any particular cut-offs of C-peptide are associated with

increased rates of hypoglycaemia/ unawareness. If this was seen to be so, if there is any difference according to diabetes diagnosis. Very important questions still remain regarding the true impact of HbA1c on hypos, and whether perhaps HbA1c targets should be different according to endogenous C-peptide level. Further study of this style, with a larger sample size, and collecting concurrent HbA1c, glucose and C-peptide levels along with concurrent hypoglycaemia questionnaire completion could help address this further.

The final thing which would be incredibly useful, is to develop a more user-friendly (and discriminatory) hypoglycaemia questionnaire, which could easily be validated in this population.

Chapter 7

Low c-peptide is associated with high glycaemic variability and hypoglycaemia in insulin-treated patients with type 2 diabetes

Summary

In order to address the developing hypothesis that insulin-treated people with a clinical diagnosis of Type 2 diabetes but differing endogenous insulin levels are at different risks of hypoglycaemia, this final study recruited pairs of patients who were matched by gender and HbA1c, but differed in endogenous levels. They underwent continuous glucose monitoring (CGM), and completed the standardised hypoglycaemia questionnaire.

Those with a low random non-fasting C-peptide (<200pmol/L) had a much greater glucose variability than those with higher rCP levels (>500pmol/L): standard deviation of glucose measurements 4.15 vs 3.01mmol/L, p=0.0005.

The number of episodes of hypoglycaemia on CGM was higher in the low C-peptide group: median (interquartile range) 5.3(1.7-7.7) vs 0(0-2.3) episodes/person/week, p=0.003. Consistent with this, the participants in the low C-peptide group reported more episodes of hypoglycaemia, the majority being asymptomatic.

Limitations

The sample size was small, although power was increased by matching of participants. A larger sample size may have been able to detect differences in other measures of glucose variability, such as MAGE or episodes of severe hypoglycaemia.

As previously discussed, rCP was used as a practical measure in this study to categorise participants into the two groups, this is the most easily available C-petpide measure in clinical practice as the sample can be taken at the point of clinical contact, and we have shown it to be well-correlated with sCP. In this study 93% of patients remained in the same category when sCP was assessed; those which did not strictly stay in the <200pmol/L group still had low sCP levels, of 221

and 443 pmol/L. Of note however the latter had an (asymptomatic) blood glucose of 2mmol/mol when the rCP sample was taken, confirming the recommendation of avoiding concurrent hypoglycaemia for accurate assessment of C-peptide levels (7).

Future work

It would be great to repeat this study with larger numbers, and to also recruit matched patients in the “middle” C-peptide range (200-500pmol/L) – in order to replicate findings, confirm an “interim” level of hypoglycaemia with the “middle” group, and hopefully to be able to demonstrate differences in other glucose variability measures. It would also be very interesting to be able to stratify people in more detail, eg according to their HbA1c, or to their insulin regimens.

It would also be extremely valuable to assess changes to treatment on the basis of endogenous insulin levels, and see if the glycaemic variability and risk of hypoglycaemia decreases.

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FINAL SUMMARY

This thesis has examined some of the challenges in managing older people with diabetes. Measurement of C-peptide, initially using the timed spot urine test UCPCR, and then the random non-fasting blood test, has been used to evaluate patients' endogenous insulin levels. We have demonstrated that patients with a clinical diagnosis of longstanding Type 2 diabetes can develop severe insulin deficiency, and that patients in this category have more marked glycaemic variability and more frequent hypoglycaemia than clinically similar patients with preserved endogenous insulin levels. Using random non-fasting C-peptide levels and questionnaires, we have also demonstrated that patients with low endogenous insulin levels are at increased risk of hypoglycaemia regardless of their clinical diagnosis of Type 1 or 2 diabetes. We have also shown data which suggests that hypoglycaemia is not always recognised in older patients with diabetes.

Further work is needed, but it seems that random non-fasting C-peptide could easily be integrated into routine clinical practice in order to help evaluate older patients with diabetes who may potentially be at risk of hypoglycaemia, in order to help detect those at highest risk. This would help in getting the right strategies in place for minimizing hypoglycaemia and maximizing quality of life for these individuals, as well as helping target the right resources to the right people.

There is much scope for further work in this area – eg further work to help clarify appropriate individual diabetes goals for older patients, which may include work on frailty, weight loss, cognition and HbA1c to name but a few; and perhaps looking at possible use of C-peptide

measurement to review treatment in older patients. I hope to be able to take some of this work forward from here.

I would once again like to thank wholeheartedly all the patients who have inspired me; those who have participated in this research, and shared their experiences and stories with me. I would also like to thank my inspirational and generous colleagues and friends in the Clinical Research Facility and the Diabetes & Vascular Research Centre.

APPENDIX

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FEATURE ARTICLE

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Hypoglycemia in the elderly

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Abstract

Hypoglycemia is a common, under-recognized complication of the management of type 2 diabetes. Elderly individuals have a higher burden of co-morbidities, cognitive impairment, physical dysfunction and frailty, which makes them more vulnerable to complications of hypoglycemia, such as falls, fractures, cognitive impairment and cardiovascular events, than younger patients. Furthermore, with ageing comes impairment of autoregulatory responses, which means the symptoms of hypoglycemia are often less specific, and are therefore either missed or incorrectly diagnosed as transient ischemic attacks or other cerebrovascular events. Older adults with diabetes have a greater risk of hypoglycemia associated with the physiological decline of ageing, and the extended duration of diabetes and insulin treatment. The elderly are also more prone to the effects of hypoglycemia such as the increased risk of accidents, falls and fractures, hospitalizations, in-hospital mortality, and long-term impairment of cognition. Using individualized treatment targets to base treatment strategies around individual circumstances may reduce the risk of hypoglycemia.

Keywords: *diabetes, elderly people, hypoglycemia*

Introduction

Type 2 diabetes is one of the most common chronic conditions in older adults, and the number of elderly individuals with diabetes is growing worldwide. For example, of the 2.6 million people in the UK with diabetes, at least half are over 65 years old.[2] The prevalence of diabetes in the elderly is more than 10% compared with 4.1% in the general adult population,[1] and approaches 25% in care home residents.[2] The management of elderly patients presents unique challenges. Episodes of hypoglycemia are a major complication of the treatment of diabetes with insulin and some oral medications. The consequences of hypoglycemia may be much greater in the frail older population than in younger adults.

This older population with diabetes represents a heterogeneous group, ranging from those who have been diagnosed recently (mainly with type 2 diabetes) to those with longstanding type 2 diabetes or type 1 diabetes, and from fit and active people to frail institutionalized individuals. Treatment of elderly patients also varies considerably. Once diabetes is established, the principal aims of 'good diabetes care' comprise blood glucose lowering, managing cardiovascular risk and identifying and treating long-term complications.[3] As glycemic control tends to deteriorate with disease progression, stepwise intensification of treatment is usual. This often includes prescription of sulfonylureas (SUs) and insulin, the agents most likely to precipitate hypoglycemia.

The utilization of these agents in order to achieve strict glycemic control is facing increasing scrutiny. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) study[4] demonstrated increased mortality with intensive treatment using strategies based around the use of SUs and insulin. Other studies have not shown this increased mortality with stricter glycemic control, but they have also failed to show any improvement in all-cause mortality.[5-7] Meta-analysis of five relevant randomized controlled trials[4-6, 8, 9] that examined the effect of intensive glycemic control on major outcomes in type 2 diabetes, has demonstrated that stricter glycemic control (an average of 0.9% reduction in HbA1c maintained over 5 years from a mean baseline of 7.8%) can lead to a 17% reduction in events of non-fatal myocardial infarction, and a 15% reduction in events of coronary heart disease.[7] In the Action in Diabetes and Vascular disease: preterAx and diamicroN MR Controlled Evaluation (ADVANCE) study,[5] severe hypoglycemia was associated with increased risk of macrovascular events, microvascular events, and death from both cardiovascular and non-cardiovascular causes,[10] although not in a clear exposure-outcome or dose-response manner. The link between hypoglycemia and other conditions, which is also relevant to quality of life in older patients, such as diminishing cognitive function, necessitates a better understanding of the precipitants of hypoglycemia and its avoidance in older people with diabetes.

This review examines how hypoglycemia can affect an older population, and highlights the need for increased attention to avoid hypoglycemia completely in elderly people.

Prevalence

The true prevalence of hypoglycemia in the elderly is unknown. Most studies that have tried to address this question rely on recall of hypoglycemic episodes by participants. Accurate recall of hypoglycemia is notoriously difficult in any age group, and none more so than in an elderly population. For epidemiological purposes 'severe' hypoglycemia is usually defined as that requiring external assistance for treatment. This is easier to measure in terms of prevalence as it is usually more dramatic and accuracy of recall is more robust for up to a year in type 1 and type 2 diabetes.[11, 12] Episodes of severe hypoglycemia can also be corroborated with documentary evidence from the medical emergency services.

The difficulties in accurate patient recall of episodes of hypoglycemia was addressed by a carefully designed prospective observational study over 9–12 months in the UK.[13] Participants were required to return a data-collection sheet every time they experienced a severe hypoglycemia episode. The annual prevalence of SU-associated severe hypoglycemia was 7%, similar to that observed in people with type 2 diabetes treated with insulin for <2 years. This compared to a prevalence of 25% in patients with type 2 diabetes who had received insulin treatment for >5 years, and 46% in those with long-standing type 1 diabetes (>15 years). However, the highest mean age of any of the subgroups included in this study was only 62 years and all had good glycemic control (HbA1c <8%). In the retrospective assessment of an older population over the age of 70 years, taking oral glucose-lowering agents, which relied on participant recall, Bramlage *et al*[14] found that only 1% reported episodes of symptomatic hypoglycemia in which external assistance had been required.

Different definitions, varying ability to recognize hypoglycemia and varying ability to recall preceding episodes all contribute to disparate estimates between studies. Mild episodes of hypoglycemia – usually defined as those that can be self-treated – are much more difficult to estimate. It has been shown that recall of mild episodes is unreliable beyond one week in people with type 1 diabetes.[15] It may be poorer still in the older population with type 2 diabetes in whom cognitive function is often diminished. In the year prior to inclusion in their study, Bramlage and colleagues found that 12.8% of the participants aged over 70 years and on oral treatment reported any episode of hypoglycemia, compared with 10.1% aged 60–69 years, and 9% aged under 60 years.[14] Over one year in the prospective UK Hypoglycaemia Study[13] with its intensive concurrent data collection, 39% of those with SU-treated type 2 diabetes reported at least one episode of mild (self-treated) hypoglycemia, compared with 64% in those with type 2 diabetes who had been treated with insulin for >5 years, and 85% of those with long-standing type 1 diabetes. Furthermore, this represented a middle-aged cohort, so the prevalence might well differ in an older population, and it may be lower if clinicians are more pragmatic with glycemic targets and choice of treatment. However, in the INdividualised Treatment targets for ElderRly patients with type 2 diabetes using Vildagliptin Add-on or Lone therapy (INTERVAL) study, where clinicians were encouraged to set individualized treatment targets for elderly patients, taking account of age, frailty, and co-morbidities, physicians still set HbA1c targets in the region of 7.0% (55 mmol/mol),[16] making this premise unlikely. Conversely, more hypoglycemia might be anticipated in elderly patients who eat less and are not confident about altering the dose of their medications. In addition, hypoglycemia may be missed in older patients when their non-specific symptoms are attributed to other age-related ailments, or the neurological symptoms are misinterpreted as transient ischemic attacks (TIAs) or other cerebrovascular events.[17]

Knowledge of symptoms of hypoglycemia in patients of this age group is often poor.[18, 19] Mild episodes of hypoglycemia are under-recognized by patients, relatives or carers, and their healthcare providers. Studies have shown poor correlation between recall of hypoglycemia by relatives and patients,[20-22] with relatives tending to recall more episodes. Furthermore, the recognition of mild hypoglycemia is made more difficult as the hypoglycemia symptom profile changes with age, as do the glycemic thresholds for symptom generation and cognitive impairment.[23] Even if hypoglycemia is recognized, patients are known to under-report this to their doctors.[24]

Symptoms, physiology and recognition

The symptoms of hypoglycemia derive from the physiological response to the change in glucose.[25] Although symptoms may differ between people, in the younger adult these are usually easy to perceive. The Edinburgh Hypoglycaemia score was developed from analyzing the most common symptoms reported by people experiencing hypoglycemia,[26, 27] and comprises autonomic symptoms (such as sweating and pounding heart), neuroglycopenic symptoms (such as confusion and light-headedness), and non-specific symptoms (such as malaise). Considerable variability in symptoms occurs between hypoglycemic events, even within the same person.[28] In older people, the symptoms of hypoglycemia are notably less intense during hypoglycemia than in younger adults,[17, 29] and there is an overall reduced subjective awareness of hypoglycemia with increasing age.[30] In younger people, autonomic symptoms of hypoglycemia tend to be more prominent than neuroglycopenic symptoms, although the latter also occur. These autonomic symptoms of hypoglycemia become less

prominent with increasing duration of diabetes and also in older patients with diabetes.[29, 31] It has been postulated that this change in symptoms may be related to a reduced end-organ response in older people.[29] The attenuation of autonomic symptoms, and change in glycemic threshold at which they are generated, crucially restricts the 'protective window' for action between the recognition of symptoms and the onset of cognitive dysfunction.[23, 32] This may be particularly dangerous in an elderly person, who may therefore progress to severe neuroglycopenia.

The Edinburgh Hypoglycaemia score can be adapted to include the neurological symptoms that are common in older people (studied in those aged over 70 years); light-headedness and unsteadiness were found to be particularly frequent.[17] The non-specific nature of symptoms and their lower intensity in the elderly person with diabetes can make self-recognition of hypoglycemia difficult; a non-specific episode of confusion can be caused by numerous conditions prevalent in older patients, such as infection, early cognitive impairment, cerebral hypoperfusion resulting from postural hypotension or a TIA. If an episode is not thought to be significant enough to 'worry the doctor' it may not be recorded as a hypoglycemic event or even treated, and even if it is mentioned to medical attendants, the chances of it being recognized as hypoglycemia are not high, because of conflicting differential diagnoses. The treatment of the patient's diabetes may therefore remain unchanged, and unrecognized hypoglycemia may continue to occur. As with younger patients with diabetes, repeated episodes of hypoglycemia can lead to impaired hypoglycemia awareness.[27, 33] In insulin-treated people with type 2 diabetes, patients with impaired hypoglycemia awareness had a 17-fold higher frequency of severe hypoglycemia events than those with normal awareness.[33] Furthermore, newer methodologies, such as continuous glucose monitoring (CGM), have demonstrated that hypoglycemia is more common than previously appreciated.[34]

As symptoms of hypoglycemia are varied and non-specific in the older population,[33] the most pertinent pragmatic question is how to identify those at greatest risk?

Risk factors – including comorbidities and frailty

Elderly people have multiple potential risk factors for hypoglycemia. These risk factors are similar to those observed in young adults, but in people of advanced age these risk factors are cumulative and have a greater impact.

In type 1 diabetes, duration of insulin therapy, loss of endogenous insulin secretion, and a previous history of severe hypoglycemia are predictors for an increased risk of severe hypoglycemia.[17, 35] Other than treatment with insulin and SUs,[8] the predictors for an increase in incidence of hypoglycemia in type 2 diabetes are more varied, consistent with the heterogeneity of type 2 diabetes, and advanced age increases the potential for serious morbidity. One important risk factor is the duration of insulin treatment.[8, 22, 27, 36, 37] Other observed associations vary between studies, and include older age,[38] longer duration of diabetes *per se*,[22, 27] increased comorbidities (especially chronic kidney disease),[38, 39] impaired hypoglycemia awareness,[22, 27, 33] intensive therapy and strict glycemic control,[27, 40] and behavioral factors, such as irregular eating,[41] exercise,[39, 42] and errors in timing of medication.[42]

The observed association between increased frequency of hypoglycemia with increased duration of diabetes is linked with increasing age and increasing loss of endogenous insulin secretion.[43] Certainly in type 1 diabetes, the Diabetes Care and Complications Trial (among others) showed that the lower the C-peptide the higher the rate of severe hypoglycemia.[17, 35, 44] Surprisingly, few studies have examined the role of endogenous insulin secretion in type 2 diabetes, and results have been conflicting: the UK Hypoglycaemia Study Group found an association with frequency of hypoglycemia and C-peptide levels,[13] whereas a Danish study by Akram *et al*[22] did not.

There is increasing evidence that people with cognitive impairment may be at higher risk of experiencing hypoglycemia.[45-49] Of the 11,140 patients with type 2 diabetes in the ADVANCE study,[45] 212 were classed as having 'severe' cognitive impairment (scoring <24/30 on the Mini-Mental State Examination), and this subgroup had double the risk of severe hypoglycemia (hazard ratio [HR] 2.10, 95% CI 1.14–3.87; p=0.018), than those with 'mild' or no cognitive impairment. Similarly, in 497,900 veterans with diabetes aged 65 years or over,[46] the adjusted odds ratios for experiencing hypoglycemia that required medical assistance over the course of 1 year were 1.58 (95% CI 1.53–1.62) for those with dementia. Over a median 3.25 years of follow-up, post-hoc analysis of 2,956 patients with type 2 diabetes aged over 55 years in the ACCORD trial,[47] showed that poorer scores on a battery of cognitive tests were predictive of a first episode of hypoglycemia requiring medical assistance (HR 1.13, 95% CI 1.08–1.18). Yaffe at al[49] have recently demonstrated that over 12 years of follow-up, 14.2% of patients with diabetes who developed dementia, subsequently experienced an episode of severe hypoglycemia, compared with 6.3% of those who did not develop dementia (multivariate-adjusted HR 3.1, 95% CI 1.5–6.6; p<0.001).

People with diabetes who live in residential homes, where the estimated prevalence of diabetes is 20–25%,[2, 50] are perhaps at particular risk. Reasons for this include advanced age,[38] duration on insulin treatment,[8, 22, 27, 36, 37] comorbidities,[38, 39] reduced ability to manage their food consumption,[41]

reduced cognition,[45-49] impaired mobility, limited facilities to resolve fluctuations in glucose levels, and progressive impairment of hypoglycemia awareness.[22, 27, 33] Holstein *et al*[38] found that 34% of German patients with type 2 diabetes who required emergency medical services for severe hypoglycemia were nursing home residents or were being cared for by home nursing services. The residential home population has not, however, been systematically evaluated.[33, 51] Education about diabetes among care home staff is often patchy or absent.[52]

Effects of hypoglycemia on quality of life, morbidity and mortality

So why does it matter that older people are exposed to hypoglycemia? Hypoglycemia has a major adverse impact on quality of life,[53-55] which has been under-appreciated by healthcare professionals for many years.[56] Patients fear hypoglycemia more than the long-term consequences of diabetes.[57] Hypoglycemia has been linked to poor outcomes pertinent to an older population: increased risk of accidents,[58] falls and fractures,[58-60] hospitalizations,[58] in-hospital mortality,[61] frailty,[62] long-term impairment of cognition[48, 63] and a two-fold increased risk of developing dementia.[49, 64] It is also associated with electrophysiological changes, particularly prolongation of the QT interval, a known precipitant of cardiac dysrhythmia, which may persist for up to 48 hours after the hypoglycemic event.[65, 66]

The risk of accidents resulting in hospital visits among people with type 2 diabetes on medications excluding insulin, was assessed retrospectively in a large US health insurance database.[58] Hypoglycemia was associated with significantly increased hazards for any accident (HR 1.39, 95% CI 1.21–1.59; p<0.001), accidental falls (HR 1.36, 95% CI 1.13–1.65; p<0.001) and motor vehicle accidents (HR 1.82, 95% CI 1.18–2.80; p=0.007). Diabetes *per se* is associated with an increased risk of osteoporosis[67] and a large retrospective observational study in the USA[60] of more than 360,000 patients with type 2 diabetes aged over 65 years, found 4.7% who had a documented hypoglycemic episode over the course of 1 year (resulting in an outpatient medical claim) had a 70% higher chance of having a fall-related fracture (odds ratio 1.7, 95% CI 1.58–1.83); the odds still remained high even after correcting for potential confounders, such as presence of diabetic peripheral neuropathy.

These far-reaching and still under-estimated consequences of hypoglycemia have many financial as well as human costs, which are difficult to quantify. Hospital admissions resulting from hypoglycemia in type 2 diabetes are longer than those with type 1 diabetes, reflecting older age, more comorbidities and polypharmacy.[68]

It has been repeatedly observed that dementia is more common in those affected by diabetes,[69-72] although the precise mechanism(s) are still not established. Acute hypoglycemia impairs many aspects of cognition: immediate verbal and visual memory, working memory, delayed memory, visual-motor skills, visual-spatial skills, and global cognitive dysfunction.[71, 73, 74] It has been suggested that this transient impairment is associated with long-term cognitive defects. Severe hypoglycemia could result in neuronal cell death, which might conceivably accelerate the development of dementia.[75] One might postulate that an episode of severe hypoglycemia may be more likely to have a long-term effect on cognition in an older and more vulnerable brain, or that repeated episodes of hypoglycemia (even if apparently less severe) may have a deleterious effect.

One large scale epidemiological study that suggested severe hypoglycemia may lead to dementia, was a longitudinal cohort study in the USA by Whitmer and colleagues.[64] They found a graded increase in risk of dementia with increasing numbers of previous hypoglycemic events requiring hospitalization – even after adjustment for age, education, comorbidities, duration of diabetes, diabetes treatment, years on insulin, and 7-year mean HbA1c. This was based on the electronic hospital records of 16,667 patients with a diagnosis of type 2 diabetes (mean age 65 years): 8.8% (1,465) had documented at least one episode of severe hypoglycemia (requiring hospitalization) between 1980 and 2002, and 11% (1,822) had a diagnosis of dementia by follow-up. The fully adjusted HR for dementia having had one episode of hypoglycemia requiring hospitalization was 1.26 (95% CI 1.1–1.49), having had two episodes was 1.8 (95% CI 1.37–2.36), and for three or more episodes was 1.94 (95% CI 1.42-2.64). Similar HRs were found when considering emergency department admissions for hypoglycemia. This appeared to amount to a 2.3% increase in absolute risk of dementia per year of follow-up for patients with a history of severe hypoglycemia.

In a broadly similar study design based on the Taiwanese National Health Insurance Research Database, Lin and Sheu[76] found that of over 15,000 people with type 2 diabetes, mean age of 64.2 years and no documentation of a dementia diagnosis at recruitment, 7.2% developed dementia over 7 years of follow-up. From coding (hospital or ambulatory episodes), approximately 2% of the cohort were found to have an episode of hypoglycemia recorded over a 3-year period. An episode of hypoglycemia predicted an almost 3-fold increase in the risk of dementia (29.9 people developing dementia per 1,000 person-years [95% CI 22.1–39.2] versus 11.1 per 1,000 person-years [95% CI 10.3–11.8]), giving a crude risk ratio of 2.76 (95% CI 2.06–3.70; p<0.001). After adjustment for age and sex the risk ratio for developing dementia after hypoglycemia was 1.60 (95% CI 1.19–2.14; p=0.002), and this was a graded increase in risk according to the number of episodes of hypoglycemia experienced.

Both of these studies[64, 76] can be criticized for potential selection bias, lack of correction for certain potentially significant confounders, and inability to accurately assess for cognitive function.[77] By only recording the most severe hypoglycemic events (those requiring medical assistance), when most episodes of hypoglycemia are self-treated in the community, those patients identified may be those least able to look after their own diabetes and potentially may be those most at risk of being cognitively impaired (eg, with subclinical cerebrovascular disease) at the time of their severe hypoglycemia episode, which could neither be measured nor corrected for. Other potentially significant comorbid conditions such as a history of alcoholism, epilepsy, psychiatric illness or head injury could also not be corrected for.[77] Additionally, patients who experience hypoglycemia needing hospitalization are often considered to be an atypical group of patients; they are often severely ill (eg, with sepsis), which may provide other causes precipitating subsequent cognitive decline.[77] The authors considered that because the sub-analysis of data from emergency department attendances was as robust as that from hospital episodes, this made this scenario less likely – plus the up-to 15 year lag from hospital episode of hypoglycemia to diagnosis of dementia was likely to dispel the effect of any other comorbid conditions from the hospital admission.[78] While it is acknowledged by the authors[78] that no observational study can completely eliminate all confounders, the strength of the data raises legitimate concerns that hypoglycemia may precipitate the onset of dementia. This calls for some circumspection when treating frail elderly to strict glycemic targets – and calls for the need for prospective studies in this area.

The Edinburgh Type 2 Diabetes Study avoided some of the methodological concerns of the above study[64] and also supported the suggestion of an association between severe hypoglycemia and subsequent development of dementia.[48] In this study, a cross-sectional methodology was used, with 1,066 participants aged 60–75 who had type 2 diabetes, being asked to complete a validated questionnaire to assess their frequency of severe hypoglycemia in the previous year, and over their lifetime. Cognitive function was assessed both at the time of the study (using age-sensitive psychological tests to derive a 'late-life cognitive ability factor'), and projected prior cognitive ability (using vocabulary tests that are stable during ageing). In those reporting at least one severe hypoglycemic event (113 patients, 10.6%), a slightly lower mean vocabulary score was observed, but was not statistically significant ($p=0.13$), ie, there was seemingly no major difference in premorbid cognitive ability. However, a clear difference was found in their 'late-life general cognitive ability factor' ($p<0.001$), and this difference persisted even after adjustment for various potential confounders such as duration of diabetes, smoking, HbA1c and vascular disease. Additionally, although those having experienced severe hypoglycemia scored higher on the Hospital Anxiety and Depression Scale, the cognitive associations remained significant after being corrected for this. The temporal relationship between hypoglycemia and cognitive decline cannot be determined accurately with this cross-sectional design,[48] and it is interesting to note that 76% of the patients reporting at least one episode of hypoglycemia had experienced an episode in the year preceding recruitment. However, on analysis no significant difference was observed in the overall strength of the association with cognition when hypoglycemia in the year preceding recruitment was used versus lifetime history.

The Fremantle Diabetes Study in Australia[79] found an association between previous severe hypoglycemia and subsequent cognitive impairment and dementia, when the cognition of 302 individuals was assessed and their previous exposure to severe hypoglycemia estimated retrospectively. However, a small prospective arm was included in an attempt to address the question of temporal decline. The study was probably underpowered to answer this question, and the authors did not find an association between severe hypoglycemia and evidence of premature dementia in 205 individuals over 70 years old without cognitive impairment who were followed over a comparatively short period of 4 years.

A recently published prospective study by Yaffe *et al*[49] provides more convincing evidence and lends weight to the causality of dementia in relation to hypoglycemia exposure. The authors found a two-fold increased risk of developing dementia in people who had experienced an episode of severe hypoglycemia requiring hospitalization. A total of 783 older adults (mean age 74 years) with diabetes but no evidence of cognitive impairment at recruitment (determined by a baseline Modified Mini-State Examination), were followed for 12 years. During this time, 7.8% (61 patients) had a severe hypoglycemic event requiring hospitalization, and 18.9% (148 patients) developed dementia (determined by a dementia-related hospital event or prescription of a dementia medication, and confirmed by cognitive assessment). Of those who had experienced a hypoglycemic event, 34.4% developed dementia, compared with 17.6% who did not ($p<0.001$), with a multivariate-adjusted HR of 2.1 (95% CI 1.0–4.4). A bidirectional association was observed; those who developed dementia had a greater risk of subsequently experiencing hypoglycemia (14.2%) compared with those who did not develop dementia (6.3%, multivariate-adjusted HR 3.1, 95% CI 1.5–6.6; $p<0.001$).

Overall, an increasing body of evidence ould support a putative association between hypoglycemia and dementia – in bidirectional fashion – and given the increasing prevalence and burden both of dementia and of diabetes in the older population, this is an area that deserves much more attention. Causes of dementia are

still poorly understood; if reducing hypoglycemic events in the older population can help to reduce the likelihood of the development of dementia, physicians should tangibly address this possibility.

HbA1c targets

Strict glycemic control and intensive therapy are associated with an increased incidence of severe hypoglycemia.[5, 6, 80] A meta-analysis of randomized controlled trials[81] – which included over 28,600 patients with type 2 diabetes – found the relative risk of severe hypoglycemia was increased by 30% in the groups undergoing strict glycemic control. The frequency of mild hypoglycemia is also likely to be increased, as are acute daily glucose fluctuations, which are increasingly recognized as being associated with poor outcomes, such as effects on cognition.[63] A retrospective cohort study using data from nearly 28,000 patients over the age of 50 years and with type 2 diabetes sourced from the UK General Practice Research Database, found a U-shaped association between HbA1c and all-cause mortality and cardiac events,[82] with the lowest risk at an HbA1c of 7.5%.

Targets for glycemic control in elderly patients have become more controversial and pragmatic.[83] Guidelines are starting to reflect a need for more individualized treatment, but the evidence base is very limited. To date only one clinical study has even attempted to utilize individualized treatment targets,[16] and no study has used clinically meaningful outcomes for elderly patients, such as falls, progression of frailty and quality of life. Disappointingly, in the INTERVAL study, despite being asked to individualize glycemic targets around patients' age, frailty and co-morbidities, the participating physicians only considered baseline HbA1c and gender, and they set a HbA1c target of 7.0%. Individualizing the treatment target was associated with lower than anticipated side effects, including hypoglycemia, and good tolerability of the strategy. Indeed, 27% of the population achieved their targets with nothing more than lifestyle change and increased contact with the care-providers. The American Diabetes Association (ADA) and the European Association for the Study of Diabetes have issued a joint position statement suggesting a more patient-centered approach for the treatment of type 2 diabetes.[84] For older adults, they have relaxed the HbA1c target to <7.5% or even 8% if stricter targets are more difficult to achieve. In 2012, the ADA and American Geriatrics Society also issued a consensus report on diabetes in older adults,[85] which suggested more pragmatic glycemic targets for older adults than those previously published (Table 1).

Table 1. The ADA/American Geriatrics Society consensus guidelines for setting HbA1c targets based on patient baseline characteristic[85]

Patient type	Examples of patient features	HbA1c target
"Healthy"	Few coexisting chronic illnesses; cognitive & functional status intact	<58.5 mmol/mol (7.5%)
"Complex" or "intermediate"	Multiple coexisting chronic illnesses; >2 activities of daily living impairments; mild-to-moderate cognitive impairment	<64 mmol/mol (8%)
"Very complex" or "in poor health"	Long-term condition/end-stage chronic illnesses; moderate-to-severe cognitive impairment; >2 activities of daily living dependencies	<69 mmol/mol (8.5%)

However, more relaxed HbA1c targets do not eradicate hypoglycemia. Munshi *et al*[86] used CGM to estimate the frequency of hypoglycemia in an older population (>69 years) with a 'more relaxed' glycemic target of HbA1c of >8%, and found that 65% experienced at least one episode of hypoglycemia (glucose <70mg/dl; 3.9 mmol/l) during the 72 hours of monitoring. They concluded that relaxing glycemic control to >8% was not necessarily sufficient to prevent hypoglycemia in this population. They did not compare the frequency of hypoglycemia events in this population with the frequency of events in patients with stricter glycemic targets; the frequency of hypoglycemia in patients with strict glycemic targets would be expected to be even higher.

In practice, inadequate recognition of hypoglycemia may not alert patients or clinicians to the need to re-evaluate individual treatment targets. With the increased recognition of the adverse effects of hypoglycemia and glucose variability, an increasing number of older people on insulin, and continued strict glycemic targets, hypoglycemia will become increasingly important. A stronger evidence base for individualized treatment is needed.

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Assessment of Practical Classification Guidelines for Diabetes in insulin-treated patients

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Practical Classification Guidelines for Diabetes in patients treated with insulin:

a cross-sectional study of the accuracy of diabetes diagnosis

Abstract

Background
Differentiating between type 1 and type 2 diabetes is fundamental to ensuring appropriate management of patients, but can be challenging, especially when treating with insulin. The 2010 UK Practical Classification Guidelines for Diabetes were developed to help make the differentiation.

Aim
To assess diagnostic accuracy of the UK guidelines against 'gold standard' definitions of type 1 and type 2 diabetes based on measured C-peptide levels.

Design and setting
In total, 601 adults with insulin-treated diabetes and diabetes duration ≥5 years were recruited in Devon, Northamptonshire, and Leicestershire.

Method
Baseline information and home urine sample were collected. Urinary C-peptide creatinine ratio (UCPCR) measures endogenous insulin production. Gold standard type 1 diabetes was defined as continuous insulin treatment within 3 years of diagnosis and absolute insulin deficiency (UCPCR<0.2 nmol/mmol ≥5 years post-diagnosis); all others classed as having type 2 diabetes. Diagnostic performance of the clinical criteria was assessed and other criteria explored using receiver operating characteristic (ROC) curves.

Results
UK guidelines correctly classified 86% of participants. Most misclassifications occurred in patients classed as having type 1 diabetes who had significant endogenous insulin levels (57 out of 601; 9%); most in those diagnosed ≥35 years and treated with insulin from diagnosis, where 37 out of 66 (56%) were misclassified. Time to insulin and age at diagnosis performed best in predicting long-term endogenous insulin production (ROC AUC = 0.904 and 0.871); BMI was a less strong predictor of diabetes type (AUC = 0.824).

Conclusion
Current UK guidelines provide a pragmatic clinical approach to classification reflecting long-term endogenous insulin production; caution is needed in older patients commencing insulin from diagnosis, where misclassification rates are increased.

Keywords
diabetes mellitus; C-peptide; general practice; insulin-treated diabetes; type 1/type 2 classification; type 1/type 2 diagnosis.

INTRODUCTION

Correctly classifying patients with diabetes with type 1 or type 2 is fundamental to ensuring they receive correct management.[1–3] In clinical practice this can be challenging, with 7–15% patients misclassified in England, and large variations in practice.[4–7]

Historical lack of clear clinical guidelines for diabetes classification is likely to have contributed to this variation. International guidelines from the World Health Organization[8] and the American Diabetes Association[9] base classification on underlying aetiology, with type 1 described as a destruction of beta cells leading to absolute insulin deficiency. However, these guidelines do not provide clear criteria or classification pathways for clinical use.[8,9] A pragmatic classification algorithm (Figure 1) was thus developed in 2010 by key diabetes stakeholders in the UK, and published by the Royal College of General Practitioners (RCGP) and (the previously existing) NHS Diabetes in their *Coding, Classification and Diagnosis of Diabetes* document.[4] This uses age at diagnosis and time to commencing insulin treatment from diagnosis as its diagnostic criteria. The efficacy of this algorithm has not yet been tested on a large cohort of patients with diabetes.

The fundamental difference between type 1 and type 2 diabetes is the rapid development of absolute insulin deficiency in type 1, forming the basis of their different treatment and management. Patients with type 1 require accurate insulin dose replacement;[10,11] patients with type 2 continue to produce substantial amounts of their own insulin, responding to non-insulin therapy, or if insulin is needed good control can be achieved with non-physiological insulin regimens.[12,13] Measuring endogenous insulin secretion (using C-peptide, a component of the insulin pro-hormone secreted in equimolar amounts to insulin) in longstanding diabetes may be a useful 'gold standard' marker of endogenous insulin production, confirming a diagnosis of type 1 versus type 2 diabetes. Development of the spot urine test urinary C-peptide creatinine ratio (UCPCR)[14–17] has enabled practical testing in a community setting. UCPCR is well-correlated with mixed meal tolerance test measures[16,17] and a UCPCR cut-off

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How this fits in

Correct classification as type 1 or type 2 diabetes is fundamental to appropriate diabetes management. *The UK Practical Classification Guidelines for Diabetes* published by the Royal College of General Practitioners and (the previously existing) NHS Diabetes are pragmatically based on age at diagnosis and time from diagnosis to commencing insulin treatment.
This the first study testing the UK classification guidelines in a large cohort of insulin-treated patients against a gold standard classification of diabetes subtype based on presence or absence of retained endogenous insulin secretion (measured using C-peptide) >5 years post-diagnosis.
The UK classification criteria correctly classified 86% of patients, with age at diagnosis and time to insulin being the best clinical predictors of long-term endogenous insulin production.

of 0.2 nmol/mmol gives a sensitivity and specificity of 100% and >95% for detecting severe insulin deficiency[16,17] as defined by the gold-standard mixed meal test 90-minute C-peptide level of 200 pmol/L.[18]

Therefore, this study aimed to determine the reliability of the 2010 *UK Practical Classification Guidelines*[4] to correctly classify diabetes in a large cohort of insulin-treated participants against 'gold-standard' classification based on measurement of C-peptide, in those with diabetes of ≥5 years' duration. Although UCPCR can be used at any stage in diabetes to confirm endogenous insulin levels, in the current study ≥5 years' duration was chosen to avoid misclassifying people with early type 1 who may have been still producing their own insulin.

METHOD

Participants

Adults with insulin-treated diabetes centred in/around three UK centres (Exeter, Northampton and Leicester) were sent letters before attending routine diabetes appointments or retinal screening (in primary care, both urban and rural, and secondary care). Those expressing an interest in participating either by returning an expression of interest form in advance, or when arriving for their routine appointment, were formally consented on the same day, and provided the research team with data on:

- age at diagnosis;
- weight at diagnosis;
- current age;
- weight and height;
- treatment;
- time to insulin from diagnosis; and
- ethnicity.

Body mass index (BMI) at diagnosis and recruitment were calculated where possible; weight at diagnosis for those diagnosed as children converted to the adult equivalent using the *UK Child Growth Reference Standards*.[19] Participants were also given a boric acid-containing urine specimen pot and padded stamped addressed envelope. They were asked to collect a urine sample for UCPCR[14] 2 hours after their largest meal of a day, and post the next morning (within 24 hours) for analysis in the Exeter biochemistry laboratory. UCPCR is stable in boric acid at room temperature for at least 3 days.[14] There was no financial incentive for participating.

Classification of diabetes

Participants were classified as having type 1 or type 2 diabetes using the UK guidelines,[4] (Figure 1). The authors developed 'gold-standard' criteria: type 1 diabetes: continuous insulin treatment within the first 3 years of diagnosis and absolute insulin deficiency (UCPCR <0.2 nmol/ mmol ≥5 years post-diagnosis);[16] type 2 diabetes: UCPCR >0.2 nmol/mmol, or UCPCR <0.2 nmol/mmol but not treated with insulin for first 3 years after diagnosis.

Statistical analysis

Proportions of patients correctly classified by the UK guidelines according to the 'gold standard' C-peptide-based definition were calculated, and differences in clinical characteristics between those correctly and incorrectly categorised were explored using the Mann-Whitney test.

Diagnostic performance of continuous variables (age at diagnosis, time to insulin,

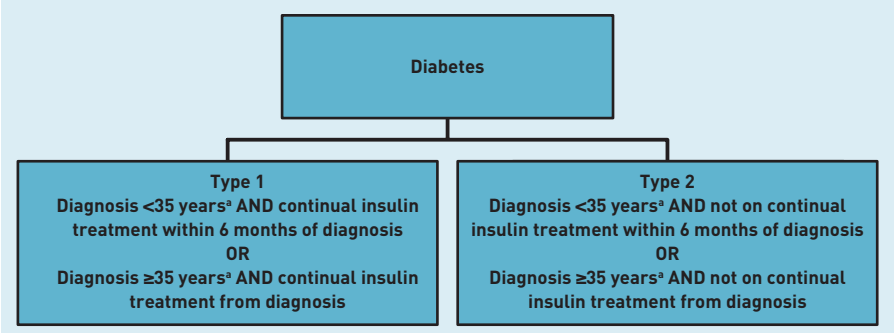

Figure 1. **UK Practical Classification Guidelines for Diabetes** ***(extract showing algorithm of classification guidelines for type 1 and type 2 diabetes).[4]***

Table 1. Participant characteristics

	Overall	Gold standard type 1 diabetes	UK guidelines type 1 diabetes	Gold standard type 2 diabetes	UK guidelines type 2 diabetes
Age at recruitment, median years (IQR)	64 (53–73)	54 (41–64)	53 (41–64)	68 (60–74)	68 (61–75)
Sex, % male	58.2	48.7	52.7	62.8	61.4
BMI at recruitment, median (IQR)	28.7 (25.3–33.3)	26.5 (23.1–29.3)	26.8 (23.8–29.7)	29.7 (26.6–34.5)	30 (26.6–34.1)
Age at diagnosis, median years (IQR)	45 (30–56)	24 (12–36)	25 (13–39)	50 (42–59)	50 (43–58)
BMI at diagnosis, median (IQR)	27 (23.9–32.0)	21.8 (19.8–26.3)	22.9 (20.0–27.6)	28.4 (25.4–32.9)	28.3 (25.2–33.6)
Latest HbA1c, % (IQR)	8.0 (7.3–8.8)	8.1 (7.4–8.9)	8.0 (7.3–8.9)	7.9 (7.2–8.8)	7.9 (7.3–8.8)
Insulin, IU/kg/24 hours (IQR)	0.64 (0.44–0.90)	0.61 (0.50–0.84)	0.61 (0.49–0.88)	0.65 (0.42–0.93)	0.64 (0.43–0.92)
UCPCR, nmmol/mmol, median (IQR)	0.6 (0.03–1.60)	0.019 (0.019–0.03)	0.019 (0.019–0.22)	1.19 (0.59–2.25)	1.1 (0.4–2.1)

BMI = body mass index. IQR = interquartile range. UCPCR = urinary C-peptide creatinine ratio.

BMI at diagnosis and recruitment) was assessed using receiver operating characteristic (ROC) curves. Optimal cut-offs for these variables (with maximum specificity and sensitivity for discrimination) were calculated, and this study explored whether use of these optimal cut-offs led to improvements in classification over and above the RCGP algorithm using net reclassification improvement.[20]

Detailed subgroup analysis could not be carried out on the Asian patients due to small numbers. Analysis was carried out on Stata (version 13.1) and R (version 3.1.2).

RESULTS

In total, 601 white European and 30 Asian patients who had had diabetes for ≥5 years responded. Table 1 shows the characteristics of participants per classification

UK guidelines versus gold standard

The UK clinical classification criteria were compared with the gold standard C-peptide based criteria for defining type 1 and type 2 diabetes in the cohort of 601 white European patients. In total, 514 (86%, 95% confidence interval [CI] = 83 to 88) patients overall were correctly classified by the UK guidelines when compared with the gold standard criteria.

Figure 2 shows 163 out of 193 patients (84%, 95% CI = 79 to 89) were correctly classified with type 1 diabetes, and 351 out of 408 (86%, 95% CI = 82 to 89) with type 2 diabetes. The extent of the agreement between the classifications of diabetes type using the UK guidelines compared with the gold standard is evident in Figure 3.

In the Asian group, the criteria (taking note of the age cut-off of 30 years for high-risk ethnicities) performed less well, classifying only 21 out of 30 (70%) correctly (P = 0.02

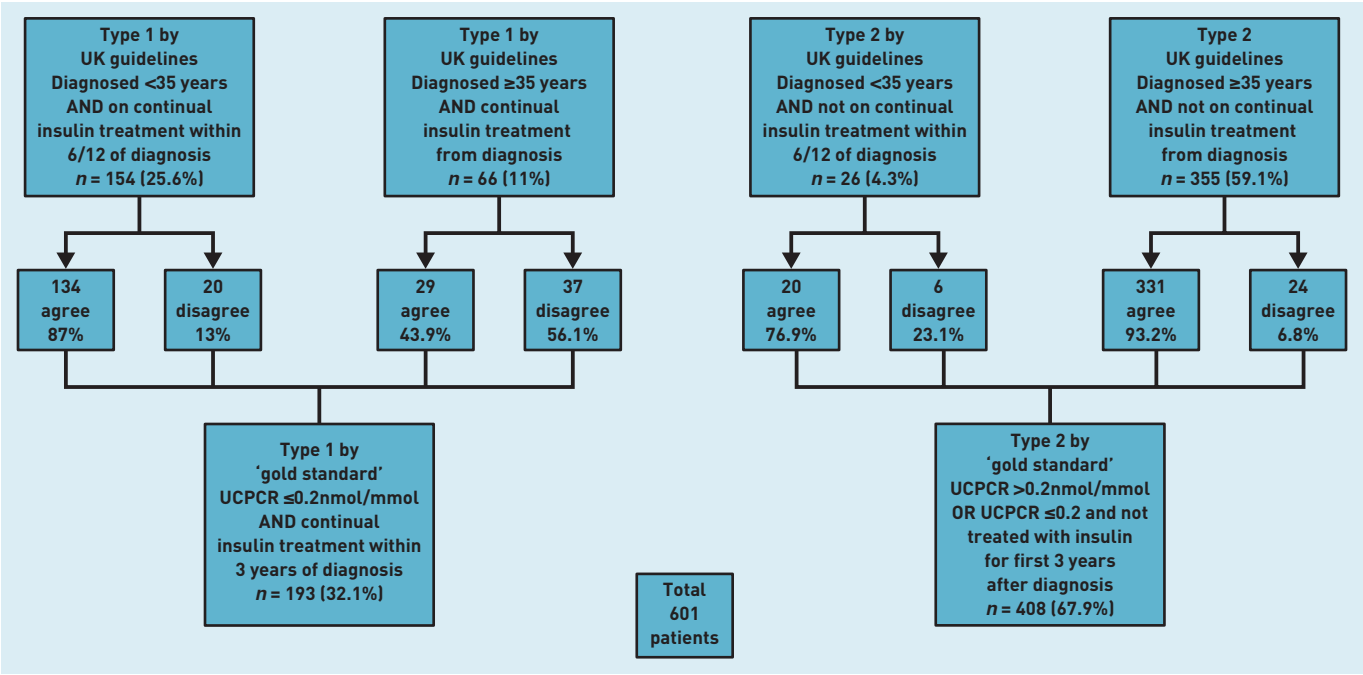

Figure 2. Classification of type of diabetes according to UK guidelines' clinical criteria compared to 'gold standard' C-peptide-based criteria.

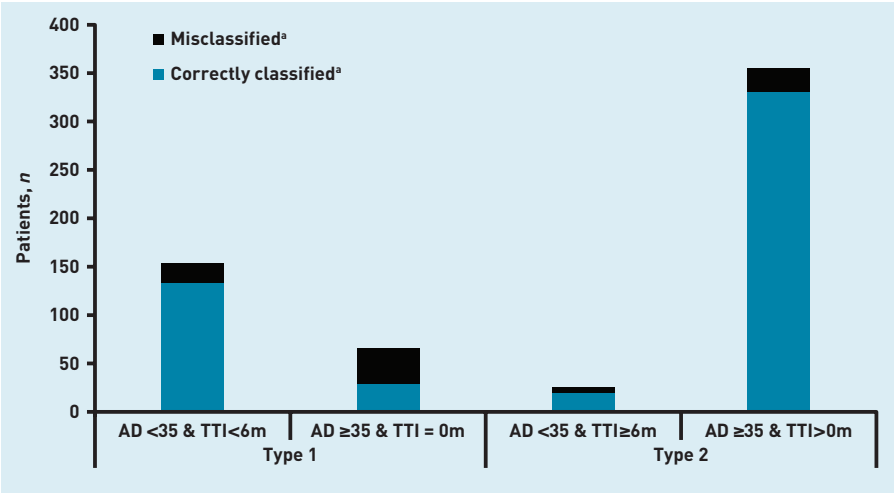

Figure 3. Proportion of patients classified as having type 1 or type 2 diabetes according to the UK guidelines. [a]According to C-peptide-derived gold standard definition. AD = age at diagnosis. TTI = time to insulin from diagnosis.

for comparison with white Europeans) (data not shown). Three out of four (75%) were correctly classified with type 1 diabetes, and 18 out of 26 (69%) with type 2 diabetes.

Misclassifications

Of patients misclassified by the UK guidelines' clinical criteria in comparison with the gold standard (n = 87), most (n = 57, 66%, Figure 2) were misclassified as having type 1 diabetes and were producing substantial endogenous insulin ≥5 years post diagnosis (data not shown). Thirty out of 87 patients (34%) were misclassified as having type 2 diabetes (Figure 2); these individuals were severely insulin deficient and had started insulin treatment within 3 years of diagnosis.

The majority of misclassifications (eight out of nine) in the Asian group were also cases in which the UK guidelines' criteria suggested type 1 diabetes (using the UK guidelines' age cut-off of 30 years for high-risk ethnicities) but the patients were still producing their own insulin (data not shown). Most patients who were misclassified as having type 1 diabetes were diagnosed aged ≥35 years, and were given insulin immediately. According to UK guidelines, 66 patients had type 1 diabetes by these criteria, however 37 of these (56%) had a UCPCR of >0.2 nmol/mmol and so, by gold standard criteria, had type 2 diabetes (Figure 2).

Those misclassified as having type 1 diabetes were older than those correctly classified (median age 44 years [interquartile range {IQR} 30–59 years] versus 20 years [IQR 11–30 years], P<0.001) and had a higher BMI at diagnosis (26.4 kg/m^2 [IQR 23–30.3 kg/m^2] versus 21.8 kg/m^2 [IQR 18.9–25.4kg/m^2], P = 0.002) (data not shown).

In contrast, those who were insulin deficient but were incorrectly classified by UK guidelines as having type 2 diabetes commenced insulin treatment more quickly than those correctly classified as having type 2 diabetes (time to insulin from diagnosis 12 months [IQR 2–18 months]) versus 84 months [IQR 42–138 months], P<0.001), had lower BMI (22.5 kg/m^2 [IQR 21.1–26.3 kg/ m^2] versus 28.1 kg/m^2 [IQR 25.4–33.3 kg/m^2], P<0.001), and were younger at diagnosis (44 years [IQR 35–56 years] versus 51 years [IQR 43–59 years], P= 0.014).

Optimal clinical criteria

ROC curves were used to examine the discriminative ability of key clinical criteria: time to insulin, age at diagnosis, BMI at diagnosis, and BMI at recruitment (Figure 4). They were also used to identify the best cut-offs for classification based on the 'gold standard' criteria. An area under the curve (AUC) equal to 1 represents the perfect discrimination between types of diabetes, and an AUC of >0.8 is generally deemed clinically useful.

The most discriminatory individual characteristic was months from diagnosis to insulin treatment (AUC 0.904, 95% CI = 0.88 to 0.93), with the optimal cut-off at 12 months. In total, 91.5% patients were correctly classified as having type 1 diabetes and 82.1% were correctly classified as having type 2 diabetes (data not shown).

Age at diagnosis was also a useful discriminator between type 1 and type 2 diabetes (AUC 0.871, 95% CI = 0.84 to 0.90), with the optimal cut-off being ≤39 years for type 1 diabetes. This correctly classified 81.9% of patients with type 1 and 84.3% of those with type 2 diabetes (data not shown).

BMI at diagnosis gave an AUC of 0.824 (95% CI = 0.77 to 0.87; data were available in 359 of 601 [59.7%] patients only), with the optimal cut-off being ≤23.1 kg/m^2. However, although this correctly classified 89.4% of

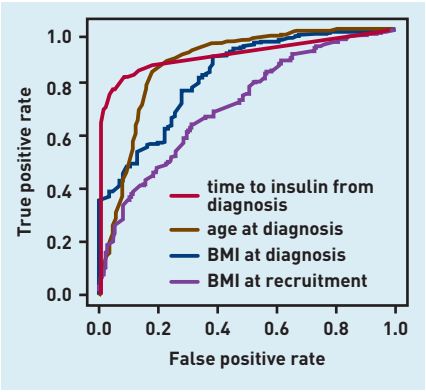

Figure 4. Receiver operating characteristic curve for discriminating between type 1 and type 2 diabetes (Based on the gold standard definition).

those with type 2 diabetes, it only classified 65.7% of patients with type 1 correctly .

BMI at recruitment was even less discriminatory, with an AUC of 0.72 (95% CI = 0.67 to 0.76) and an optimal cut-off of 28.0 kg/m^2; this correctly classified 66.8% of people with type 2 diabetes, and 61.8% of people with type 1 diabetes.

Modifying the guidelines' clinical criteria

The UK guidelines use age at diagnosis and time to insulin as the classification criteria to differentiate between type 1 and type 2 diabetes. On the basis of the ROC curve data, the optimal cut-offs for time to insulin (12 months), age at diagnosis (39 years), BMI at diagnosis (23.1 kg/m^2), and recruitment (28.0 kg/m^2) were incorporated into modified criteria in various combinations to see whether they improved diagnostic accuracy. Aiming for a sensitivity and specificity of >80% (equivalent to an ROC AUC of >0.8), none were superior to the UK guidelines as improvements in sensitivity led to greater decreases in specificity and vice versa.

The best-performing alternative was the combination of an age cut-off of 39 years and time to insulin of 12 months; this improved the correct classification of those with type 2 diabetes to 94%, but reduced to 78.3% those correctly classified with type 1 diabetes. In general, adding BMI at diagnosis or time of recruitment improved the proportion of those with type 2 diabetes that were correctly classified, but markedly reduced the proportion correctly classified with type 1 diabetes.

DISCUSSION

Summary

The study results show that the UK guidelines are an accurate method of predicting long-term endogenous insulin production and perform well in correctly classifying patients with insulin-treated diabetes based on the development of absolute insulin deficiency using endogenous insulin levels and time to insulin from diagnosis. This supports the guidelines' use as a beneficial, pragmatic way of classifying patients. When all patients with diabetes are considered, the authors hypothesise that the performance of the UK guidelines will be even better because the vast majority of patients who are not treated with insulin will be correctly classified as having type 2 diabetes.

Patients diagnosed at an older age (≥35 years) in whom insulin treatment commenced at diagnosis are at the highest risk of being misclassified when using the UK guidelines.

In clinical practice, emphasis is often placed on BMI to help differentiate between type 1 and type 2 diabetes but the study findings presented here indicate that, among patients treated with insulin, time to insulin and age at diagnosis are better predictors of diabetes subtype than BMI. Median BMI at diagnosis of those with type 1 diabetes by the gold standard criteria was lower than in those with type 2 diabetes: 21.8 kg/m^2 versus 28.1 kg/m^2 (P<0.001 but the interquartile ranges overlapped (19.8–26.3 kg/m^2 and 25.4–32.9 kg/m^2). By the time of recruitment (that is, ≥5 years from diagnosis), the difference in BMI between those with type 1 and type 2 diabetes was smaller (26.5 kg/m^2 [23.1–29.3 kg/m^2] versus 29.7 kg/m^2 [26.6–34.5 kg/m^2]) although still significant (P<0.001), and the ROC AUC was low, highlighting the reduced discriminative ability of this as a clinical marker to differentiate between type 1 and 2 diabetes once the patient was receiving insulin.

Strengths and limitations

This study comprised patients who had had diabetes for ≥5 years. If considering all patients with diabetes, the misclassification rate of 14% is likely to be much lower: patients who are treated with tablets or diet who were diagnosed ≥5 years ago are likely to have been correctly diagnosed with type 2 diabetes. In patients with a diabetes duration of <5 years, some patients with type 1 diabetes may be still producing insulin (the 'honeymoon' period) and not yet treated with insulin, although it is rare for patients with type 1 diabetes to be without insulin for prolonged periods. Due to recruitment locations and difficulty in recruiting Asian patients,[21] the majority of the recruited patients were white European; only 30 Asian patients participated. Take-up rates in the white European population were high, and participants drawn from urban and rural populations, and thus the authors consider the results in this group are likely to be fairly representative for insulin-treated patients ≥5 years from diagnosis. In comparison, the authors cannot comment on the reliability of the UK guideline criteria for populations in which the prevalence of diabetes is high; further work is needed in these groups.

Limited data on BMI were available at diagnosis, due to a combination of participants not knowing their weight at diagnosis and/or missing details in GP records in patients having been diagnosed with diabetes ≥5 years ago. Improved recording of such details in those newly diagnosed with diabetes over the last few years means the authors consider this

information is likely to be more available in any future studies.

The gold standard criteria used a UCPCR cut-off of 0.2 nmol/mmol, which has a sensitivity and specificity of 100% and >95% respectively to detect absolute insulin deficiency.[16,17] It is the best gold standard available in this context, being practical for use in large numbers of adults living in the community. Insulin treatment has the potential to suppress endogenous insulin,[22–24] but the findings presented here show that this rarely affects diabetes classification.[24] In addition, it should be noted that the small possibility of an overdiagnosis of type 1 diabetes is a safer direction of error than the opposite.

Comparison with existing literature

Previous reports on the misclassification of diabetes[4,6,25,26] were mainly based on contraindications in coding rather than on gold standard definitions of insulin deficiency.[18,27,28] These reports have attempted to assess accuracy of recorded diagnosis on the basis of electronically recorded data. Although this may detect patients who are miscoded, for example as having type 1 diabetes but are not on insulin 10 years postdiagnosis, it is less likely to detect patients who are misdiagnosed, for example in receiving insulin despite high endogenous insulin levels several years after diagnosis. *The UK Practical Classification Guidelines for Diabetes*[4] use very simple clinically available information to classify patients from scratch, and the authors have assessed their accuracy using a gold-standard diagnosis based on endogenous insulin levels and time to insulin.

A recently published systematic review identified diagnostic accuracy studies in the literature, which compared clinical criteria with C-peptide cut-offs.[7] Age at diagnosis, time to insulin, and BMI are the clinical characteristics most frequently used to classify type 1 and type 2 diabetes, but few studies have addressed clearly which are most strongly associated with long-term C-peptide secretion.[7] Where strength of association has been measured, time to insulin and age at diagnosis appear stronger than BMI. Again as found in the current study, combining time to insulin and age at diagnosis improved diagnostic accuracy, with BMI adding little.[7]

Implications for research and practice

Correct classification of type 1 and type 2 diabetes is important so the appropriate treatment and management guidelines are followed;[3,29] this will relate to treatment, education (for example, about dose adjustment for normal eating for those with type 1), and the monitoring of complications, all of which are based on the presence or absence of endogenous insulin.

The clinical problem facing GPs and other health professionals is that classification can be tricky at the time of diagnosis and all guidelines — including the UK classification guidelines assessed in this study — rely on information that is only available further down the line (for example, time to insulin). The gold standard classification using UCPCR ≥5 years from diagnosis, by definition, cannot completely solve this conundrum: UCPCR of >0.2 nmol/mol within 5 years of diagnosis may represent someone with type 1 diabetes who is still in the 'honeymoon' phase, or someone with type 2 diabetes; a UCPCR of <0.2 nmol/mmol within 5 years of diagnosis can diagnose type 1 diabetes however. Studies designed to improve classification at diagnosis, for example by using islet antibodies, are needed to address this problem.

This study has shown that the UK guidelines based on time to insulin and age at diagnosis are accurate and pragmatic for classifying patients with type 1 or type 2 diabetes. Time to insulin is subject to many influences — physician or patient factors, or guidelines for treatment in a particular area or patient population — but the high rate of correlation of diagnosis with the gold standard suggests overall timing of insulin initiation may be reasonably consistent. Clinically, where the type of diabetes is unclear, giving insulin from diagnosis is a rational decision to avoid the potential consequences of untreated type 1 diabetes, such as ketoacidosis. This study however demonstrates high rates of misclassification as type 1 diabetes in those diagnosed >35 years of age, and thus revisiting the diagnosis in these patients may be worthwhile. The authors suggest that, if there is diagnostic uncertainty, the diagnosis be reviewed, specialist advice sought, and further investigations (for example, C-peptide and islet autoantibodies) be considered.

It could be interesting to follow up those patients identified as misclassified, and those diagnosed with type 2 and still producing insulin beyond 5 years to ascertain whether some of them may be able to withdraw successfully from insulin.

The authors have concentrated on the two main types of diabetes, but recognise

that there are alternative subgroups such as genetic forms of diabetes. Although rare, these are also covered by the UK guidelines and have their own criteria for diagnosis.[30]

It is important that clinicians take into account other factors that may indicate these. The term 'latent autoimmune diabetes in adults' (LADA) is sometimes proposed for adults with islet autoantibodies who eventually (>12 years) become severely insulin deficient, but do not require insulin for at least the first 6 months.[31–34] However, LADA is not included in international guidelines for classification/treatment.

Finally, nothing was found to indicate that modification of the criteria used or the cut-offs proposed would improve their diagnostic performance. This study, like others such as that of Shields *et al*,[7] suggest that age of diagnosis is a better clinical predictor of type 1 diabetes than BMI, which is often used clinically to determine diabetes subtype when it is not clinically obvious; this supports the fact that more emphasis should be placed on age of diagnosis in uncertain cases. This is perhaps particularly relevant in a time when the BMI of the average population is increasing.[35,36]

This study demonstrates that the UK Practical Classification Guidelines for Diabetes are an accurate means for differentiating between type 1 and type 2 diabetes in most instances, with time to insulin and age at diagnosis being the most discriminatory clinical characteristics. As patients aged ≥35 years who were treated with insulin from diagnosis had the highest rate of misclassification (56% classed incorrectly as having type 1 diabetes), further investigation should be considered in this subgroup.

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Ethical approval

This study was approved by the Devon and Torbay Research Ethics Committee; all participants gave written informed consent (08/H0202/167).

Provenance

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Competing interests

The authors have declared no competing interests.

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Urinary C-peptide creatinine ratio detects absolute insulin deficiency in Type 2 diabetes

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Research: Epidemiology

Urinary C-peptide creatinine ratio detects absolute insulin deficiency in Type 2 diabetes

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Abstract

Aims To determine the prevalence and clinical characteristics of absolute insulin deficiency in long-standing Type 2 diabetes, using a strategy based on home urinary C-peptide creatinine ratio measurement.

Methods We assessed the urinary C-peptide creatinine ratios, from urine samples taken at home 2 h after the largest meal of the day, in 191 insulin-treated subjects with Type 2 diabetes (diagnosis age ≥45 years, no insulin in the first year). If the initial urinary C-peptide creatinine ratio was ≤0.2 nmol/mmol (representing absolute insulin deficiency), the assessment was repeated. A standardized mixed-meal tolerance test with 90-min stimulated serum C-peptide measurement was performed in nine subjects with a urinary C-peptide creatinine ratio ≤ 0.2 nmol/mmol (and in nine controls with a urinary C-peptide creatinine ratio >0.2 nmol/mmol) to confirm absolute insulin deficiency.

Results A total of 2.7% of participants had absolute insulin deficiency confirmed by a mixed-meal tolerance test. They were identified initially using urinary C-peptide creatinine ratio: 11/191 subjects (5.8%) had two consistent urinary C-peptide creatinine ratios ≤ 0.2 nmol/mmol; 9 of these 11 subjects completed a mixed-meal tolerance test and had a median stimulated serum C-peptide of 0.18 nmol/l. Five of these 9 had stimulated serum C-peptide <0.2 nmol/l and 9/9 subjects with urinary C-peptide creatinine ratio >0.2 had endogenous insulin secretion confirmed by the mixed-meal tolerance test. Compared with subjects with a urinary C-peptide creatinine ratio >0.2 nmol/mmol, those with confirmed absolute insulin deficiency had a shorter time to insulin treatment (median 2.5 vs. 6 years, P=0.005) and lower BMI (25.1 vs. 29.1 kg/m^2, P=0.04). Two out of the five patients with absolute insulin deficiency were glutamic acid decarboxylase autoantibody-positive.

Conclusions Absolute insulin deficiency may occur in long-standing Type 2 diabetes, and cannot be reliably predicted by clinical features or autoantibodies. Absolute insulin deficiency in Type 2 diabetes may increase the risk of hypoglycaemia and ketoacidosis, as in Type 1 diabetes. Its recognition should help guide treatment, education and management. The urinary C-peptide creatinine ratio is a practical non-invasive method to aid detection of absolute insulin deficiency, with a urinary C-peptide creatinine ratio > 0.2 nmol/mmol being a reliable indicator of retained endogenous insulin secretion.

Diabet. Med. 30, 1342–1348 (2013)

Introduction

Most older patients with diabetes have Type 2 diabetes, which is typically a disease where endogenous insulin persists. Progressive β-cell dysfunction occurs in Type 2 diabetes [1–4], but it is unclear if this leads to absolute insulin deficiency. By contrast, in Type 1 diabetes absolute insulin deficiency is usual outside the initial 'honeymoon period', the period soon after diagnosis when some residual β-cell function may persist [5].

Some patients may present clinically later in life as having Type 2 diabetes, but have the autoimmune destructive process as seen in Type 1 diabetes. These patients can be recognised by pancreatic autoantibodies, known as latent autoimmune diabetes of adulthood (LADA) [6]. People with LADA may develop absolute insulin deficiency [7–10]. In practice, however, autoantibody levels are rarely measured in

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patients presenting with adult-onset diabetes: a clinical diagnosis of Type 2 diabetes is usually made and seldom revisited, and so later subsequent development of absolute insulin deficiency is rarely suspected or tested for.

Absolute insulin deficiency in patients with Type 2 diabetes is likely to carry similar risks to those associated with Type 1 diabetes, such as fluctuant blood glucose levels, high hypoglycaemia risk and diabetic ketoacidosis [11]. The patient with Type 2 diabetes, however, is unlikely to be offered a similar level of education to deal adequately with these, such as the Dose Adjustment for Normal Eating (DAFNE) programme [12]. Frail older people, in particular, may be ill-equipped to cope with such complications, with less functional reserve both physically and cognitively, and in terms of their social support. The development of absolute insulin deficiency in Type 2 diabetes will alter treatment: oral hypoglycaemic agents (especially sulphonylureas) will not be effective, the newer agents, e.g. glucagon-like peptide (GLP)-1 receptor analogues and dipeptidyl peptidase (DPP)4 inhibitors, are not suitable, and the most appropriate insulin regimen may be basal-bolus rather than background long-acting insulin. With an estimated 870 000 people with insulin-treated Type 2 diabetes in the UK, the development of absolute insulin deficiency in even a small proportion could have significant impact on both individuals and society.

Endogenous insulin levels are rarely measured in routine clinical practice, even in secondary care, owing to practical limitations, including the need for rapid laboratory analysis of blood tests. The majority of patients with Type 2 diabetes are cared for in primary care where this is even less practical. Recently, a simple urine test, the urinary C-peptide creatinine ratio (UCPCR) [13], has been shown both in Type 1 diabetes and Type 2 diabetes, to be excellently correlated with the 'gold standard' measure of endogenous insulin secretion, the formal mixed-meal tolerance test (MMTT), and a sensitive and specific test for absolute insulin deficiency [5,14]. The UCPCR test has the advantages of being widely available, and stable at room temperature for 3 days, so offering the potential for widespread non-invasive testing which may be particularly useful for a more frail, older population. The aim of the present study was to use the UCPCR to test for absolute insulin deficiency in older people with insulin-treated Type 2 diabetes.

Methods

Subjects

A total of 191 insulin-treated subjects with Type 2 diabetes (clinical diagnosis of Type 2 diabetes, diagnosis at ≥ 45 years of age, insulin treatment not started within 1 year of diagnosis) were recruited from primary care at the time of their routine retinal screening appointment, and written consent was obtained for participation in the study. Baseline data collected included duration of diabetes, current treatment, BMI and most recent HbA_{1c} concentration.

Urine collection and analysis

Participants were asked to provide an initial urine sample, collected at home, 2 h after their largest meal of the day. The urine sample was collected in a standard mid-stream urine boric acid-containing specimen pot, and returned by post to the routine pathology laboratories for UCPCR analysis. UCPCR $\leq$ 0.2 nmol/mmol is equivalent to a stimulated serum C-peptide (sSCP) of 0.2 nmol/l in an MMTT [15,16], representing an absence of clinically significant insulin secretion [11]. This level is associated with unstable glycaemia, increased risk of hypoglycaemia and microvascular complications (as well as absolute insulin requirement) in Type 1 diabetes [11,16].

All patients identified as insulin-deficient were asked to provide a repeat sample to confirm their initial result, as were a random group of those with a UCPCR >0.2 nmol/mmol.

Mixed-meal tolerance test

In those patients with consistent UCPCR results ≤0.2 nmol/mmol, we performed a formal MMTT with their insulin excluded, to confirm the absolute insulin deficiency [5]. A comparison group of age-matched participants with UCPCR >0.2 nmol/mmol also underwent the standardized MMTT [17]. In brief, subjects fasted from midnight, and omitted their morning medications including insulin. Fasting serum and urine samples were taken before participants consumed 6 ml/kg Ensure Plus HP (Abbott Laboratories, Abbott Park, IL, USA). A blood sample for sSCP was taken 90 min later, and a urine sample for UCPCR at 2 h. As above, a sSCP concentration of <0.2 mmol/l was used to represent absolute insulin deficiency [5,18].

Sample analysis

Urine and serum samples were analysed for C-peptide using an electrochemiluminescence immunoassay (intra-assay coefficient of variation <3.3%; interassay coefficient of variation <4.5%) on a Roche Diagnostics E170 analyser (Roche, Mannheim, Germany) by the biochemistry department at the Royal Devon and Exeter NHS Foundation Trust. Urine creatinine was analysed on the Roche P800 platform using creatinine Jaffé reagent (standardized against isotope dilution mass spectrometry) to obtain a urinary C-peptide creatinine ratio. Blood samples for all patients completing the MMTT were analysed for glutamic acid decarboxylase (GAD)65 and islet antigen 2 (IA2) autoantibodies, using the Biokit automated Elisa System (BEST 2000; Biokit, Barcelona, Spain) following the manufacturer's instructions. The cut-offs used were those based on the 99th centile for 500 individuals

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without diabetes; for GAD65 the reference-positive value was >64 units/ml, for IA2 the reference-positive value was >15 units/ml.

Data analysis

The data were not normally distributed, and so are presented as medians and interquartile ranges (IQRs). Results were analysed primarily by comparing the clinical characteristics of those with confirmed absolute insulin deficiency on MMTT and those with endogenous insulin secretion, using Mann–Whitney *U*- and chi-squared tests (using Predictive Analytic Software: PASW 17.0). The full group of 167 participants with an initial home UCPCR >0.2 nmol/mmol was used to represent those with significant insulin secretion, given the consistency of repeat UCPCR and MMTT results in subgroups drawn from these (see Results and Fig. 1).

Ethics approval was obtained from the Southwest Research Ethics Committee.

Results

A total of 191 participants, with a median (IQR) age 73.5 (67–78) years and of whom 37% were women, provided an initial urine sample for UCPCR measurement. They had a median (IQR) age at diagnosis of 58 (50–65) years, duration of diabetes of 13.5 (9–19) years, and BMI at recruitment of 29 (25.9–33.54) kg/m^2. Their median (IQR) time to insulin treatment from diagnosis was 6 (3.5–11) years.

Figure 1 shows the flow of patients through the study. Of the 191 participants screened, 24 (12.5%) had UCPCR ≤ 0.2 nmol/mmol. Of these, 21 provided a repeat sample, and 11/188 (6% of the whole cohort) had two consistent UCPCR results of ≤ 0.2 nmol/mmol.

Table 1 shows the MMTT results of the two groups selected on the basis of their UCPCR. These two groups were similar in age, duration of diabetes, time to insulin from diagnosis, and BMI. As expected the sSCP concentration was lower in those with a low UCPCR than in those with a high UCPCR (median 0.18 vs. 2.0 nmol/l, respectively, $P = 0.002$). Five of the nine participants with a low UCPCR had a sSCP of <0.2 nmol/l, representing absolute insulin deficiency [18], in contrast to none with a high UCPCR had a sSCP <0.2 nmol/l. This suggests a minimum prevalence of absolute insulin deficiency in insulin-treated Type 2 diabetes of 3% [5/186, excluding the five subjects who were unable to provide repeat urine samples or participate in the MMTT (Fig. 1)].

Notably, the UCPCR results obtained in both groups were substantially higher after the MMTT than after the home

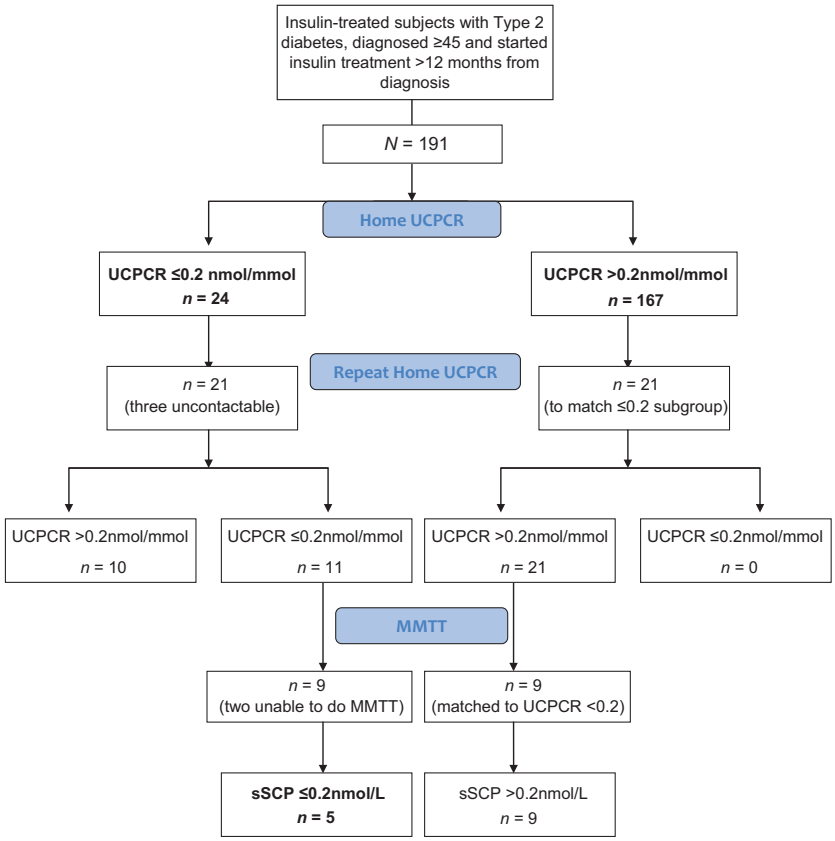

FIGURE 1 Flow of participants through the study. UCPCR, urinary C-peptide creatinine ratio; MMTT, mixed-meal tolerance test; sSCP, stimulated serum C-peptide.

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Table 1 Urinary C-peptide creatinine ratios and stimulated serum C-peptide values in nine subjects with two home UCPCRs of ≤0.2 nmol/mmol, compared with nine matched subjects with two home UCPCRs of >0.2 nmol/mmol

	UCPCR ≤0.2 nmol/mmol	UCPCR >0.2 nmol/mmol	*P*
UCPCR (home), nmol/mmol	<0.02 (<0.02–0.2)	1.7 (0.8–7.1)	<0.001
UCPCR (MMTT) nmol/mmol	0.07 (<0.02–0.7)	2.6 (1.9–5.6)	0.001
fSCP, nmol/l	0.13 (0.08–0.35)	0.59 (0.45–0.88)	0.003
sSCP, nmol/l	0.18 (0.08–0.64)	2.0 (1.53–2.52)	0.002

Data are shown as median values (interquartile range).
UCPCR, urinary C-peptide creatinine ratio; MMTT, mixed-meal tolerance test; fSCP, fasting serum C-peptide; sSCP, stimulated serum C-peptide.

meal. For those four patients with two home UCPCRs ≤ 0.2 nmol/mmol but an sSCP >0.2 nmol/l, the post-MMTT UCPCR results were also >0.2 nmol/mmol. This suggests the MMTT provided more β-cell stimulation than did the meals consumed at home.

The five patients with confirmed absolute deficiency on MMTT had a lower BMI (BMI 25.1 vs. 29.1 kg/m^2, *P*=0.04), and commenced insulin treatment more rapidly after diagnosis (2.5 vs. 6 years, *P*=0.005), although there was substantial overlap for both these measures between those with (*n*=5) and without (*n*=167) absolute insulin deficiency. There was no difference in age of diagnosis, duration of diabetes, glycaemic control or insulin dose (Table 2).

Two of the five participants with absolute insulin deficiency were GAD-positive (titre in both >2000 units/ml); one of these was also IA2 positive (titre 74.9 units/ml). In addition, one patient who had two low UCPCR measurements from home but an sSCP of 0.37 nmol/l was GAD-positive (titre >2000 units/ml). None of the nine participants from the comparison MMTT group, i.e. with home UCPCR demonstrating residual endogenous insulin secretion and confirmed on MMTT, were positive for GAD or IA2 antibodies.

Notably, only two of the five participants with absolute insulin deficiency were on a basal-bolus regimen, and two were treated with oral agents in combination with insulin.

Discussion

A total of 2.7% of insulin-treated patients with a clinical diagnosis of Type 2 diabetes in the present study were found to have absolute insulin deficiency. Patients who may have had absolute insulin deficiency were detected using the simple non-invasive testing method, the UCPCR, and a MMTT was used to confirm findings. These patients cannot be solely identified on the basis of clinical characteristics, or by testing of GAD antibodies.

Table 2 Clinical characteristics of those with absolute insulin deficiency as confirmed by a mixed-meal tolerance test, vs those with endogenous insulin secretion (urinary C-peptide creatinine ratio >0.2 nmol/mmol)

	Absolute insulin deficiency	Endogenous insulin secretion	*P*
n	5	167	
Age at diagnosis, years	63 (54–72)	58 (50–66)	0.28
Duration of diabetes, years	12 (9.5–19.5)	13 (9–17)	0.87
BMI, kg/m^2	25.1 (22.8–28.8)	29.1 (26.3–33.6)	0.04
HbA_{1c}, mmol/mol	72 (57–85)	62 (55–69)	0.24
HbA_{1c},%	8.7 (7.4–9.9)	7.8 (7.2–8.5)	
Time to insulin from diagnosis, years	2.5 (1.5–3)	6 (3–10.75)	0.005
Insulin/kg/24 h, units/kg/24 h	0.72 (0.54–0.88)	0.51 (0.31–0.84)	0.26
No. of subjects on oral hypoglycaemic agent, in addition to insulin (%)*	2/5 (40)	115/167 (69)	0.17
No. of subjects on basal-bolus regime (%)*†	2/5 (40)	19/167 (11)	0.05

Data shown as medians (interquartile range).
*Chi-squared tests; all others Mann–Whitney *U*-test. †Basal-bolus regime: four or five injections of insulin a day.

Prevalence and aetiology of absolute insulin deficiency in Type 2 diabetes

Our prevalence of absolute insulin deficiency of 2.7% (5/186) is similar to the 2.3% (3/133) found at 10 years from diagnosis in an observational study by Niskanen *et al.* [7]. This looked at adult patients over the age of 45 years with new-onset non-insulin-dependent diabetes, and measured sSCP and GAD titres at 0, 5 and 10 years. By including only insulin-treated patients in our study, one might have expected a more insulin-deficient group and hence a comparatively higher proportion of patients with absolute insulin deficiency than in the study by Niskanen *et al.* The aim for tighter glycaemic control (and hence earlier initiation of insulin) in the post-Diabetes Control and Complications Trial/United Kingdom Prospective Diabetes Study era may provide an explanation for why this was not seen. Additionally, the 2.7% prevalence in our study population is a minimum: there were five additional participants with an initial UCPCR suggestive of absolute insulin deficiency who were either uncontactable or unable to undergo a MMTT (Fig. 1). If all these participants had confirmed sSCP <0.2 nmol/l, the prevalence would have risen to 5.2% (10/191).

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In subjects with high titres of GAD antibodies and reasonably long diabetes duration (10–12 years), prospective longitudinal studies have shown that many (but not all) develop absolute insulin deficiency [7,9]. When combined with the clinical features of adult-onset diabetes not immediately requiring insulin treatment, the presence of pancreatic autoantibodies is known as LADA [7,9,10]. Two of the five participants with absolute insulin deficiency in our study fit these criteria, having high GAD titres (>2000 units/ml, reference value >64 units/ml); however, with three participants with a confirmed absolute insulin deficiency not exhibiting GAD antibodies, it suggests that the presence of these antibodies is not a sensitive test for detecting the development of absolute insulin deficiency in those with long-standing diabetes.

Our study has hence identified three people with apparent non-autoimmune Type 2 diabetes and confirmed absolute insulin deficiency. Of the three patients developing absolute insulin deficiency in the study by Niskanen *et al.* [7], one was GAD-antibody-negative. This was the only other case we found in the literature of absolute insulin deficiency confirmed using sSCP in non-autoimmune Type 2 diabetes [7]. It is possible that the cross-sectional measurement of pancreatic autoantibodies in our study may have missed some patients who were antibody-positive at an earlier stage, but lost this positivity over time; however, numerous studies have found that high GAD titres persist [7,9,19,20]. The cross-sectional design of the present study meant we were able to look a wide range of durations of diabetes, longer than those looked at before in Type 2 diabetes, and this may help explain why we have detected absolute insulin deficiency where others have not. No previous studies we have found were designed to look for absolute insulin deficiency in Type 2 diabetes; the majority have looked at the significance of GAD antibodies on the deterioration in β-cell function over time.

Urinary C-peptide creatinine ratio testing

The urinary C-peptide creatinine ratio was used in this study as a practical test in a large number of individuals, and was able to detect patients at risk of absolute insulin deficiency. The gold standard MMTT was used to confirm findings. Those with evidence of endogenous insulin secretion on an initial UCPCR test had consistent results, both on repeat UCPCR and on MMTT. As would be expected by regression to the mean when selecting a low cut-off, those with an initial low UCPCR suggesting absolute insulin deficiency had a tendency to higher results upon repeat testing, taking some above the designated 0.2 nmol/mmol threshold. In addition, some practical issues were identified which may have led to erroneously low UCPCR results on initial testing: these included patients tipping out the boric acid preservative from the sample pots, and postal delays. Additionally, in those with low endogenous insulin levels, variation in meal stimulus may have contributed to a low UCPCR on one occasion vs. a UCPCR over the 0.2 nmol/mmol threshold on another occasion. This is supported by the finding that, in four patients, despite two home UCPCR results suggestive of absolute insulin deficiency, a higher UCPCR and measurable (though low) sSCP levels were seen under controlled MMTT conditions. This suggests the MMTT was more stimulating than the home meals of these patients and they were still able to mount an insulin response when maximally stimulated. Nevertheless, insulin secretion with their normal diet may be more clinically relevant.

The screening method did identify individuals with genuine absolute insulin deficiency. With clear instructions on how optimally to take a sample for UCPCR testing, and advice to repeat a low UCPCR in the first instance, it is a very easy and practical test which has the advantage of being widely available, avoiding the need for venepuncture, and being able be carried out at home and posted in. Since the completion of the present study, it has been shown that the previously widely perceived practical limitations in measurement of C-peptide in blood may be to some extent overcome by using ethylenediaminetetraacetic acid (EDTA) sample tubes: these can improve the stability of C-peptide concentrations to > 24 h at room temperature (average 19.5°C) [21]. This would also make measurement of C-peptide in blood a viable test in the outpatient/primary care setting.

In the increasingly complex climate of diabetes management options, confirmation (or not) of insulin deficiency should help guide treatment, education and management decisions, which will be valuable in optimizing care for any patient, but perhaps particularly for the more frail, older patient. We would suggest that a measure of C-peptide, such as UCPCR, may have an important role when clinical features, such as marked variation in blood glucose values, suggest absolute insulin deficiency.

Clinical characteristics

In our study, those with confirmed absolute insulin deficiency had started insulin sooner after diagnosis than those with retained endogenous insulin (2.5 vs. 6 years), and had lower BMIs (25 vs. 29 kg/m^2). In terms of other easily available and measurable baseline patient characteristics, there was little else to distinguish them.

Although two of the five patients with confirmed absolute insulin deficiency were on basal-bolus regimens, the three others, and several of those with low endogenous insulin levels, were on unusual regimens more suited to patients with endogenous insulin secretion. Two of the five were still on oral hypoglycaemic agents, and none had had any training, such as the DAFNE course [12], to help them understand and manage their diabetes better.

Theoretically despite a clinical diagnosis of Type 2 diabetes, patients with absolute insulin deficiency may be at risk of the complications seen in Type 1 diabetes. This was reflected in all of the patients with absolute insulin deficiency

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– and those with low endogenous insulin levels - reporting difficulty in managing their blood glucose levels owing to seemingly unpredictable fluctuations in blood glucose levels, and one patient having had an episode of ketoacidosis.

Implications for clinical practice

Identification of absolute insulin deficiency in patients with a clinical diagnosis of Type 2 diabetes may enable optimization of their treatment such as basal-bolus regimens, management and education such as DAFNE courses [12], and recognition of potential complications such as higher risks of hypoglycaemia or ketoacidosis. All these have not been traditional considerations in many patients with Type 2 diabetes, and recognition should help improve the quality of life of these individuals.

The UCPCR is a practical and useful test to detect absolute insulin deficiency in Type 2 diabetes and should be used in individuals with Type 2 diabetes developing ketoacidosis, severe hypoglycaemia or having a large fluctuation in blood glucose values, to help inform optimum diagnosis and/or management. A UCPCR suggestive of endogenous insulin production is reliable, and in this clinical context may suggest other explanations for the clinical features (such as compliance). A low UCPCR suggestive of insulin deficiency should be repeated in the first instance, but may help guide management and education as described above.

Conclusion

In conclusion, we have shown that absolute insulin deficiency is present in 3% of insulin-treated subjects with Type 2 diabetes and may be detected using UCPCR. Clinical features such as GAD antibodies, starting insulin sooner after diagnosis, and having a lower BMI are pointers to help recognize those at risk, but are not diagnostic. Those with absolute insulin deficiency are at risk of more fluctuant blood glucose levels, hypoglycaemia and ketoacidosis, which may adversely affect quality of life as well as potentially have more severe consequences, especially in the older population. Recognition of absolute insulin deficiency is thus important as it will aid the optimum management of these individuals, and the UCPCR is a useful test that can be used in general practice or in outpatients to confirm a clinical suspicion of insulin deficiency.

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Competing interests

None declared.

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Random non-fasting C–peptide: bringing robust assessment of endogenous insulin secretion to the clinic

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Short Report: Care Delivery

Random non-fasting C–peptide: bringing robust assessment of endogenous insulin secretion to the clinic

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Abstract

Background Measuring endogenous insulin secretion using C–peptide can assist diabetes management, but standard stimulation tests are impractical for clinical use. Random non-fasting C–peptide assessment would allow testing when a patient is seen in clinic.

Methods We compared C–peptide at 90 min in the mixed meal tolerance test (sCP) with random non-fasting blood C–peptide (rCP) and random non-fasting urine C–peptide creatinine ratio (rUCPCR) in 41 participants with insulin-treated diabetes [median age 72 (interquartile range 68–78); diabetes duration 21 (14–31) years]. We assessed sensitivity and specificity for previously reported optimal mixed meal test thresholds for severe insulin deficiency (sCP < 200 pmol//l) and Type 1 diabetes/inability to withdraw insulin (< 600 pmol//l), and assessed the impact of concurrent glucose.

Results rCP and sCP levels were similar (median 546 and 487 pmol//l, $P = 0.92$). rCP was highly correlated with sCP, $r = 0.91$, $P < 0.0001$, improving to $r = 0.96$ when excluding samples with concurrent glucose < 8 mmol//l. An rCP cut-off of 200 pmol//l gave 100% sensitivity and 93% specificity for detecting severe insulin deficiency, with area under the receiver operating characteristic curve of 0.99. rCP < 600 pmol//l gave 87% sensitivity and 83% specificity to detect sCP < 600 pmol//l. Specificity improved to 100% when excluding samples with concurrent glucose < 8 mmol//l. rUCPCR (0.52 nmol/mmol) was also well-correlated with sCP, $r = 0.82$, $P < 0.0001$. A rUCPCR cut-off of < 0.2 nmol/ mmol gave sensitivity and specificity of 83% and 93% to detect severe insulin deficiency, with area under the receiver operating characteristic curve of 0.98.

Conclusions Random non-fasting C–peptide measures are strongly correlated with mixed meal C–peptide, and have high sensitivity and specificity for identifying clinically relevant thresholds. These tests allow assessment of C–peptide at the point patients are seen for clinical care.

Diabet. Med. 00, 000–000 (2016)

Introduction

Assessment of endogenous insulin secretion using C–peptide is useful in clinical practice to assist in the classification and treatment of diabetes [1]. Assessment of a stimulated blood C–peptide level following a standardized stimulus such as a mixed meal (mixed meal tolerance test, MMTT) provides a gold standard measure of endogenous insulin secretion, but is impractical for clinical use [2]. Other C–peptide measures such as fasting blood C–peptide [3], or a post-home meal urinary C–peptide creatinine ratio (UCPCR) [4–6], give a reasonable approximation to the gold standard, and high sensitivity and specificity in classifying diabetes [7–10]. However, for routine clinical care, the most practical test would be a spot 'random' non-fasting sample, sent when a patient is seen in an outpatient or primary care clinic.

Random non-fasting blood C–peptide (rCP) has been shown to have superior performance to both post-glucagon and fasting blood C–peptide assessment in differentiating clinically well-defined Type 1 and Type 2 diabetes [7,8], and to have clinical utility in detecting Maturity Onset Diabetes of the Young (MODY) [11,12]. However, rCP has never been formally validated against a gold-standard MMTT C–

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What's new?

- Measuring endogenous insulin secretion using C–peptide can assist diabetes management, but standard stimulation tests are impractical for clinical use.
- This study assessed whether a random non-fasting C–peptide can be used to assess endogenous insulin secretion.
- Random blood C–peptide and urine C–peptide creatinine ratio (UCPCR) were both highly correlated with mixed meal tolerance test C–peptide and were sensitive and specific measures for clinically useful mixed meal test thresholds.
- A random non-fasting C–peptide taken when a patient is seen in clinic can be used to assess endogenous insulin secretion in clinical practice.

peptide assessment. Although UCPCR changes little from 2 to 4 h post meal in those with Type 2 diabetes (McDonald T. J., unpublished), utility of a random non-fasting UCPCR sample has never been assessed.

We aimed to compare non-fasting random blood C–peptide and UCPCR with 'gold standard' blood C–peptide assessment at 90 min in the MMTT.

Methods

Participants

Forty-one participants with insulin-treated diabetes were recruited to the GREAT study (https://clinicaltrials.gov, NCT02506296). To ensure a range of C–peptide values, participants were selected on the basis of prior C–peptide assessment to include participants with and without severe insulin deficiency (under/over 200 pmol//l post-MMTT blood C–peptide or equivalent [1]). All participants had a clinical diagnosis of Type 2 diabetes, and an estimated glomerular filtration rate (eGFR) > 30 ml/min/1.73 m^2. Ethical approval was obtained from the NRES Committee South West, and all participants gave written informed consent.

Mixed meal tolerance test

Participants fasted from 10 p.m., then attended the following day prior to 11 a.m., having not taken their morning medication prior to arrival. Baseline bloods for glucose and C–peptide were taken, morning insulin given [13] and 160 ml of Fortisip Compact (Nutricia, Trowbridge, UK) drunk within 10 min (content per 100 ml: carbohydrate 29.7 g, protein 9.6 g, fat 9.3 g). Bloods for C–peptide and glucose analysis were repeated every 30 min, up to and including 180 min post-mixed meal. Samples were immediately centrifuged after collection and stored at –80°C, for later batched analysis.

Non-fasting tests

On a separate occasion (within 8 days of the MMTT), blood was taken between 9 a.m. and 5 p.m., within 5 h of a meal, and without restriction on snacks or other intake. Whole-blood samples collected in potassium–EDTA (C–peptide) and fluoride oxalate (concurrent glucose) tubes were sent at room temperature to be processed routinely at the Royal Devon & Exeter Hospital Blood Sciences department. Participants were also asked to provide a spot urine sample. This was frozen at –80°C before later batch analysis.

Sample analysis

C–peptide was analysed using the automated Roche Diagnostics (Manheim, Germany) E170 immuno-analyser (limit of detection: 3.3 pmol//l; inter- and intra-assay coefficients of variation: < 4.5% and < 3.3%, respectively). Urinary creatinine was analysed on the Roche P800 platform to obtain UCPCR (nmol/mmol).

Analysis

We compared the median rCP with the median blood C–peptide at 90 min in the MMTT (sCP) using Wilcoxon's signed rank test, and correlation coefficient between both rCP and random non-fasting UCPCR (rUCPCR) with sCP using Spearman's rank correlation.

We then assessed the utility of rCP and rUCPCR in correctly classifying participants in relation to previously described clinically relevant MMTT C–peptide thresholds using receiver operating characteristic (ROC) curves, with corresponding specificities and sensitivities:

1. MMTT sCP < 200 pmol//l: absolute insulin deficiency [1,14];
2. MMTT sCP < 600 pmol//l: Type 1 diabetes/inability to withdraw insulin [1].

Finally, we assessed the influence of concurrent glucose repeating the above analyses excluding hypoglycaemia (concurrent glucose < 4 mmol//l), and a previously suggested cut-off of < 8 mmol//l [1,8,15].

Results

Participant characteristics

Twenty-eight of the 41 participants (68%) were men. Participants had a median age of 73 years [interquartile range (IQR), 68–78], diabetes duration 21 (14–31) years, BMI 26.8 (25–29.9) kg/m^2 and HbA_{1c} 68 (58–75) mmol/mol [8.4% (7.5–9.0%)].

Twelve of the 41 participants (29%) had severe insulin deficiency (sCP < 200 pmol//l). C–peptide was detectable

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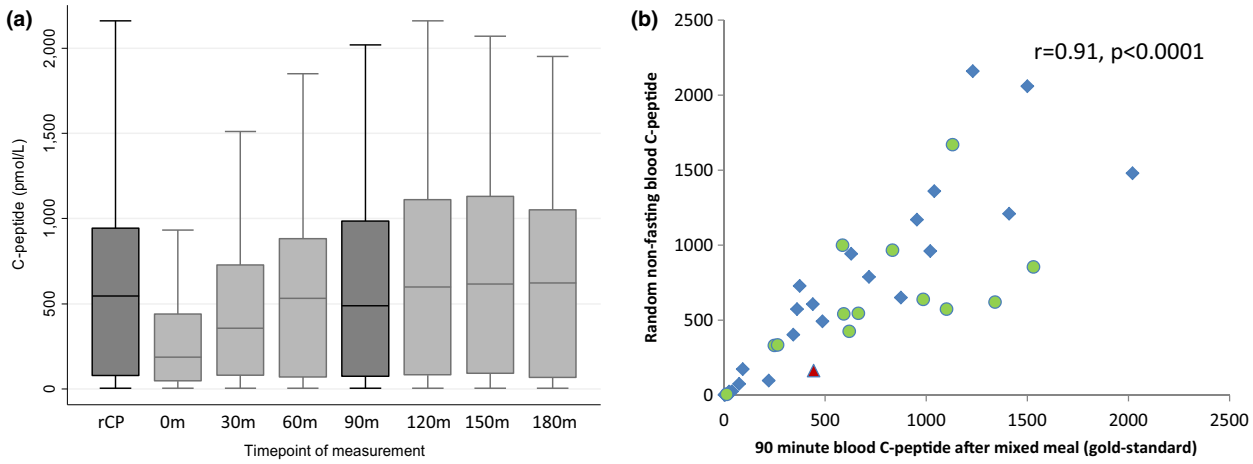

FIGURE 1 (a) Blood C–peptide levels on random sampling and in the mixed meal test. rCP, random non-fasting; time points reflect minutes post mixed meal ingestion, 0 m: fasting sample. (b) Random non-fasting C–peptide vs. 90–min C–peptide in the mixed meal tolerance test. Level of blood glucose measured concurrently with rCP shown by blue diamonds > 8 mmol/l; green circles > 4–8 mmol/l; red triangles: < 4 mmol/l.

(> 2.9 pmol//l) at all time-points, fasting and stimulated, in 40 of 41 participants.

C–peptide was stable 1–3 h after meal stimulation

There was little change in the C–peptide from 60 min to 3 h post MMTT: median C–peptide ranged from 487 to 622 pmol//l across these five time points (Fig. 1a). Mean individual coefficient of variation over the 1–3–h post-MMTT period was 14.3%.

Random non-fasting blood C-peptide level is strongly correlated with the gold standard 90–min mixed meal test C–peptide

The median rCP of 546 pmol//l (IQR 76–943) was similar to sCP at 90 min, 487 pmol//l (75–985), $P = 0.92$ (Fig. 1a).

rCP was strongly correlated with sCP: Spearman's rho correlation coefficient = 0.913, $P < 0.0001$ (Fig. 1b). When only participants who had a concurrent lab glucose value of ≥ 8 mmol//l were included (66% participants), the correlation coefficient increased to 0.96.

As expected, the results showed more variation in the higher C–peptide range (Fig. S1).

Random non-fasting bood C-peptide is a highly sensitive and specific test for severe insulin deficiency

rCP was a highly sensitive and specific test for severe insulin deficiency (sCP < 200 pmol//l), with an area under the ROC curve (AUC ROC) of 0.99 [95% confidence interval (95% CI): 0.91–1; Table 1]. An rCP cut-off of < 200 pmol//l gave a sensitivity of 100% (74–100) and specificity of 93% (77–99) for severe insulin deficiency, with 95% (83–99) of

Table 1 Ability of random non-fasting blood C–peptide (rCP) and UCPCR (rUCPCR) to define absolute insulin deficiency [90-min mixed meal tolerance test C–peptide (sCP) < 200 pmol/l] and Type 1 diabetes/insulin dependence (sCP < 600 pmol/l) using equivalent thresholds, with and without exclusion based on concurrent glucose (blood C–peptide only)

Mixed meal test C–peptide threshold	Concurrent glucose cut-off (mmol/l)	n	AUC	AUC 95% CI	Specificity 95% CI (%)	Sensitivity 95% CI (%)	Correctly classified 95% CI (%)
Random non-fasting blood C–peptide							
< 200 pmol/l	All	41	0.99	0.91–1.0	93 (77–99)	100 (74–100)	95 (83–99)
	≥ 4	39	1.0	0.91–1.0	96 (82–100)	100 (72–100)	97 (87–100)
	≥ 8	27	0.99	0.87–1.0	94 (73–100)	100 (66–100)	96 (80–100)
< 600 pmol/l	All	41	0.94	0.84–0.99	83 (59–96)	87 (66–97)	85 (71–94)
	≥ 4	39	0.94	0.79–0.98	83 (59–96)	86 (64–97)	85 (69–94)
	≥ 8	27	0.99	0.87–1.0	100 (74–100)	87 (60–98)	93 (76–99)
Random non-fasting UCPCR							
< 200 pmol/l	All	40	0.98	0.87–1.0	93 (76–99)	83 (52–98)	90 (76–97)
< 600 pmol/l	All	40	0.90	0.76–0.97	83 (59–96)	82 (60–95)	83 (67–93)

Sensitivity, specificity and % correct classification are given for numerically equivalent thresholds (rCP: 200 and 600 pmol/l; UCPCR: 0.2 and 0.6 nmol/mol) because these were close to optimal on ROC analysis. 95% CI, 95% confidence intervals.

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participants correctly classified. This did not alter significantly with concurrent glucose (Table 1).

rCP was also able to identify participants with sCP < 600 pmol//l (Type 1 diabetes/inability to withdraw insulin): AUC ROC 0.94 (95% CI: 0.84–0.99). An rCP value < 600 pmol//l gave a sensitivity of 87% (66–97) and specificity of 83% (59–96) to detect sCP< 600 pmol//l – with 85% (71–94) correctly classified. Excluding concurrent glucose values < 8 mmol//l improved specificity to 100% (74–100) without altering sensitivity (Table 1).

rUCPCR is also strongly correlated with the gold standard blood C–peptide measure and a sensitive and specific test for severe insulin deficiency

rUCPCR [median 0.52 nmol/mmol (IQR 0.095–1.57 nmol/mmol)], was well-correlated with sCP, $r = 0.82$, $P < 0.0001$ ($n = 40$). rUCPCR was also a sensitive and specific test for detecting the clinically relevant thresholds of sCP < 200 and < 600 pmol//l: ROC AUC 0.98 (0.87–1.0) and 0.90 (0.76–0.97), respectively (Table 1). For identifying severe insulin deficiency (sCP < 200 pmol//l), an rUCPCR cut-off of < 0.2 nmol/mmol gave a sensitivity and specificity of 83% (52–98) and 93% (76–99), with 90% (76–97) participants being correctly classified. An rUCPCR cut-off of < 0.6 nmol/mmol had a sensitivity and specificity of 82% (60–95) and 83% (56–96) for detecting sCP< 600 pmol//l.

Discussion

Our results show that random non-fasting blood C-peptide and UCPCR measurements taken when a patient attends clinic are highly correlated with the gold standard mixed meal test assessment of endogenous insulin secretion, and are sensitive and specific tests for clinically relevant thresholds. These tests, combined with the demonstration of stability at room temperature of blood C–peptide for > 24 h (in EDTA) [16] and UCPCR for > 72 h (in boric acid) [17], offer a practical way of assessing endogenous insulin excretion when contact is made for clinical care.

Our findings are consistent with previous research demonstrating that a random non-fasting blood C–peptide offers similar performance to C–peptide in a formal glucagon stimulation test when classifying clinically well-defined Type 1 and Type 2 diabetes [8], is superior to fasting C–peptide when identifying autoimmune diabetes [7] and has high clinical utility for detecting patients with undiagnosed monogenic diabetes [11]. This is the first study to formally evaluate use of a random non-fasting C–peptide sample against a gold standard in a mixed meal test. The use of a random non-fasting UCPCR has not been previously assessed.

Limitations of our study include that our modest sample size limits our ability to assess the impact of concurrent glucose on random non-fasting C–peptide testing. In addition, our population may not be representative of the patients in whom C–peptide testing has most utility (difficult to classify diabetes) in that they are older patients who have been selected on the basis of a clinical diagnosis of Type 2 diabetes with or without discordant C–peptide.

Our results suggest that a random non-fasting blood C–peptide or UCPCR could be used to assess endogenous insulin secretion in clinical practice. This would have major practical advantages in that the test can be conducted when a patient is seen for clinical care. Although our sample size is too small to robustly assess the impact of concurrent glucose, our results suggest this has only modest impact. Although a high value in the presence of any glucose is likely to be robust it may be prudent to treat random non-fasting C–peptide values below a clinical threshold where concurrent glucose is < 8 mmol//l with caution, and consider a repeat sample.

Conclusion

Random non-fasting blood and urine C–peptide measures seem to be strongly correlated with the gold standard C–peptide test and have high sensitivity and specificity in identifying clinically relevant C–peptide thresholds. A larger study would confirm findings, but these tests could allow assessment of C–peptide at the point patients are seen for clinical care.

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Competing interests

None declared.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Bland–Altman plot showing the difference between 90-minute C–peptide (sCP) and random non-fasting C–peptide (rCP).

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