Prediction and Detection of Hypoglycaemia in Type 1 Diabetes

Sankalpa Neupane

Wolfson College Cambridge

University of Cambridge

July 2018

This dissertation is submitted for the degree of

Doctor of Philosophy

This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text.

It does not exceed the prescribed word limit set by the Degree Committee of Clinical Biochemistry.

Abstract

‘Prediction and Detection of Hypoglycaemia in Type 1 Diabetes’

Sankalpa Neupane

Patients with Type 1 Diabetes still suffer from recurrent and troublesome hypoglycaemia despite improvements in glucose monitoring, insulin delivery and structured education.

The key objectives of my thesis were to explore ways to identify patients at greater risk to develop hypoglycaemia and to investigate novel ways to prevent hypoglycaemic episodes by detecting this early and with ease. This might ultimately translate into reduction in overall risk of hypoglycaemia occurrence in those with Type 1 Diabetes.

I investigated potential factors that could predict persistent presence of severe hypoglycaemia in the HypoCOMPaSS study, a multi-centre UK study examining clinical strategies to reduce burden of hypoglycaemia. Looking at a subset of these participants who underwent detailed hypoglycaemic clamp studies, I found no obvious parameters predicting persistent risk of severe hypoglycaemia.

I then examined whether individual genetic factors might contribute to risk of persistent severe hypoglycaemia, exploring the association between polymorphisms in Angiotensin Converting Enzyme ACE gene and severe hypoglycaemia in 77 participants in HypoCOMPaSS study. Interestingly, I found that the homozygous DD ACE gene polymorphism was associated with a significantly increased risk of severe hypoglycaemia.

Considering then practical approaches to trying to minimise hypoglycaemia risk, I examined the efficacy and safety of a novel implantable Continuous Glucose Monitoring Senseonics CGM System in 10 subjects with Type 1 Diabetes from the Cambridge cohort of the pivotal European PRECISE 1 study. In keeping with the global data, I found in Cambridge participants that this novel system was safe and its efficacy was comparable to commercially available Continuous Glucose Monitoring. This device was useful in detecting hypoglycaemia early with high device satisfaction among the users.

I also explored alternative non-invasive methods to measure blood glucose and detect hypoglycaemia easily using breath sample in Type 1 Diabetes participants during experimental hypoglycaemia. The concentration of Isoprene exhaled in breath was significantly raised during hypoglycaemia.

Finally, I looked at the stability of ‘diluted insulin aspart (NovoRapid®)’ in ambient temperature and CSII over 30 days. Both neat and diluted insulin aspart were stable beyond 30 days and could potentially be used by patients with T1D requiring very low insulin doses to avoid hypoglycaemia.

Acknowledgements

I am grateful to my supervisor Dr Mark Evans for the support and guidance throughout my clinical research fellow period, at the write up period and overall guidance on career as a whole. I am also grateful to Dr Roman Hovorka for supervision during PRECISE I study. I am grateful to funders for research grant and salary support – the NIHR Cambridge Biomedical research centre, Diabetes UK, University of East Anglia and Senseonics Inc. I am equally grateful to Dr Frank Waldron-Lynch (formerly of Department of Medicine University of Cambridge) for partly supporting my university fees.

I wish to thank fellow laboratory and research colleagues Dr Lalantha Leelarathna, Dr Hood Thabit, Dr Martin Tauschman, Christine Riches, Dr Kavita Kumareswaran and staff at WTCRF for their support and help during various stages of the research studies. I particularly wish to thank Dr Lalantha Leelarathna for his contribution to HypoCOMPaSS study and ACE genotype sub study.

I would like to thank study participants for their time for research, without whom none of the clinical studies described here would have been possible to complete.

I am indebted to my parents for their support throughout my life. I could not reach this stage in my career without my parents Chandra Mani Neupane and Indira Neupane's belief and support. My twin brother Bikalpa has also been my source of inspiration.

Above all, none of this would have been possible without the love and patience of my family. I dedicate this dissertation to my wife Prajita who is my strength and also to children who have sacrificed their own play/family time. My son Safal and my daughter Smarika never understood why I needed to be in the computer studying all the time, but I am sure they will understand this later in their own life and I hope to be the source of inspiration for them.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ACE	Angiotensin Converting Enzyme
ADA	American Diabetes Association
CGM	Continuous Glucose Monitoring
CBGT	Capillary Blood Glucose Testing
CEG	Clarke Error grid
CL	Closed Loop
CI	Confidence Interval
CRF	Clinical Research Facility
CSII	Continuous Subcutaneous Insulin Infusion
CV	Coefficient of Variation
DAFNE	Dose Adjusted for Normal Eating
DCCT	Diabetes Control and Complication Trial
HbA1c	Glycosylated Haemoglobin
HCP	Health Care Professionals
HypoCOMPaSS	Comparison of Optimised MDI versus Pumps with or without Sensors in Severe Hypoglycemia
HAAF	Hypoglycaemia associated autonomic failure
IDF	International Diabetes Federation
IAH	Impaired Awareness of Hypoglycaemia
ISF	Interstitial Fluid
JDRF	Juvenile Diabetes Research Foundation
LGS	Low Glucose Suspend

MARD	Median Absolute Relative Difference
MDI	Multiple Daily Injections
NICE	National Institute for Health and Care Excellence
POC	Point of Care
OGTT	Oral Glucose Tolerance Test
QOL	Quality of Life
RAS	Renin-Angiotensin System
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomised Control Trial
RT-CGM	Real Time Continuous Glucose Monitoring
SAP	Sensor-Augmented Pump
SH	Severe Hypoglycaemia
SD	Standard Deviation
SEM	Standard Error of Mean
SMBG	Self-monitoring of Blood Glucose
T1D	Type 1 Diabetes Mellitus
T2D	Type 2 Diabetes Mellitus
WHO	World Health Organisation
WTCRF	Wellcome Trust Clinical Research Facility
UKPDS	United Kingdom Prospective Diabetes Study
VOCs	Volatile Organic Compounds

1 Introduction

1.1 Epidemiology of Type 1 Diabetes

The global incidence of diabetes is increasing rapidly. According to a 2015 estimate from the International Diabetes Federation, 415 million adults aged 20-79 have diabetes worldwide, including 193 million who are undiagnosed. It is estimated that by 2040, the number of people affected by diabetes could be as high as 642 million[1].

Diabetes Mellitus is classified in 4 broad categories –Type 1 diabetes (T1D), Type 2 Diabetes Mellitus (T2D), Gestational Diabetes (GDM) and diabetes due to other causes for e.g. diabetes due to genetic defects, drug induced diabetes, diabetes due to endocrinopathies etc.[2].

T1D previously encompassed by the terms insulin-dependent diabetes or juvenile-onset diabetes, results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas, accounts for 5–10% of those with diabetes[2]. It is one of the commonest autoimmune disorders in children. Around 86,000 children develop T1D each year worldwide[1]. The UK has the world's fifth highest rate of T1D diagnosis in children aged 0 to 14 based on estimates from the International Diabetes Federation as 24.5 per 100,000 children in this age group are diagnosed with the condition every year[3].

T1D can occur at any age, with a peak incidence around puberty[4]. In many populations, the incidence is somewhat higher in males than in females, and a 1.3- to 2.0-fold male excess in incidence after about 15 years of age exists in most populations. The incidence of childhood-onset T1D varies markedly

among countries. East Asian and native American populations have low incidences (approximately 0.1–8 per 100,000/year), while the highest rates are found in Finland (>60 per 100,000/year), Sardinia (40 per 100,000/year), and Sweden (47 per 100,000/year). The risk is highest in European-descent. About 10 %–20 % of newly diagnosed childhood cases of T1D have an affected first-degree relative. Those with an affected sibling or parent have a cumulative risk of 3 %–7 % up to about 20 years of age, as compared with <1 % in the general population. The cumulative incidence among the monozygotic co-twins of persons with T1D is less than 50 %[4].

1.2 Pathophysiology and Natural History of Type 1 Diabetes

T1D is a common metabolic condition occurring as a consequence of the autoimmune destruction of pancreatic beta cells[5]. This is characterised by an absolute or relative insulin deficiency leading to hyperglycaemia. Hyperglycaemia presents as polyuria, polydipsia, and in extreme situations if untreated, may lead to diabetic ketoacidosis which is potentially fatal. It may also co-exist with other autoimmune disorders like primary hypothyroidism, Addison's disease and pernicious anaemia.

This chronic autoimmune condition is caused by T-cell mediated inflammation and destruction of pancreatic β-cells. Classically, auto antibodies to antigens such as glutamic acid decarboxylase GAD (GADAs), insulin [Insulin Auto antibody (IAAs)], Zinc transporter 8 (ZnT8As) and protein tyrosine [Insulinoma Antigen 2 (IA-2) (IA-2As)] may be present. Antibodies can be seen in 80-90% of the people with T1D[6]. However antibodies are often not seen in some

populations for example people from Afro-Caribbean descent [7]. Although the exact reason is not known, some speculate that T1D in African populations is mediated by novel autoantibodies that may have not been uncovered[8]. Furthermore those diagnosed with T1D clinically but with negative auto antibodies may actually have been non-Type 1 diabetes for example Maturity Onset Diabetes of the Young (MODY)[9].

It is not known if pancreatic beta cell inflammation is the response to other triggering factors such as viral infection. Several theories have been postulated to explain the initiation of this destructive autoimmune process. Some have explained this on the basis of molecular sharing between beta cells and an environmental factor or loss of immune tolerance[10]. The discovery of a susceptible gene locus, the Human Leukocyte Antigen (HLA) complex, has provided insight into the contribution of genetic susceptibility in type 1 diabetes[11]. Among the three classes of HLA genes, the strongest association is found with HLA class II genes. These genes are involved in antigen presentation and therefore may influence the way beta cell antigen is presented leading to auto-self-reactivity. DR and DQ genotypes have been associated with T1D. HLA-DR3 and HLA-DR4 are associated with T1D in Caucasians [12-14].

Underpinning the heritability, monozygotic twins have shown a concordance rate of 30 - 50 % compared with dizygotic twins whose rate is lower at 10%[15]. It is hypothesised that T1D may be triggered by various environmental factors in the individuals who are genetically prone to develop the condition. Various factors which have been suggested but are unproven in the activation of

autoimmunity are congenital Rubella infections, enteroviral infection and early introduction of cow's milk in infancy effect [16-19].

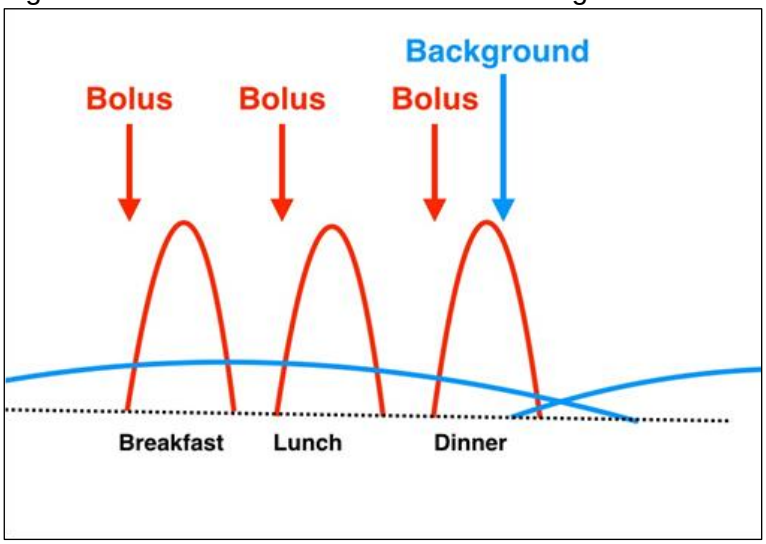
The Diabetes Prevention Trial-Type 1 (DPT-1) suggested that although metabolic changes may occur 2 years prior to presentation, significant c-peptide level changes were seen only 6 months prior to diagnosis and that insulin sensitivity may play a vital role in the period before the overt presentation leading to the diagnosis. At the time of diagnosis up to 50% of beta cell function may still be preserved[20].

Prolonged hyperglycaemia is associated with poor outcome [21, 22]. Diabetes related complications in humans can be either macrovascular or microvascular. Microvascular disease affects eye, nerve and kidney leading to retinopathy, neuropathy and nephropathy respectively. Macrovascular disease largely comprises peripheral vascular, cardiovascular and cerebrovascular disease [23-27]. Hence tight glycaemic control is being advocated by clinicians to minimise morbidity and mortality associated with poor glycaemic control [28, 29].

1.3 Management of glycaemia in Type 1 Diabetes

Many technical and healthcare changes have occurred over the last few years in T1D. New insulin analogues continue to be developed and have been widely adopted in T1D [30]. The combination of rapid acting 'bolus' insulin given before meals and/or as ad hoc doses to correct high blood glucose values, with long-acting background insulin replacement (termed 'basal' insulin) allows people to use more 'physiological' basal bolus insulin regimens [28, 31](Figure 1-1)[32].

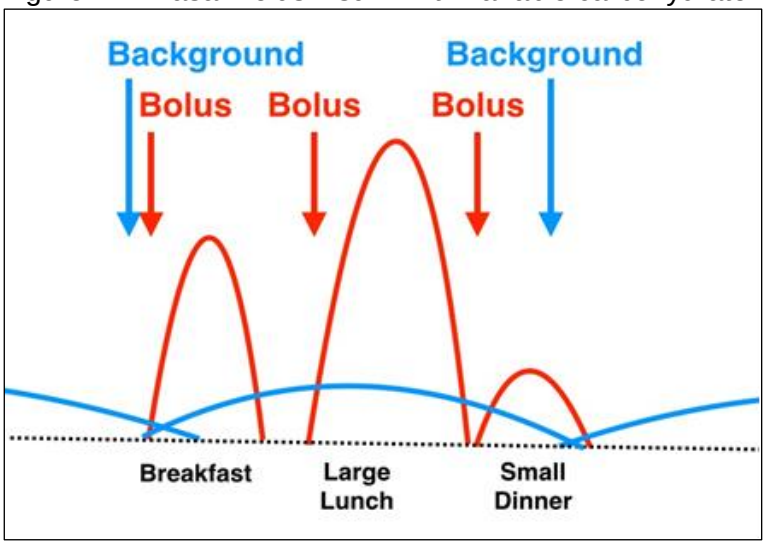
Figure 1-1: Standard Basal Bolus Insulin Regimen



A ‘basal bolus’ insulin regimen with 3 bolus injections of rapid acting insulin (red) for meals with, in this case, once daily background insulin (blue).

Variable bolus insulin is adjusted with counting carbohydrates in meals. Below is an example of larger carbohydrate containing lunch and smaller carbohydrate dinner (Figure 1-2)[32].

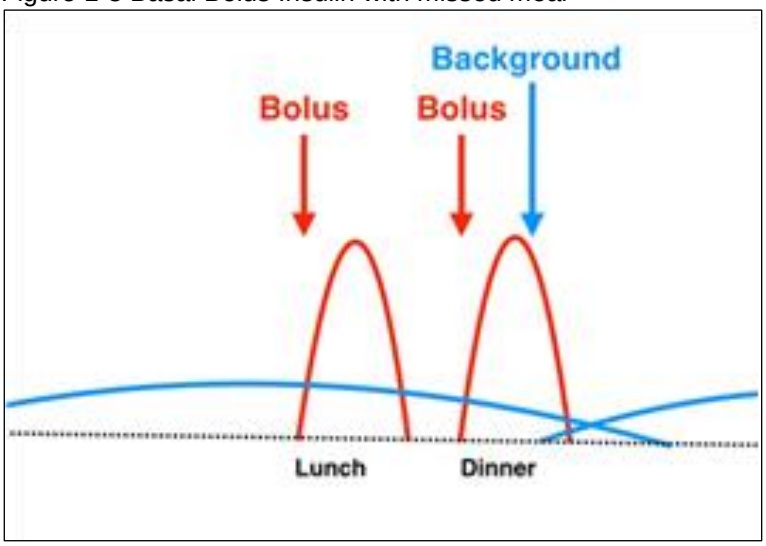
Figure 1-2: Basal Bolus insulin with variable carbohydrate intake



Rapid-acting insulin (red) for meals with, in this case, twice daily background insulin (blue)

This regimen is flexible and patient can miss meal in contrast to those on twice daily mixed insulin regime where people are required to eat to give insulin injection as shown in Figure 1-3 [32].

Figure 1-3 Basal Bolus Insulin with missed meal



Example of missed (or carbohydrate free) breakfast. Rapid acting insulin (red) for meals with, in this case, once daily background insulin (blue).

1.3.1 *Structured patient education*

The flexibility offered by modern variable insulin regimens presents a challenge for people with T1D, who need to learn and use many self-management skills beyond simply checking blood glucose concentration and administering insulin [32-34]. For example, many people with T1D adjust doses of meal-time rapid-acting insulin depending on the carbohydrate content of food, meaning that they need training to count carbohydrates accurately. Concept of flexible meal related insulin dose adjustment was reported as early as the 1930s. This became practicable since home blood glucose monitoring was possible[35]. Diabetes Teaching and Treatment Programme (DTTP) developed in Germany was one of the first few education programmes in the modern era. This was a residential 1-week programme.[35, 36]. Educational packages have emerged in the UK over the last one and half decades [37-41]. The largest and best established of these in the UK is the DAFNE programme (Dose Adjustment For Normal Eating), described below, but a number of broadly similar smaller structured education programmes are also being used in UK services [39, 42, 43]. The DAFNE course is based on a patient education model developed in Germany. This was modified from Germany's Diabetes Teaching and Treatment Programme (DTTP) as a 5-day non-residential course. This programme was first integrated in T1D care in the UK in 2002[35]. In a randomized controlled trial, DAFNE improved blood glucose control and patient well-being [37]. As per early 2018 data, 6,445 DAFNE courses have been delivered in the UK resulting in 43,880 DAFNE graduates and 41 DAFNE courses were planned for February 2018[44]. It has also been adopted in Australia, New Zealand and

Singapore[35]. DAFNE continues to be effective with improved glycaemic and patient-reported outcomes [39, 41, 45]. People experience not only lower average blood glucose concentrations, reflected by lower HbA1c values, but also less hypoglycemia and improvement in their ability to detect a falling blood glucose sooner [35, 40, 42, 45, 46].

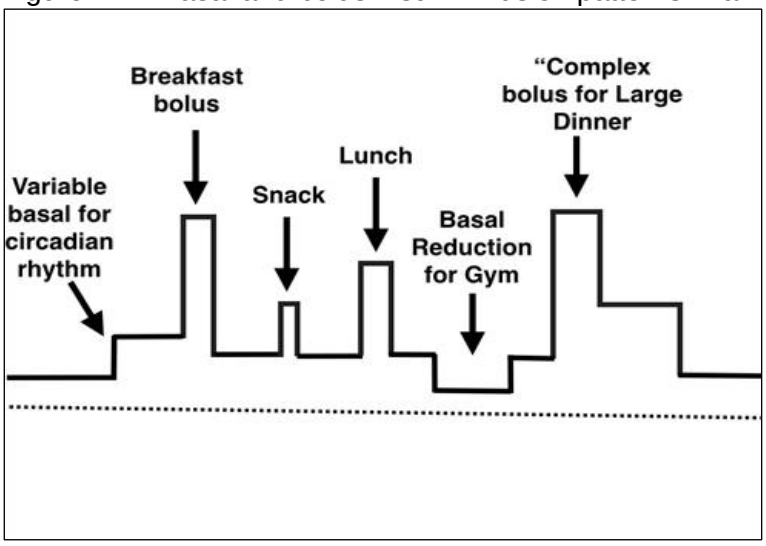
DAFNE and similar programmes provide education in skills required to self-adjust insulin doses, such as carbohydrate counting, adjustment for exercise/activity, illness, stress, alcohol, travel and the menstrual cycle [40-43].

1.3.2 *Continuous subcutaneous insulin infusion (CSII /'insulin pumps') therapy*

Dr. Arnold Kadish developed the first insulin pump in the early 1960s, which was as big as a Marine backpack. Since then, insulin pumps have become much more refined and have decreased to the size of a pager [47, 48].

With continuous subcutaneous insulin infusion (CSII), rapid-acting insulin is pumped in constantly as a basal insulin replacement via an indwelling subcutaneous infusion cannula, self-inserted typically into abdomen, buttock, thigh or arm. The infusion set is removed typically every 3 days and a fresh set inserted at a different site[49]. People can then use the pump to deliver an insulin bolus to cover meals and/or corrections for high glucose values as needed. Figure 1-4 illustrates a daily profile with changes in insulin infusion to cover circadian patterns, meals (including snacks and large complex meals) and activity.

Figure 1-4 : Basal and bolus insulin infusion patterns in an insulin pump (CSII)



Early CSII models were cumbersome and unreliable but the last decade has seen increased uptake in many developed countries in both children and adults [48, 49]. Used judiciously, CSII can improve glucose control [50-57] but they are more complex technically and medically and thus unlikely to replace injections as standard treatment for insulin-requiring diabetes in the near future [54, 58-61].

Currently in the UK, NICE (National Institute for Care Excellence) guidance is that, in adults, CSII should be considered in those with HbA1c value of greater than 69 mmol/mol (8.5%) in spite of optimized therapy with injections and /or if hypoglycaemia is a significant problem [62].

1.3.3 *Continuous Glucose Monitoring*

Continuous glucose monitoring (CGM) has been commercially available since 2000 [63]. They measure interstitial glucose continuously. The CGM system

usually consists of a self-inserted subcutaneous sensor linked wirelessly to a receiver.

Amperometric CGM sensors measure interstitial glucose. The sensor typically is a needle type electrode that contains enzyme. Glucose is oxidized into hydrogen peroxide by glucose oxidase at the working electrode to generate an electrical current. The sensor then measures glucose concentration by detecting changes in the electric current in the interstitial fluid[64, 65]. There is a delay of 5-15 minutes between the blood and interstitial glucose depending on the specific technology used in the CGM system[60].

CGM receivers can be stand-alone handheld devices or linked to an insulin pump that acts as the receiver. Users need to calibrate the system by performing SMBG testing [66]. These CGM systems could be real-time CGM (RT-CGM) or indeed could be blinded for professional use. In the former system, receivers can display real-time information about both absolute blood glucose values and indicate the direction and speed of change. The system can be set to alert the patient if values are outside target ranges or, with predictive algorithms, to sound an alarm if glucose values are changing rapidly and/or predicted to move outside the target range [67].

Accuracy of various CGM systems had historically been inferior to blood glucose meters for measurement of capillary blood glucose such that there was increased risk of error in the clinical application of CGM values [68, 69]. Accuracy of CGM systems have without a doubt improved over the last 2 decades[70]. However, this is still frustratingly inaccurate for some [60, 70-73]. The sensors are also susceptible to pharmacological interventions including

commonly prescribed medications. For example, paracetamol can falsely elevate CGM glucose values. Recently advanced CGMs are less susceptible to these interferences [74-76]. I have discussed this further in chapter 4. Furthermore, lag time particularly when glucose is falling rapidly remains a problem [77, 78]. Some people do also find this painful where as others are fatigued by alarms and the wear time of CGM reduces as a result. Perhaps as a consequence of these initial trial evidence suggested only modest benefits in glycaemic outcomes from CGM. The use of CGM in clinical practice is still relatively low not only due to above factors but also because of the costs of the CGM systems [60, 61, 70, 73, 78-80].

Latest CGM systems however are shown to be more advanced, accurate and usable with improved patient related outcomes[60, 65, 70, 71, 81, 82]. Two recently published studies have shown effectiveness of CGM in T1D treated with MDI. DIAMOND (Effect of Continuous Glucose Monitoring on Glycemic Control in Adults With Type 1 Diabetes Using Insulin Injections) and GOLD (Continuous Glucose Monitoring vs Conventional Therapy for Glycemic Control in Adults With Type 1 Diabetes Treated With Multiple Daily Insulin Injections) randomised studies used Dexcom system (Dexcom,Inc., San Diego, CA) to demonstrate improved HbA1c in people with T1D using CGM in addition to SMBG compared to those using SMBG only[81, 83, 84]. The benefit of CGM was also demonstrated in pregnant women with T1D. The CONCEPTT randomised controlled trial (Continuous glucose monitoring in pregnant women with type 1 diabetes) showed that use of CGM during pregnancy was associated with

improved neonatal outcomes and the women spent more time in target during CGM use[85].

People using CGM are required to do SMBG tests for insulin dosing on a day to day basis and also to confirm glycaemic excursions when using most of the currently available commercial systems with the exception of some latest CGM sensors[64, 70, 86]. Recently real time CGM systems have been approved in Europe and the United States as non-adjunct to glucose meter testing i.e. users are allowed to use glucose values observed from CGM to guide day to day insulin dosing without the need to perform adjunct SMBG test.[69, 70, 86-88]. G5 Mobile CGM systems (Dexcom,Inc., San Diego, CA) was approved by FDA in 2016 for non-adjunctive use for insulin dosing [87]. However users still need to do SMBG testing twice a day to calibrate the sensor and also in situations where users experience symptoms that do not match the CGM data[89] .

Since then their technology has further advanced. Sixth generation Dexcom G6 system is factory calibrated and does not require SMBG testing to calibrate the sensor or to aid insulin dosing (non- adjunct use)[90-92].

Abbott's FreeStyle Libre Flash glucose monitoring system is a different category of glucose monitoring system. This system uses wired enzyme technology to measure interstitial glucose concentration and is factory calibrated with sensor life up to 14 days. Users do not need to do calibrations with blood glucose meter readings to maintain sensor accuracy [89, 93]. This particular feature is convenient to people. However, this system is not real time and does not push the data although users can view 8 hours of glucose history. Users are required to scan the reader to observe glucose readings[93]. Accuracy and effectiveness in improving glycaemic control of this system is shown to be comparable with

other real time CGM system [65, 94-98]. Second version of this Flash Glucose monitoring system has been approved by European regulatory body and this comes with optional real time alarms[99].

I have further discussed CGM later in this chapter.

1.3.4 *Pancreas / Islet transplant*

Beta cell replacement in the form of Islet or pancreas transplantation is one of the therapeutic options for people with T1D who suffer from recurrent severe hypoglycaemia that has not responded to other treatments or for those with suboptimal glycaemic control who also have had a renal transplant and are currently on immunosuppressive therapy [62].

I have further discussed islet transplantation for management of hypoglycaemia in hypoglycaemia sub section of this chapter.

Whole organ pancreas transplantation has a reasonable success rate for graft survival and insulin independence[100]. In the UK as in many countries, this is largely combined with kidney transplantation, but pancreas / islet alone transplantation may be performed where indicated – usually for recalcitrant severe hypoglycaemia [62, 100, 101]. Similarly, islet transplantation involves a lesser procedural complexity but, like whole-organ transplantation, the risks of anti-rejection therapy including immunosuppression must be weighed against the benefits [100-104].

1.4 Hypoglycaemia in Type 1 Diabetes

Risk of long-term complications in T1D can be minimised by good glycaemic control through intensive insulin therapy aimed to lower average blood glucose levels on a daily basis[24]. However, for many people with T1D who are on insulin therapy, this blood glucose lowering comes at a cost of increasing risk of suffering from “overshoot” episodes of low blood glucose known as hypoglycaemia[105]. Hypoglycaemia is the main complication of treatment with insulin. Hypoglycaemia is usually defined as a plasma glucose level <3.9 mmol/L (70 mg/dL) [106].

Historically there has been no universal definition of clinical hypoglycaemia [107, 108]. Various biochemical cut off values including 3.9 mmol/L, 3.5 mmol/L, 3 mmol/l, 2.8 mmol/l have been used both in clinical management and reporting of clinical trials[108, 109]. Cut-off value of 3.9 mmol/l to report hypoglycaemia was proposed by American Diabetes Association work group in 2005 to bring uniformity in reporting biochemical hypoglycaemia in clinical trials [110]. This work group categorised, and defined hypoglycaemia as described below.

***1*) Severe hypoglycemia.**

An event requiring assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions. These episodes may be associated with sufficient neuroglycopenia to induce seizure or coma. Plasma glucose measurements may not be available during such an event, but neurological recovery attributable to the restoration of plasma glucose to normal

is considered sufficient evidence that the event was induced by a low plasma glucose concentration.

***2*) Documented symptomatic hypoglycemia.**

An event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration ≤3.9 mmol/l.

***3*) Asymptomatic hypoglycemia.**

An event not accompanied by typical symptoms of hypoglycemia but with a measured plasma glucose concentration ≤3.9 mmol/l.

***4*) Probable symptomatic hypoglycemia.**

An event during which symptoms of hypoglycemia are not accompanied by a plasma glucose determination (but that was presumably caused by a plasma glucose concentration ≤3.9 mmol/l). Since many people with diabetes choose to treat symptoms with oral carbohydrate without a test of plasma glucose, it is important to recognize these events as “probable” hypoglycemia. Such self-reported episodes that are not confirmed by a contemporaneous low plasma glucose determination may not be suitable outcome measures for clinical studies that are aimed at evaluating therapy, but they should be reported.

***5*) Relative hypoglycemia.**

An event during which the person with diabetes reports any of the typical symptoms of hypoglycemia, and interprets those as indicative of hypoglycemia, but with a measured plasma glucose concentration >3.9 mmol/l. This category reflects the fact that patients with chronically poor glycemic control can experience symptoms of hypoglycemia at plasma glucose levels >3.9 mmol/l as plasma glucose concentrations decline toward that level. Though causing distress and interfering with the patient’s sense of well-being, and potentially

limiting the achievement of optimal glycemic control, such episodes probably pose no direct harm and therefore may not be a suitable outcome measure for clinical studies that are aimed at evaluating therapy, but they should be reported.

There has been controversy around using ≤3.9 mmol/l of glucose concentration as a cut off value to define hypoglycaemia. Some argue that this is of no clinical significance and may well falsely inflate frequency of hypoglycaemia[107-109]. Recently, International Hypoglycaemia working group has recommended defining hypoglycaemia as glucose concentrations below 3 mmol/L when reporting clinical trials[111].

For many people fear of hypoglycaemia is the main factor that limits improvement in glycaemic control as attempts to achieve recommended glycaemic targets results in unacceptable episodes of hypoglycaemia[105]. Severe Hypoglycaemia (SH) is defined as any such episodes that requires third party assistance to recover from hypoglycaemia [110]. The Diabetes Control and Complications Trial (DCCT) demonstrated that intensive glycaemic control with insulin therapy in T1D prevents progression and development of complications but this came with a three-fold rise in SH [24, 112, 113]. People with T1D typically experience 2 episodes of mild hypoglycaemia a week and can self-treat these minor episodes with carbohydrate intake and thus prevent severe hypoglycaemia [114]. The rate of Severe Hypoglycaemia in T1D is variable in different patient populations with one study reporting an annual incidence of 1.0 – 1.7 episodes per patient per year [115]. The risk of Severe Hypoglycaemia is skewed with a small proportion of people with T1D

representing vast episodes of SH. For e.g.in a study of 1076 people with T1D, 5% represented 54% of all SH episodes in the whole cohort [107, 116].

SH is potentially fatal as it can be associated with falls, injury, arrhythmias and seizures. Even without physical harm, it results in increased anxiety among people with diabetes and their care givers [117-119]. Fear of hypoglycaemia is higher in those who have already experienced SH[120].

In addition to the effects of aggressive insulin therapy on its own, risk of hypoglycaemia is compounded for some people who lose their defensive responses against hypoglycaemia classified as Impaired Awareness of Hypoglycaemia (IAH), increasing further the risk of suffering from severe hypoglycaemic episodes [105, 116, 121, 122] .

Glycaemic goals in diabetes is a trade-off between glycaemic control and iatrogenic hypoglycemia. People with T1D have individualised glycaemic targets so they achieve lowest possible HbA1c without significant burden of hypoglycaemia on a day to day basis[117].

1.4.1 *Physiologic defence mechanisms against Hypoglycaemia*

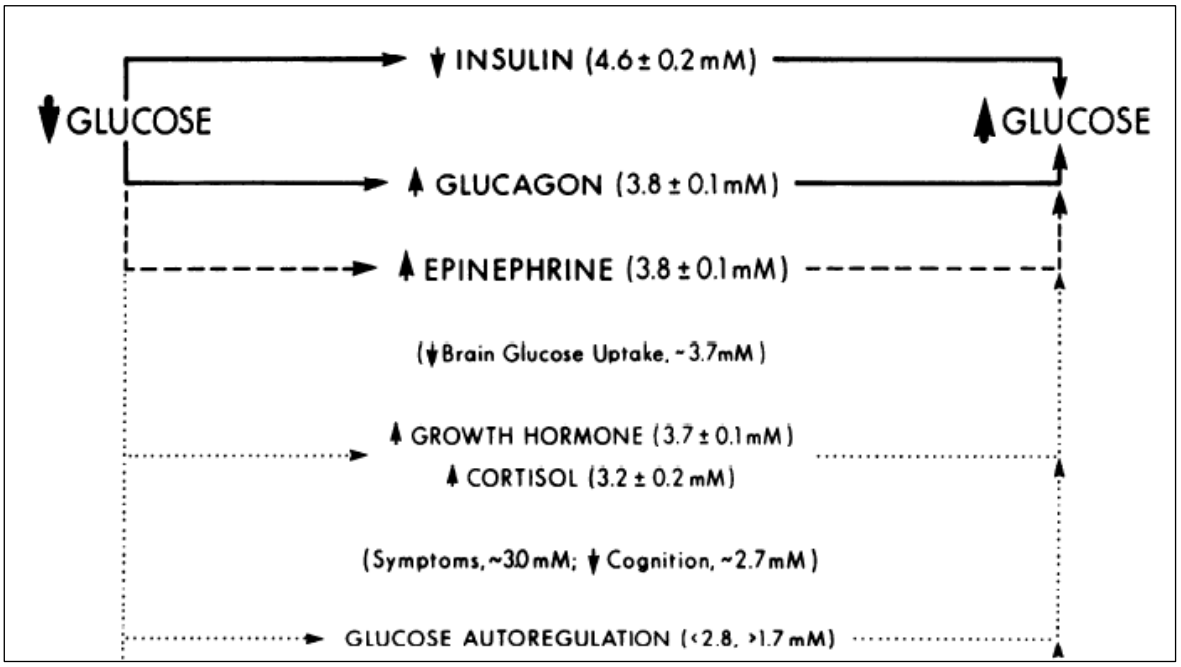
There are several physiological defence mechanisms against hypoglycaemia in human subjects without diabetes [123]. Brain uses glucose as its fuel [124]. Brain is limited in several ways when it relies on glucose to function. Firstly, brain cannot synthesize glucose in brain and so has to rely on peripheral organs to supply glucose to brain. Secondly, it cannot store glucose to any significant degree. Thirdly although brain can use alternative energy sources in the form of lactate and ketones, these alternative fuels cannot be generated in minutes if blood glucose is falling rapidly [123, 125]. So brain is one of the first organs to be affected by falling blood glucose levels [106]. As a result, when blood

glucose starts falling, several physiological pathways gets activated to prevent glucose level falling below a level where brain glucose supply gets compromised and these pathways aim to raise blood glucose levels. These mechanisms/pathways are also termed hypoglycaemia counter regulation [125, 126].

The first response to a falling blood glucose in non-diabetes is suppression of insulin release from pancreatic beta cells [125]. This starts at a plasma glucose of around 6 mmol/L and is maximally supressed below 4mmol/L[125]. Thus, insulin mediated suppression of hepatic glucose production and peripheral glucose utilisation is reduced even before hypoglycaemia is reached in healthy individuals[125, 127, 128].

If glucose level falls below the physiological range, glucagon and epinephrine secretion are triggered, typically at around 3.8 mmol/L. Epinephrine is not critical for counter regulation in healthy subjects but becomes important in Type 1 diabetes where glucagon responses to hypoglycaemia become deficient in the first few years after diagnosis.[125]. Both glucagon and epinephrine are fast acting in defence against hypoglycaemia. Glucagon increases glucose production through gluconeogenesis and hepatic glycogenolysis. Epinephrine on the other hand can also stimulate renal glucose production and reduce insulin mediated glucose uptake in the periphery in addition to increasing hepatic glucose production[129]. Other counter regulatory hormonal responses to hypoglycaemia include increased release of norepinephrine (at sympathetic nerve terminals but spilling over into the blood stream), cortisol and growth hormone. Growth hormone and cortisol have modest role in acute

hypoglycaemia but may have a more significant role in prolonged hypoglycaemia [129, 130]. Diagram below illustrates the counter regulation mechanism in humans[125].



Adapted from Cryer, P.E.[125], Glucose counter regulation: prevention and correction of hypoglycemia in humans

In addition to neurohumoral responses, symptoms are generated which can alert the individual to a falling glucose. These warning symptoms of hypoglycaemia are typically categorised in two broad categories: autonomic and neuroglycopenic. Autonomic symptoms include sweating, palpitations, shaking and hunger whereas confusion, speech difficulty, drowsiness, odd behaviour and incoordination are predominantly neuroglycopenic symptoms due to cerebral dysfunction caused by brain glucose deprivation [125, 127, 131]. Symptoms of hypoglycaemia prompt individuals to take corrective action

(carbohydrate /glucose intake) to prevent glucose from further drop. If glucose falls below 2.8 – 3 mmol/L cognitive impairment starts to develop [106, 132].

1.4.2 *Impaired defence against Hypoglycaemia in Type 1 Diabetes*

Insulin suppression as a first defence against iatrogenic hypoglycaemia is absent in T1D subjects. Loss of endogenous insulin leads to loss of cessation of paracrine signalling of α cells to release glucagon thus increasing the risk of hypoglycaemia in people with T1D. Furthermore, not only glucagon response is lost within 5 years of diagnosis in T1D subjects, catecholamine counter regulation is also diminished in those with longer duration of diabetes[132] with the glycaemic threshold for sympathoadrenal activation being shifted to lower glucose concentrations [126, 132, 133].

The attenuated epinephrine response in the presence of defective glucagon response with no endogenous beta cell insulin results in defective counter regulatory mechanisms in T1D[132]. Associated with this, people fail to recognise a declining plasma glucose and develop loss or impairment in ability to recognise hypoglycaemia, known as impaired awareness of hypoglycaemia (IAH). They do not experience typical symptoms of hypoglycaemia and miss the opportunity to treat their symptoms to prevent further decline in plasma glucose and at greater risk to develop severe hypoglycaemia (SH) [133]. In people with IAH, threshold of autonomic and sympatho adrenal response falls to or below threshold for cognitive impairment [107, 134]. IAH affects 25 to 40 % of the people with diabetes and carries a 6-fold increased risk of SH occurrence [107, 135].

The imperfection in the exogenous insulin physiology means people are already prone to hypoglycaemia. The drive to achieve perfect glycaemia increases the risk even further and some people with T1D experience either frequent mild hypoglycaemia on a regular basis or severe hypoglycaemia which is potentially fatal[21, 112].

Although the exact mechanisms leading to IAH and impaired counter-regulation remain to be determined, there is no doubt that antecedent biochemical hypoglycaemia itself reduces symptoms and counter regulatory hormone responses to subsequent hypoglycaemia in both T1D and non-diabetic subjects [136, 137]. This potentially creates a vicious cycle of recurrent hypoglycaemia. This concept of 'Hypoglycaemia associated autonomic failure' (HAAF) was postulated by Cryer[136]. This concept of HAAF is extended to include sleep and exercise related HAAF[126]. Hypoglycaemia in itself is a major contributor of HAAF. Antecedent hypoglycaemia, exercise and sleep significantly increases the burden of hypoglycaemia[127].

Cranston et al. showed that restoration of awareness of hypoglycaemia is possible by rigorous avoidance of hypoglycaemia for 3 weeks. This was independent of disease duration or initial metabolic control[138].

In day to day clinical practice, various other factors are implicated in the causation of hypoglycaemia. These include missed meals; carbohydrate inaccuracy leading to overestimation of bolus insulin; exercise; alcohol; behavioural problems; liver disease; renal impairment; extremes of age; co existing autoimmune conditions particularly hypothyroidism and Addison's disease etc. [106, 107, 129, 131].

Various hypothesis has been postulated to explain IAH on the basis of central mechanisms. Some of these are:

1.Brain Glucose metabolism hypothesis

Certain parts of brain are shown to exhibit decreased glucose uptake in response to hypoglycaemia in those with IAH. Fluorodeoxyglucose (FDG) uptake in sub thalamic region, amygdale and ventral striatum were diminished in those with IAH compared to those who had preserved hypoglycaemia awareness [139-141].

Mangia et al. demonstrated that recurrent hypoglycaemia in T1D was associated with reduced cerebral circulation to thalamus and hypothalamus region[139, 142]. It is speculated that IAH is caused by reduced neuronal responses in certain parts of the brain due to habituation of higher behavioural responses to hypoglycaemia and hence the failure to trigger counter regulatory responses [139].

2.Brain Glycogen supercompensation

Some preliminary studies indicated that increase in brain glycogen aimed to obtain additional metabolic substrates from local sources contributed to impaired sympatho adrenal response during hypoglycaemia thus contributing to IAH in T1D[115, 139, 143, 144]. However brain glycogen levels are very low and not so reliable additional reserve[115]. Subsequent studies failed to back this glycogen ‘super compensation’ hypothesis[145].

3.Brain fuel Hypothesis

Brain may be able to maintain metabolic activity during hypoglycaemia by increasing uptake of non-glucose fuels for e.g. lactate and ketones. Increased blood brain barrier monocarboxylic acid (MCA) transporter promoting increased lactate uptake could potentially contribute to IAH[139, 146].

4.Brain neuronal communication hypothesis

Neuronal communication in the brain is dependent on the release of classical neurotransmitters for e.g. potent inhibitory neurotransmitter Gamma-Aminobutyric Acid (GABA). This is normally decreased in hypoglycaemia. Recurrent hypoglycaemia is found to be associated with failure to decrease this GABA in interstitial fluid in Ventromedial Hypothalamus (VMH) and consequent reduction in epinephrine & glucagon response resulting in HAAF [139, 147, 148].

Neuro imaging studies have provided some insight into differences in brain responses to hypoglycaemia between individuals with preserved hypoglycaemia awareness and those with IAH [107] . Hypoglycaemia is shown to lead to activation and deactivation of certain regions of brain. This includes activation of thalamus (autonomic response), anterior cingulate (stress response), insula (food seeking response) and deactivation of hippocampus (memory), visual cortex (vision) and lateral orbito-frontal cortex)([107, 149, 150]). Functional studies of the brain in T1D people with IAH is associated with decreased activation in stress areas of the brain possibly caused by repeated episodes of hypoglycaemia[140, 151]. Reports suggest that those with IAH may

not perceive hypoglycaemia to be stressful and unpleasant and that these individuals may not motivated to avoid hypoglycaemia [152, 153].

A recent study examining brain responses to experimental hypoglycaemia in T1D and healthy controls using magnetic resonance imaging showed that thalamic cerebral blood flow changes correlated with autonomic symptoms and anterior cingulate cortex blood flow changes correlated with epinephrine response respectively. The findings suggested that these regions of the brain may play a part in counter regulation [154].

Given the complexity of responses to hypoglycaemia, it is likely that a number of adaptations at cellular and whole body system levels all contribute to some degree in defective counter regulation and thus IAH[115]. However, there is lack of complete understanding of central pathophysiology of IAH and the research to understand this is ongoing[152, 155].

1.4.3 ***Cardiac autonomic neuropathy :***

There have been several reports of ‘dead in bed’ syndrome in people with T1D including young individuals[156, 157]. Studies exploring underlying cause have suggested that these episodes are caused by disturbances in cardiac rate and rhythm including QT interval prolongation caused by a number of factors including acute hypoglycaemia on the background of cardiac autonomic neuropathy[156, 158]. Hypoglycaemia is also associated with raised plasma catecholamine levels and lowering of serum potassium, both of which may augment the arrhythmogenic effect of QT prolongation[159-161]. A study by

Adler et al in 12 healthy individuals showed that antecedent hypoglycaemia impaired autonomic cardiovascular autonomic function[162].

Cardiac autonomic neuropathy can have wide range of features including exercise intolerance, orthostatic hypotension, resting tachycardia, decrease heart rate variability, abnormal blood pressure regulation, dizziness, presyncope, asymptomatic myocardial ischemia and infarction [163, 164].

Autonomic neuropathy was previously suspected to be one of the factors contributing to IAH by some [165-168]. Although acute and antecedent hypoglycaemia has been implicated in impaired cardiac function, lowering of arrythmia threshold and hence sudden cardiac death; IAH as such is not found to be associated with cardiac autonomic neuropathy[162-165, 169]. One recent study found no difference in measures of autonomic function between adults with long-standing T1D adults who had IAH, and carefully matched T1D with preserved hypoglycaemia awareness[165].

1.4.4 *Clinical strategies to reduce hypoglycaemia*

1.4.4.1 **Education**

Various structured education packages such as Dose Adjustment For Normal Eating (DAFNE) have been shown to reduce hypoglycaemia in T1D[170]. Although not specifically targeted at hypoglycaemia, these programmes do achieve this by allowing people with diabetes to match better insulin dosing to varying insulin requirements. [34, 37, 41, 45, 171]. People with T1D are trained to do meticulous adjustments of insulin by Capillary Blood Glucose Testing

(CBGT) in presence of various clinical and social situations. This minimises insulin mismatch and bolus insulin dosing errors. The diabetes educator's role is pivotal in re-enforcing and consolidating the skills and understanding individual's self-management skills following their enrolments in structured education courses [171, 172].

1.4.4.2 **Psychological intervention**

Targeted education programmes aimed more specifically at hypoglycaemia to improve hypoglycaemia awareness have shown to improve SH[170]. For example, HyPOS programme trained people about hypoglycaemia symptom awareness using diaries and performing blood glucose estimation. Burden of hypoglycaemia improved 6 months post intervention along with improvement in IAH. They observed reduction in SH after 31 months follow up period although not in the first 6 months of intervention period [173, 174] . Enhanced version of Blood glucose awareness training (BGAT- II) was psycho-educational and behavioural [175] intervention programme that has been shown to be effective in training people to be able to recognize hypoglycaemia and reduce SH [176-178]. Hypoglycaemia Awareness Restoration Therapy (DAFNE–HART) incorporated diabetes education with two psychological approaches motivational interviewing & cognitive behaviour therapy. This helped restore IAH and reduce SH. This was further associated with improvement in behaviour and worry around hyperglycaemia [179, 180]. Another psycho-analytical training programme ‘‘Hypoglycemia Anticipation, Awareness and Treatment Training’’ (HAATT) also demonstrated benefit in reduction of SH in adults with T1D[181].

1.4.4.3 **Continuous subcutaneous insulin infusion (CSII / 'insulin pumps')**

CSII is now a stablished therapeutic option in the pathway of T1D management[29]. This has been endorsed by NICE as well [62, 182]. This has shown to be particularly beneficial in reduction in SH in T1D [53, 54]. A meta-analysis by Pickup et al. found that SH was significantly lower in CSII users compared to MDI users with the greatest reduction seen in those with higher burden of SH on MDI[59]. In the Cochrane database systematic review, Misso et al. similarly observed a beneficial effect of CSII on SH and glycaemic control[53]. However not all systematic review favoured CSII over MDI in terms of hypoglycaemia reduction [50, 107, 183].

1.4.4.4 **Continuous glucose monitoring (CGM)**

Use of CGM and its glycaemic benefits has already been discussed earlier in this chapter. Real time CGM with alerts and predictive alarms can alert users of impending hypoglycaemia prompting them to take corrective action to prevent severe events.[53, 79] NICE recommends real time CGM in those with significant burden of hypoglycaemia [62, 70]. CGM benefits those with IAH as well [170, 184]. The current UK use of CGM though is low, likely due to multiple factors including costs, inaccuracy, lag time, information overload, lack of time and skills among health care professionals etc. [55, 61, 70, 78, 185]. Alarm fatigue and sleeping through alarms remains a problem in CGM users[186]. One of the potential problems for CGM alone is that it requires users to take action as otherwise insulin delivery continues in presence of hypoglycaemia[187].

Previous literature was not always in favour of stand-alone CGM in clinically significant hypoglycaemia reduction[73, 80, 107, 188, 189]. Little et al. suggested that this could be largely due to patient selection and study design[107]. However, data are emerging to show that CGM reduces hypoglycaemia. A recent study showed that RT-CGM reduced the number of hypoglycaemic events in people with T1D on MDI with problematic hypoglycaemia [190]. Similarly, Reddy et al. found that CGM reduced time spent in hypoglycaemia in T1D with IAH compared with flash glucose monitoring[191].

1.4.4.5 Sensor augmented pump therapy (SAP) /Automated Insulin Delivery

Modern CSII can be integrated with CGM, also known as sensor augmented pump therapy (SAP) have shown benefits in glycaemic improvement [79, 170, 192]. RealTrend study showed that people in SAP with LGS arm of the study who were compliant with use of the sensor achieved significant reduction in HbA1c compared to those on using CSII only [188]. Eurythmics study demonstrated glycaemic improvement with SAP compared to MDI therapy[193]. These and other studies did show that SAP therapy can offer better glycaemic control compared to MDI alone or CSII alone particularly if sensor compliance is maintained. [97, 192]. SAP system has evolved, and some system comes with a feature to suspend basal insulin delivery automatically if hypoglycaemia is observed in the CGM [Low Glucose Suspend (LGS)] to prevent further drop in blood glucose and avoid SH. SAP with LGS feature suspends insulin delivery automatically for up to 2 hours [187, 194]. A study by Choudhary et al. found 3 weeks usage of Paradigm Veo (Medtronic, Inc., Northridge, CA) insulin pump with LGS function was associated with reduced nocturnal hypoglycaemia[187].

LGS also minimised anxiety regarding hypoglycaemia among people with Type 1 diabetes and their caregivers[187]. In ASPIRE (Automation to Simulate Pancreatic Insulin REsponse) study, 3-month period use of SAP therapy where basal insulin delivery was interrupted at a pre-set sensor glucose value was associated with reduced nocturnal hypoglycemia, without increase HbA1c compared to those on SAP only [195]. Similarly study in children found that those on SAP with LGS spent significantly less time in hypoglycaemia compared to those on SAP alone[196].

Latest generations SAP are more advanced and automatically suspend insulin delivery earlier when hypoglycaemia is predicted by CGM [Predictive Low Glucose Suspend (PLGS)]. For example, Medtronic MiniMed 640G pump (Northridge, CA) with SmartGuard® system automatically suspends insulin infusion not only when hypoglycaemia occurs but also when hypoglycaemia is predicted. Data are emerging to support that these SAP with PLGS reduce hypoglycaemia[197, 198]. Abraham et al. showed its benefit in reduction of biochemical hypoglycaemia and hypoglycaemia events in children and adolescent compared with sensor-augmented pump therapy (SAP) alone[198]. This feature was not associated with adverse outcomes in the form of diabetes ketoacidosis or worsening of metabolic control [197, 199]. Recently PROLG trail demonstrated that Tandem Diabetes Care Basal-IQ pump integrated with Dexcom G5 sensor and a PLGS system algorithm significantly reduced time spent in hypoglycemia in adults, adolescents and children with T1D compared to SAP therapy alone[200].

CSII technology is progressing rapidly to allow insulin delivery to be automated. Closed Loop (or “artificial pancreas”) integrates CSII with CGM controlled by an algorithm which varies insulin delivery through CSII in real time using continuous glucose data received from CGM. This has shown not only to increase time in target but also reduce time spent in hypoglycaemia [54, 201-205].

To date, Closed Loop (CL) has really not been tested in a hypoglycaemia group.

1.4.5 *Pancreas / islet transplant*

NICE recommends Islet / pancreas transplantation for people with T1D if they suffer from intractable SH that has not responded to other treatments in addition for those with suboptimal glycaemic control who are already on immunosuppressive therapy for kidney transplant [62].

Beta cell replacement is one of the treatment options for recalcitrant SH at least in theory [103, 206-208]. The issues however revolve around post-surgical complications and immunosuppression post islet / pancreas transplantation [102, 209, 210]. Islet transplantation is technically and surgically less challenging compared to pancreas transplant [104, 210, 211]. Furthermore, advances in immunosuppression therapy which are largely steroid free ('Edmonton protocol') have led to minimise complications related to immunosuppression [210, 212, 213].

Gerber et al. reported a significant reduction of SH in a median follow up of 51 months following islet transplantation in those who had failed pancreas transplantation. The benefit was seen even in those who needed exogenous insulin. Beta cell replacement has shown to improve counter regulatory response in short term as well as long term [214-216]. For example in a stepped

hypoglycaemic clamp study, Rickels et al. demonstrated recovery of glucagon secretion and improvement in epinephrine secretion 6 months post intrahepatic islet transplantation thus supporting role of islet transplantation in IAH reversal[215]. The same group had previously observed that glycaemic thresholds for activation of counter regulatory hormone and symptom responses appeared normal after islet transplantation[216].

Meyer et al. showed that islet graft function improved hypoglycaemia counter-regulation by increasing endogenous glucose production (EGP) and decreasing systemic glucose disposal. This may in part explain reduction in SH post-transplant in T1D [217].

Although pancreas / islet transplant looks encouraging in the management of debilitating hypoglycaemia, various obvious logistical challenges still remain. Paramount are the cost and skills of the centres to delivery this service, supply of islet and undesirable effects of immunosuppression [218, 219].

1.5 Outline of thesis

In summary, despite advances in glucose monitoring, insulin delivery and beta cell replacement, many people with T1D have persisting problems with recurrent hypoglycaemia, hypoglycaemia unawareness and remain at risk of severe hypoglycaemia [170, 179, 180, 220-222]. This has a significant impact not only at personal level to people with T1D and their care givers but also remains a huge challenge to health system as well [221-224].

As discussed above and as elucidated by Little et al. impaired counter-regulation, hypoglycaemia-associated autonomic failure, psychosocial and behavioural factors have been reported to be the main underlying drivers of severe hypoglycaemia in T1D [107]. However, data is limited in terms of underlying biomedical factors that could predict future occurrence of SH particularly in those with highest burden of hypoglycaemia. The HypoCOMPaSS study was a UK based multi-centre study that examined problematic hypoglycaemia and looked at interventions to improve IAH and minimise SH[225]. A proportion of these participants underwent insulin camp studies before the intervention. It would be logical to examine biomedical factors measured at baseline clamp study to explore if these factors could predict the occurrence of SH in the participants both during the study and the follow- up period.

There have been reports of association between polymorphisms in Angiotensin Converting Enzyme gene and SH in Scandinavian studies (discussed in detail in subsequent chapter), but these findings have not been convincingly replicated by other investigators beyond single group of researchers [226-230]. Underpinning mechanisms to explain this association to SH are also not clear. Study of this association in a high-risk group may provide some insight.

CGMs system have been shown to improve time in range and reduce hypoglycaemia in people with T1D as discussed above [81, 83]. But existing CGMs do have some draw backs which contribute to poor compliance and satisfaction among the CGM users[70]. One of the issues are short sensor life, sensors falling off etc. Implantable CGM system with long sensors life could potentially address some of the issues.

Monitoring blood glucose regularly is a key to minimise hypoglycaemia for people with T1D whilst maintaining good glycaemic control[231]. However, this is limited by painful and less convenient method of self-monitored blood glucose testing using capillary blood sample obtained by pricking own finger [232, 233]. Volatile Organic Compounds (VOCs) in exhaled breath have been explored as a non-invasive alternative to measuring glucose levels with a view to improve people's compliance with home blood glucose monitoring. VOCs in exhaled breath have been studied in experimental setting to measure glucose [234, 235]. However, insulin could potentially be one of the important confounders in these studies[236]. Furthermore, these studies did not examine VOCs during hypoglycaemia. So, there a need of randomized study, examining exhaled

VOCs to detect glucose levels eliminating potentially confounding effect of insulin and also the need to study the exhaled VOCs in extreme range of glucose values such as hypoglycaemia.

Accuracy of low dose insulin delivery is nor very reliable. This may be an issue for those people withT1D requiring very low doses of insulin [237-240]. Diluting insulin may offer greater accuracy. There is no published literature reporting stability of diluted insulin in CSII at 30 days or beyond.

In my thesis, I have looked at novel ways to predict and detect hypoglycaemia which would ultimately help reduce the overall burden of hypoglycaemia in T1D. Chapter 2 is the sub-analysis from the main HypoCOMPaSS study. The HypoCOMPaSS study was a UK based multi-centre study that examined problematic hypoglycaemia and looked at interventions to improve IAH and minimise SH. A sizeable subset underwent insulin clamp studies to measure hypoglycaemia responses in depth (before and/or after intervention). Here I have explored the symptoms, hormonal responses and cognitive performance in the baseline hyperinsulinaemic hypoglycaemic clamp studies, looking for predictors of favourable (or not) outcomes.

In chapter 3, I have explored biological factors that may contribute to the persisting risk of SH in HypoCOMPaSS participants. Here, I have described association between the polymorphisms in Angiotensin Converting Enzyme ACE gene and residual SH.

In chapter 4, I have described a study of a novel implantable CGM [(Senseonics CGM System), now called the Eversense® CGM System] in T1D. Here I examined the efficacy and safety of this novel system in the Cambridge cohort

from the pivotal European PRECISE 1 study. I have also discussed patient satisfaction and hypoglycaemia from Cambridge participants.

In chapter 5, I have looked at alternative methods to measure blood glucose and detect hypoglycaemia early. Here, I have described a pilot study measuring Volatile Organic Compounds (VOCS) in exhaled breath in T1D during hyperinsulinaemic clamp studies.

In chapter 6, I have described in vitro study looking at the stability of ‘diluted insulin aspart (NovoRapid®) in ambient temperature and CSII over 30 days. Use of diluted insulin is targeted for people with T1D requiring very low insulin doses to maintain glycaemia and avoid hypoglycaemia.

In chapter 7, I have concluded the overall finding of my studies and analyses.

I have listed study responsibilities and achievements during my PhD in the appendices.

2 HypoCOMPaSS sub Analysis : Examination for potential predictors of severe hypoglycaemia from baseline hypoglycaemic clamp study

2.1 Background

HypoCOMPaSS study was a 6 month Randomised Control Trial (RCT) with an 18 month post RCT extension looking at clinical interventions to reverse impaired awareness of hypoglycaemia (IAH) and thus reduce rates of severe hypoglycaemia (SH) in a cohort of 96 adults with T1D[241]. The specific clinical strategies used in the study were hypoglycaemia awareness training and optimized insulin delivery via insulin pumps or multiple injections with or without the use of real time continuous glucose monitoring (RT CGM). The rationale was to use (and compare) these clinical interventions to avoid hypoglycaemia in order to reverse impaired awareness.

The main HypoCOMPaSS RCT design and study procedures is attached as Appendix 10 [241] In the main hypo-COMPaSS study, 96 participants were randomised to four intervention groups.

Baseline characteristics of the participants who took part in main HypoCOMPaSS study is attached as Appendix 3.

At the end of 6 months RCT, awareness of hypoglycaemia improved as 64 % of participants showed an improvement in GOLD score (responder) and 39 % had resolution in Impaired Awareness of Hypoglycaemia (IAH) i.e. those with GOLD score below threshold score of 4 compared to baseline GOLD score.

In my work presented in this chapter, I have examined rates of SH as an important metric of hypoglycaemia burden. SH can be measured both by the number of people affected in a time period and by the average SH rates. In the main HypoCOMPaSS study, a total of 77% of participants were affected by SH over the 6 months preceding the study. At the end of RCT period, SH rate improved significantly by more than 10-fold. Despite this overall reduction in SH, 20 % of participants experienced SH episodes during the 6 months RCT period. In the follow up period, SH rate remained low with only 30 %, 27% and 29% of total participants experiencing SH episodes post RCT at 6, 12 and 18 months respectively. In general, comparing the SH rates during the RCT and follow up period with the rates seen during the 6 months preceding the RCT illustrated the marked baseline burden of SH in the study group, being 8.88±1.4 episodes/patient/year. This was reduced to (episodes/patient-year ± SEM)] 0.82±0.2, 0.84±0.3, 0.75±0.2, 0.81±0.3 at the end of RCT, and post RCT at 6, 12 and 18 months respectively (Table 2-1).

I have also analysed data looking at the proportion of participants who did not experience any episode of severe hypoglycaemia during the 6 months HypoCOMPaSS study intervention and 18 months follow up period shown in Table 2-2 and the proportion demonstrating Gold score Response and Resolution respectively among HypoCOMPaSS cohort at the end of RCT period as shown in Table 2-3.

Table 2-1:Severe Hypoglycaemia episodes at baseline, 6 months during HypoCOMPaSS study intervention and 18 months post RCT follow up period

	Severe Hypoglycaemia (Episodes/patient/year**)**
Pre RCT (N=96)	8.88±1.4
6 months RCT intervention period* (N=90)	0.82±0.2
6 months post RCT * (N=90)	0.84±0.3
12 months post RCT * (N=89)	0.75±0.2
18 months post RCT* (N=89)	0.81±0.3

Data shown are mean ±SEM
*Cumulative episodes inclusive of RCT intervention period

Table 2-2 :Participants who did not experience any episode of severe hypoglycaemia during 6 months HypoCOMPaSS study intervention & 18 months follow up period

	None	At least 1	Total (96)
Severe hypoglycaemia during 6months RCT			
N	72	18	90
Valid Percent	75%	18.8%	93.8%
Severe hypoglycaemia during study (6 months RCT & 18months FU)			
N	63	26	89
Valid Percent	65.6%	27.1%	92.7%

Missing values n=6

Table 2-3:Hypoglycaemia awareness status at the end of 6 months HypoCOMPaSS study intervention with Gold score response indicating improvement of score compared to baseline score and Gold score resolution indicating restoration of awareness (improvement of scores below threshold value of 4)

	Yes	No	Total
Gold Score Response			
N	54	31	85
Valid Percent	56.3%	32.3%	88.5%
Gold Score Resolution			
N	33	52	89
Valid Percent	34.4%	54.2%	88.5%

Missing values n=11

In summary, the HypoCOMPaSS study demonstrated a significant restoration of awareness of hypoglycaemia and associated reduction in severe hypoglycaemia (SH) in a UK cohort of T1D people [225]. However, the intervention(s) could not eliminate SH completely.

25 participants underwent stepped hypoglycaemic clamp sub-study at baseline before the intervention. Analysis of biomedical factors observed and measured at baseline clamp study could potentially predict occurrence of severe hypoglycaemia during the study period and also response to interventions.

2.2 Objective of this sub analysis

The primary objective of this sub-analysis from the main Hypo-COMPASS study was to explore symptoms, hormonal responses and cognitive performance in the baseline hyperinsulinaemic hypoglycaemic clamp study looking for possible determinants of response / non-response to clinical interventions. The

hypothesis that I was testing here was that biomedical factors i.e. cognitive performance, hypoglycaemic symptoms and counterregulatory hormones response observed during experimental hypoglycaemia in the baseline clamp study would predict either improvements in awareness and/or future risk and occurrence of SH in the high-risk participants. Underpinning this hypothesis is the important clinical question of whether some people with longstanding T1D and IAH just have “irreversible” deficits in defences against hypoglycaemia.

2.3 Study Design and statistical plan :

The main HypoCOMPaSS RCT design, study procedures and the results are attached as Appendix 10 [225, 242].

In short , there was a 4 week run in period as outlined in appendix 10 before the 24 week RCT period. During the RCT intervention period, participants were followed up at study visits every 4 weeks. Participants were invited to attend for further follow up 6, 12 and 18 months after the RCT.

2.3.1 *Participants*

For this sub analysis all people who underwent hypoglycaemic clamp sub-study at baseline were included.

2.3.2 *Hyperinsulinemic hypoglycaemic clamp procedure*

Clamp studies were conducted in a clinical research facility within respective institutions before and after the 24-week HypoCOMPaSS study intervention. Participants were fitted with a retrospective CGM sensor (Medtronic iPro, MiniMed, CA, USA) to be worn for five to seven days preceding the study day.

This was downloaded on the morning of the study to determine whether any antecedent biochemical hypoglycaemia had occurred over the 24-hour period prior to the clamp. Studies were rescheduled on another day if any CGM and/or self-monitored capillary glucose below 3.0 mmol/l was detected during the preceding 24 hours. For participants who required rescheduling, a further 72 hours of CGM was organized. All participants were advised to fast from 22:00 h and to avoid caffeine for 24 h before the study. Participants were admitted to the clinical research facility at 7 am on the day of the study. On arrival, an intravenous cannula was inserted in the ante-cubital vein of the non-dominant arm and blood glucose was stabilized using sliding scale insulin infusion aiming initially for blood glucose 6.0–7.0 mmol/l and then 5.0–6.0 mmol/l between 10.30 am and 11 am for clamp initiation. A second retrograde cannula was inserted into a vein on the dorsum of the non-dominant hand which was heated to 50-60 °C to arterialise venous blood. During this period of stabilization, participants practiced cognitive function tests demonstrated to be sensitive to hypoglycaemia - Four Choice Reaction Time and Stroop tests. Cognitive Functions tests during clamp studies are explained as appendix 2.[243-245].

At the start of the clamp, a primed infusion of 60 mU/m^2/min soluble human Actrapid insulin in a 4% solution of autologous blood in 0.9% sodium chloride was started via the non-dominant antecubital vein catheter. Infused in parallel, the rate of infusion of 20% dextrose was adjusted as needed, aiming to stabilize plasma glucose at 5.0 mmol/l at 40mins followed by step-wise lowering to 3.8 mmol/l, 3.4 mmol/l, 2.8 mmol/l and 2.4 mmol/l. Each step lasted 40 min allowing 20 min to achieve the new target level and 20 min for stabilization at that level.

Samples for plasma glucose were obtained every 5 min and analysed in real-time. Participants were kept blinded to their real-time glucose levels throughout the study. At the end of each clamp stage, participants were asked to complete a semi-quantitative symptom questionnaire consisting of 9 items (autonomic symptoms=pounding heart, shaking / tremor, hunger and sweating and neuroglycopenic symptoms=drowsiness, difficulty speaking, headache, clumsiness / incoordination and confusion). Each item was scored from 1 (absent) to 7 (maximal) and for ease of interpretation converted to a scale of 0 to 6 with a minimum-maximum possible score range of 0 to 54. Symptom questionnaire was followed by cognitive function tests- Four Choice Reaction Time and Stroop tests (attached as appendix 2). These tests have been demonstrated to be sensitive to hypoglycaemia [243-247].

Additional blood samples were taken at regular intervals for the later measurement of insulin and the counter-regulatory hormone response (metanephrine, growth hormone, glucagon and cortisol).

2.3.3 ***Analytical methods:***

Arterialized plasma glucose was measured in real time using Yellow Springs analyser (YSI STAT Plus, Farnborough, UK, intra- assay coefficient of variation (CV) 1.5 % and inter assay CV 2.8 %). Plasma insulin was measured by an enzyme linked immunosorbent assay (DAKO) Glostrup, Denmark; intra assay CV 1.8 % and inter assay CV 7.8 %). Glucagon was measured by an enzyme linked immunosorbent assay (Alpco Diagnostics, NH, USA, intra assay CV 1.6 % and inter assay CV 2.4 %). Cortisol level were measured using a 2 step sandwich immunoassay (Roche Modular E 170 platform, Elecsys cortisols reagents, intra assay CV 1.4 % [based on a mean value of 535 nmol/l].

Metanephrines were measured by an enzyme linked immunosorbent assay (Alpco Diagnostics, NH, USA, intra assay CV 12 % [based on a mean value of 652 pmol/l] and inter assay CV 12.2 % [based on a mean value of 350 pmol/l]). Growth hormone was measured by an enzyme linked immunosorbent assay (Alpco Diagnostics, NH, USA, intra assay CV 1.4 % and inter assay CV 4.5 %). All samples were measured in a single central laboratory in the same batch.

2.3.4 *Clamp sub study :statistical considerations*

The glucose monitoring data included area under the curve for the glucose values of <3mmol/L. Different definitions were used to define resolution of unawareness and responders for the primary end point. ‘‘Resolution’’ in IAH after the intervention was defined as improvement in Gold questionnaire score below the accepted unawareness threshold value of 4. ‘‘Non-responders’’ for IAH were defined as those with no improvement in Gold Score. ‘‘Responders’’ were defined as those with improved IAH score.

Glucose thresholds for the onset of symptoms, counter-regulatory hormone responses and impairment of cognitive function were determined according to protocols in literature [123, 132, 243-245, 248, 249]. Glucose thresholds for onset of hormone responses were defined statistically as the glucose value at the time of onset of a sustained (≥2 successive time points) increase in hormone concentrations ≥2 standard deviations (SD) above the mean of the five baseline measurements for that hormone. Thresholds for an increase in total, autonomic and neuroglycopenic symptoms were determined as the glucose value at the time at which the symptoms score increased ≥ 2 over baseline on ≥ 2 successive

time points. Where no defined changed occurred, the lowest measured glucose level during the respective clamp was used as the threshold for that individual in keeping with published literature. Incremental areas under the curve for symptoms were calculated using incremental symptom scores (subtracting the symptom score at the end of stage 1 of the clamp from the scores obtained in stages 2-5). Incremental areas under the curve for hormones were calculated by subtracting the mean hormone levels achieved during euglycaemia (first 40 minutes of the clamp), from subsequent hormone levels during the rest of the clamp. For hormones, single missing values were replaced with linear interpolation and the trapezoidal rule was used for calculation of the "area under curve".

For the Four-Choice reaction time, glucose thresholds were determined as the plasma glucose level when the reaction time first exceeded twice the coefficient of variation of the stable baseline measurements. For Stroop tests, glucose thresholds were determined as the plasma glucose level where performance first deteriorated ≥2 standard deviations below the mean baseline performance.

I examined baseline clamp parameters - symptoms responses; hormonal responses and plasma glucose (mmol/l) threshold at which cognitive function deteriorated against “response” & ‘‘resolution’’ in awareness (the main study outcome) and Severe Hypoglycaemia during RCT and follow up period.

Data were analysed using IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY: IBM Corp) and a p value less than 0.05 was considered statistically significant.

Data are presented as mean with Standard Error of Mean (SEM) or Median (IQR).

2.4 Results

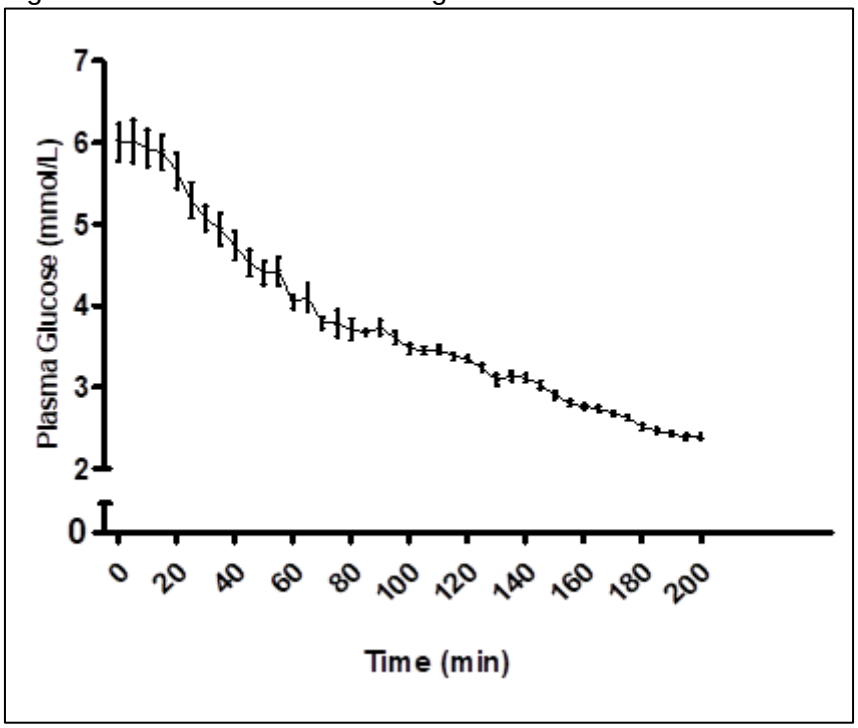
Successful clamped hypoglycaemia studies were completed in 25 participants at the start (baseline) of the 6 months RCT study.

2.4.1 *Plasma Glucose & Dextrose infusion Rates (DIR)*

Plasma glucose and Dextrose Infusion Rate (DIR) during studies is shown in the figures below (Figure 2-1 & Figure 2-2).

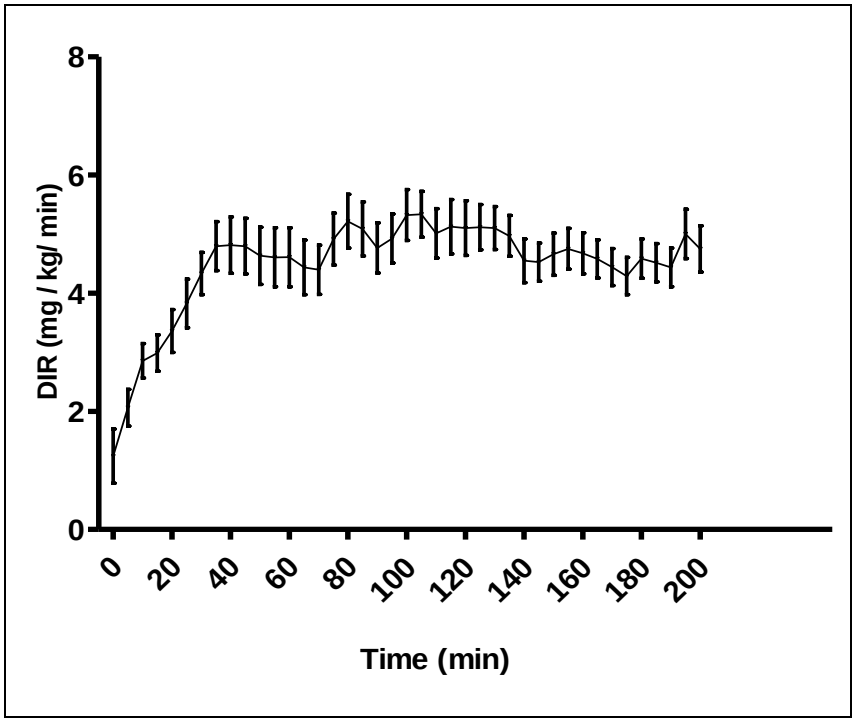
:

Figure 2-1:Plasma Glucose during studies



Data shown are mean ±SEM

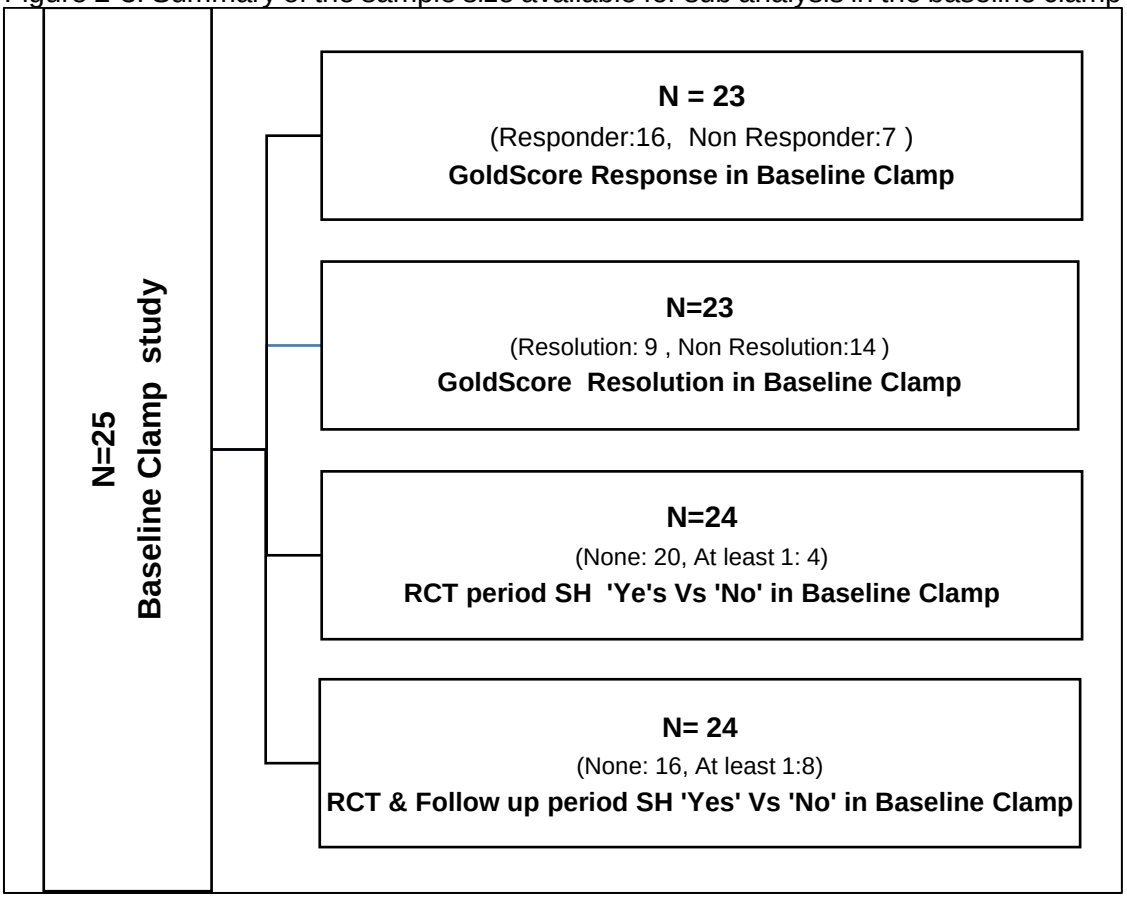
Figure 2-2 : Dextrose Infusion Rate (DIR) during studies



Data shown are mean ±SEM

Data was missing for GOLD score response and SH episodes during RCT & follow up period. Therefore, sub analysis of biomedical parameters in the baseline clamp was not available for all 25 participants. This is summarised in the Figure 2-3. The details of GOLD score response, GOLD score resolution is explained in respective section of the results below.

Figure 2-3: Summary of the sample size available for sub analysis in the baseline clamp



Each Subsection is explained in relevant section of the results

2.4.2 *Responder vs Non Responder in Baseline Clamp*

There were no differences in symptoms and hormonal responses at baseline clamped hypoglycaemia in participants who showed an improvement in GOLD score (Responder) compared to those who did not show a response (Non Responder)Table 2-4.

The plasma glucose (mmol/l) threshold at which cognitive function deteriorated was similar in ‘Responder’ and ‘Non Responder’ as measured by Stroop Colour

word [2.5(2.2,3.1) vs 2.7(2.4,3.3), P0.41] & four choice reaction time [2.4(2.2,2.9) vs 3.2(2.3,2.9), P 0.067].

Table 2-4: Symptoms and hormonal responses in baseline clamped hypoglycaemia in those who showed an improvement in GOLD score (responder) at the end of 6 months HypoCOMPaSS study intervention compared to baseline GOLD score

	Responder N=16	Non Responder N= 7	*p value
Glucose levels when felt low (mmol/l)	2.5±0.1	2.5±0.2	0.88
Symptoms AUC			
Total	660.0±124.7	525.7±96.5	0.51
Autonomic	290.0±68.3	260.0±74.6	0.79
Neuroglycopenic	370.0±83.5	265.7±60.4	0.44
Hormones AUC			
GH	1532.2(1085.1,1890.2)	2627.4(955.8,4088.6)	0.11
Normetanephrines	57461.9±7558.7	66008.6±6559.8	0.495
Glucagon	29560.5±5259.8	34284.9±11138.5	0.665
Cortisol	52585.6±5538.3	41204.3±6438.0	0.241

Data shown are mean ±SEM or Median (IQR)
* Independent sample T-Test or Mann Whitney U Test

2.4.3 ***Resolution vs Non Resolution in Baseline Clamp***

Similar to the pattern seen above for responders, there was no difference in symptoms and hormonal responses at baseline clamped hypoglycaemia in participants who showed resolution in IAH (GOLD score at end of RCT below the threshold score of 4) compared to those who did not show resolution (Non Resolution) as shown in Table 2-5.

The plasma glucose (mmol/l) threshold at which cognitive function deteriorated was similar in 'Resolution' and 'Non Resolution' as measured by Stroop Colour word [2.7(2.2,3.6) vs 2.5(2.4,3.1) ,P0.27] and four choice reaction time. [2.7(0.1, 6.4) vs 2.7(2.5, 3.3),P0.27].

Table 2-5 :Symptoms and hormonal responses in baseline clamped hypoglycaemia in those who showed resolution in Impaired Awareness of Hypoglycaemia (IAH) i.e. those with GOLD score below threshold score of 4 at the end of 6 months HypoCOMPaSS study intervention

	Resolution N=9	Non Resolution N= 14	*p value
Glucose levels when felt low (mmol/l)	2.6±0.1	2.5±0.1	0.81
Symptoms AUC			
Total	762.2±191.3	527.1±83.7	0.21
Autonomic	326.7±86.3	251.4±65.9	0.49
Neuroglycopenic	220(121.3,749.7)	260(174,.2,377.2)	0.29
Hormones AUC			
GH	1429.4±279.6	2042.5±357.8	0.23
Normetanephrines	66315.6±12162.4	56043.6±5052.7	0.38
Glucagon	34455.5±8087.4	28775.9±6234.1	0.58
Cortisol	53687.8±8435.7	46186.4±4861.4	0.41

Data shown are mean ±SEM or Median (IQR)
* Independent sample T-Test or Mann Whitney U Test

2.4.4 *RCT period Severe Hypoglycaemia Yes Vs No in Baseline Clamp*

I also examined whether baseline measures prior to the RCT could predict severe hypoglycaemia during the RCT period. Similar to findings for the Gold

score, there were no differences in symptoms and hormonal responses at baseline clamped hypoglycaemia in participants who experienced at least one episode of severe hypoglycaemia during 6 months RCT period compared to those who did not experience any severe hypoglycaemia episode (Table 2-6).

The plasma glucose (mmol/l) threshold at which cognitive function deteriorated was similar in ‘None’ and ‘At least 1’ SH as measured by Stroop Colour word [2.5(2.5,3.0) vs 2.4(0.7,4.9) P0.91] and four choice reaction time [2.5(2.5,3.0) vs 3.9(-0.2,11.3) ,P0.17].

Table 2-6: Symptoms and hormonal responses in baseline clamped hypoglycaemia in those who did and did not experience any episode of severe hypoglycaemia during 6 months HypoCOMPaSS study intervention

	None N=20	At least 1 N= 4	*p value
Glucose levels subjects felt low (mmol/l)	2.4(2.4,2.76)	2.26(2.1,2.4)	0.06
Symptoms AUC	633.0±102.7	465.0±135.7	0.49
Total			
Autonomic	256.0±57.0	340.0±116.9	0..54
Neuroglycopenic	377.0±66.2	125.0±26.3	0.10
Hormones AUC			
GH	1679.7±244.0	2160.3±846.2	0.46
Normetanephrines	58908.5±6296.0	60037.5±10500.9	0.94
Glucagon	43046.9±11091.5	21030.8±13999.5	0.40
Cortisol	48340.5±4821.3	48870.0±9850.2	0.96

Data shown are mean ±SEM or Median (IQR)
* Independent sample T-Test or Mann Whitney U Test

2.4.5 *Total study period Severe Hypoglycaemia Yes Vs No in Baseline Clamp*

Severe hypoglycaemia is an infrequent and unpredictable occurrence, even in those at risk. To increase the power to detect a difference, I also examined whether baseline clamp parameters were associated with rates of SH over the total 2 year period (6 month RCT plus 18 month follow-up). Again and importantly, I found no difference in symptoms and hormonal responses at baseline clamped hypoglycaemia in participants who experienced at least one episode of severe hypoglycaemia during total study duration (6 months RCT

and 18 months post RCT follow up period) compared to those who did not experience any severe episode (Table 2-7).

The plasma glucose (mmol/l) threshold at which cognitive function deteriorated was similar in 'None' and 'At least 1' SH groups as measured by Stroop Colour word (2.8±0.2 vs 2.7±0.2 p= 0.70) and four choice reaction time (2.7±0.1 vs 3.7±0.6 p=0.07).

Table 2-7 :Symptoms and hormonal responses in baseline clamped hypoglycaemia in those who did not experience any episode of severe hypoglycaemia during total study duration (6 months RCT and 18 months post RCT)

	None N=16	At least 1 N= 8	*p value
Glucose levels when felt low (mmol/l)	2.5±0.1	2.5±0.1	0.66
Symptoms AUC			
Total	680.0±123.5	455.0±83.7	0.23
Autonomic	295.0±66.9	220.0±75.5	0.49
Neuroglycopenic	385.0±78.9	235.0±69.7	0.23
Hormones AUC			
GH	1640.8±289.1	1997.8±448.2	0.49
Normetanephrines	60413.8±7822.1	56462.5.5±5383.1	0.74
Glucagon	44337.1±13733.2	29458.5±8733.1	0.47
Cortisol	48676.9±5762.8	47932.5±6013.2	0.93

Data shown are mean ±SEM
* Independent sample T-Test

2.5 Discussion

The hypothesis I was testing here was that differences in biomedical responses to experimental hypoglycaemia would predict the degree to which IAH and SH could be improved by subsequent clinical strategies. My findings suggest that this is not the case. There was no difference in symptoms and hormonal responses at baseline clamped hypoglycaemia in participants who showed an improvement in GOLD score (Responder) or a resolution of IAH (Resolution) compared to those who did not show a response (Non Responder and Non Resolution of IAH respectively).

Furthermore, there were no differences observed in symptoms and hormonal responses at baseline clamped hypoglycaemia in participants who experienced at least one episode of severe hypoglycaemia during total study duration (6 months RCT and 18 months follow up period) compared to those who did not experience any severe hypoglycaemia episode.

In summary, there were no obvious measurable factors or parameters during experimental hypoglycaemia that could be identified to predict persistent problematic hypoglycaemia in these high-risk people.

Age, duration of diabetes, tight glycaemic control, psychosocial issues, behavioural factors, previous SH and impaired awareness of hypoglycaemia are major risk factors to severe hypoglycaemia[241, 250]. 'Hypoglycaemia-associated autonomic failure' do contribute to IAH and thus ultimately to severe episodes [107, 126, 251]. Cranston et al. showed that avoidance of hypoglycaemia improves awareness of hypoglycaemia [138]. However, this did

look at those with severe episodes. Data is limited in terms of underlying biomedical factors that could directly predict future occurrence of SH particularly in those with highest burden of hypoglycaemia.

Findings of this analysis is difficult to compare with published literature largely because most of the prospective trials have excluded people with severe hypoglycaemia[107]. It was logical to examine biomedical factors measured at baseline clamp study to explore if these factors could predict the occurrence of SH in the participants during the study period.

There are two obvious important clinical implications of this. The first is that there is no obvious “target” to consider for potential further study or even intervention here, other than the clinical strategy of trying to avoid biochemical hypoglycaemia.

The second though is that the apparent negative findings can be regarded as clinically encouraging. There is no evidence here in this group of people with long standing diabetes and a baseline burden of hypoglycaemia for the existence of a group with “irreversibly suppressed” responses to hypoglycaemia. Based on these clamp data, the recommendation for clinicians and Health Care Professionals remains that all T1D people with problematic hypoglycaemia could benefit from targeted clinical strategies to minimise the burden of hypoglycaemia. The interpretation is limited though because of the relatively small numbers undergoing clamp studies who had continuing SH. In the following chapter, I have looked further into possible predictors of SH.

3 HypoCOMPaSS ACE genotype sub study

3.1 Background

3.1.1 *Severe Hypoglycaemia in HypoCOMPaSS study*

As previously discussed, various targeted interventions and clinical strategies aimed at reducing the burden of hypoglycaemia in a well-phenotyped UK cohort of HypoCOMPaSS participants reduced severe hypoglycaemia significantly and restored awareness of hypoglycaemia in most participants [225]. However, these extensive clinical interventions could not eliminate SH completely. At the end of HypoCOMPaSS RCT period, one fifth of participants were still suffering from SH episodes and around one third continued to experience SH during the 18 months post-RCT follow up period.

In the sub analysis of baseline clamp study from the main HypoCOMPaSS detailed in chapter 3, I could not identify factors in baseline clamp study that could predict future occurrence of SH in the high-risk participants.

Although, as presented in the last chapter, there were no obvious measurable parameters that could be identified from experimental hypoglycaemia (insulin clamp studies) to explain the lack of response in some, this still leaves open the possibility that underlying biological drivers are contributing to SH in a subset of people with T1D who are resistant to targeted clinical interventions aimed at improving IAH and minimising severe hypoglycaemia.

In this chapter, I have examined the residual risk of severe hypoglycaemia. Severe hypoglycaemia (SH) affects up to 30% of individuals with established T1D each year and remains one of the most feared complications[117-119].

Risk of hypoglycaemia varies markedly, and the distribution is skewed so that most problematic severe hypoglycaemia clusters in a subset of those with T1D[116]. In addition to IAH, many environmental and behavioural factors may contribute to mild, moderate and severe hypoglycaemia (SH) in T1D. For example, some may have preserved awareness of a falling blood glucose but be slow to take action when they start to get warning, resulting in SH rather than "moderate" self-managed hypoglycaemia. Others may have psychosocial factors which lead them to avoid hyperglycaemia, ignore or minimise risk or just accept as normal. A current RCT is examining whether psychological factors can be successfully targeted [252]. As well as these "behavioural" factors though, the question remains as to whether certain people have an underlying biological predisposition to hypoglycaemia, for example with brains that are more sensitive to the effects of an interruption in fuel supply.

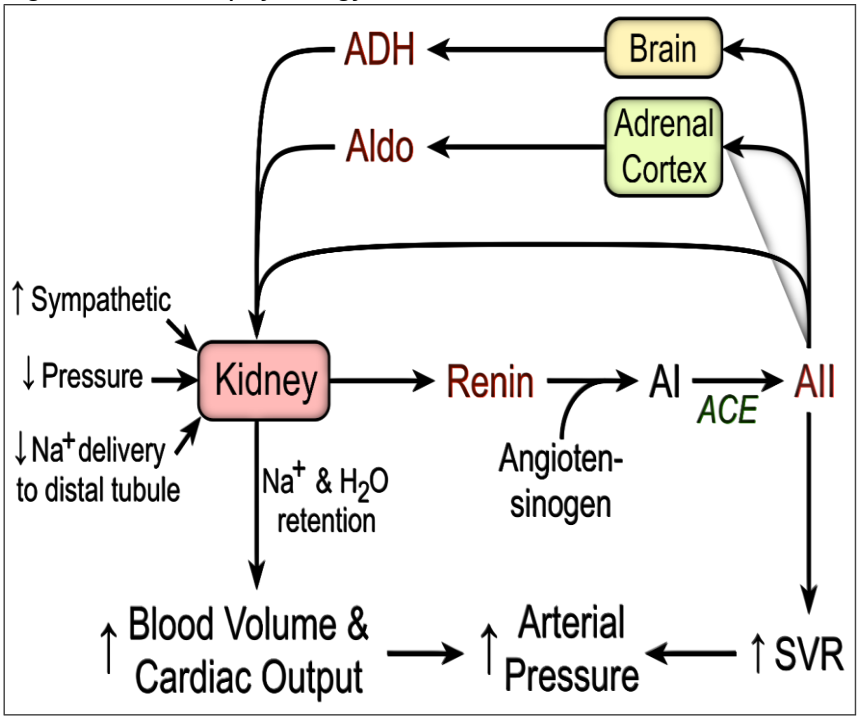
Increasing data are emerging to suggest that genetic factors contribute significantly to SH risk. In support of this, a number of (largely Scandinavian) studies have reported an association between hypoglycaemia risk and genetic variants in the Renin Angiotensin system (RAS) [226-228, 230, 253-255].

3.1.2 *Renin Angiotensin System (RAS) and SH*

Renin-Angiotensin System (RAS) was first described by Stanley Peart in 1975. Since then there have been numerous studies on the physiological effects of this system in the human body. There are three important components to this system: 1) renin, 2) angiotensin, and 3) aldosterone (Figure 3-1). Renin, which is released primarily by the kidneys, stimulates the formation of angiotensin in blood and tissues, which in turn stimulates the release of aldosterone from the adrenal cortex. RAS plays an important role in regulating blood volume and

systemic vascular resistance, which together influence cardiac output and arterial pressure (Figure 3-1). The role of RAS in the development of cardiovascular disease has been well established [256, 257].

Figure 3-1 : RAS physiology in human



Ref : [257]

The RAS has a major influence on electrolyte and fluid balance. The primary substrate of the RAS system is Angiotensinogen, which is cleaved by renin to form angiotensin I. This is then cleaved by Angiotensin Converting Enzyme (ACE) to yield active angiotensin II[256]. ACE inhibitors produce vasodilation by inhibiting the formation of angiotensin II and are used as therapeutic agents

for prevention and treatment of hypertension, heart failure and coronary artery disease [257, 258].

Within the ACE gene, frequently occurring polymorphisms can alter the activity of the RAS system. The ACE gene in humans is located on chromosome 17q23. This consists of 26 exons and 25 introns spread over ~24 kb. Intron 16 contains a restriction fragment length polymorphism based on the presence (insertion I) or absence (deletion D) of a 287-base pair (bp) non-sense DNA domain alu repeat sequence forming 3 genotypes II & DD homozygotes and ID heterozygote[259]. In 1990, Rigat et al. showed that this insertion/deletion (I/D) polymorphism in the ACE gene accounted for half of the variance of the serum ACE enzyme levels[260]. Serum ACE concentrations were significantly higher in homozygotes with the shorter deletion allele (DD) than in heterozygotes (ID) or in homozygotes with the longer insertion allele (II)[261]. In addition to ACE concentration, serum ACE activity is determined by the I/D polymorphism of ACE gene with the D allele conferring higher tissue and serum ACE activity[228]. The Insertion /Deletion polymorphism in the ACE gene, serum ACE activity and other polymorphisms in RAS have been reported to be associated with SH. The homozygous DD ACE genotype has been associated with risk of severe hypoglycaemia compared to ID & II [226-228, 253, 254].

Serum ACE concentrations were shown to be significantly higher in homozygotes with the shorter deletion allele (DD) than in heterozygotes (ID) or in homozygotes with the longer insertion allele (II) absence of ‘I’ allele in ID & DD [261]. In addition to ACE concentration, serum ACE activity is determined by the I/D polymorphism of ACE gene with the D allele conferring higher tissue and serum ACE activity[228].

Homozygous DD ACE genotype has been associated with risk of severe hypoglycaemia compared to ID & II [226-228, 253, 254]. Presence of 'I' allele results into lower ACE activity and 'D' allele leading to higher activity, thus homozygous DD (absence of 'I' allele) conferring higher tissue and serum ACE activity[228].

ACE genotype has been implicated in a number of health conditions[262]. The D allele has been associated with hypertension, preeclampsia, heart failure, cerebral infarction, diabetic retinopathy and nephropathy[262]. Interestingly, the 'I' allele is found at increased frequency in endurance athletes such as long-distance runners, climbers, rowers. The Insertion / Deletion polymorphism in the ACE gene, serum ACE activity and other polymorphisms in RAS have been reported to be associated with SH. Specifically; the homozygous DD ACE genotype has been associated with risk of severe hypoglycaemia compared to ID & II [226-228, 253, 254].

The association suggests that low ACE activity might be favourable for performance conditions with limited substrate availability for fuelling body metabolism, perhaps including brain functioning? Such conditions occur in endurance athletes during competition and, perhaps also by analogy, in people with diabetes during hypoglycaemia [255, 262, 263].

A study noted local RAS system in various organs in the body including brain[264]. This brain system modulates local metabolism in the brain when substrate availability limited with a view to increase efficiency and limit release of noxious stimuli so that brain can maintain function longer[255, 265]. Hypoglycemia is similar situation with low substrate (glucose) availability in the brain[255]. To date, these associations in T1D between SH and polymorphisms

in RAS & serum ACE activity have been reported in Scandinavian studies, largely reported by a single research group, but have not been replicated convincingly elsewhere [229, 230].

There have been some case reports of familial increases in circulating ACE levels due to intronic mutations in ACE gene[266-268]. Interestingly there was no evidence of functional consequences in these individuals for e.g. RAAS activation or cardiovascular end organ damage. These supported the pre-existing hypothesis that membrane-bound ACE rather than circulating ACE are responsible for Angiotensin II generation and its cardiovascular consequences[266, 267].

In this chapter I have examined ACE genotype and enzyme activity to see whether there was an association with risk of severe hypoglycaemia (SH). I examined (1) SH data from the RCT and (2) data from the post-RCT clamps, reasoning that any effects of RAS were likely to be seen more easily once educational/ behavioural features had been optimised. In particular, for clamp studies, I anticipated that any effects of ACE genotype might manifest as increased cognitive deterioration in those with DD genotype during experimentally controlled hypoglycaemic challenges.

3.2 Objectives

My hypothesis was that following clinical optimisation, the presence of the “at risk” DD genotype in the ACE gene would be associated with (1) persisting severe hypoglycaemia in UK HypoCOMPaSS participants with T1D and (2) greater cognitive decline during experimental (clamped) hypoglycaemia at the end of the RCT.

3.3 Study Design

The study protocol, participant information sheets and consent forms were approved by an independent Research Ethics Committee and the study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant for the main study. Participants signed an additional consent form for this sub-study and also provided additional blood samples. Clamp protocol design is attached in the appendix 5.

3.4 Study Procedure ACE genotype sub study

3.4.1 *Participants :ACE genotype sub study*

For this sub study all people participating in the Hypo-COMPASS study were invited. 77 out of 96 participants agreed to participate in this sub study.

3.4.2 *Analytical methods: ACE genotype sub study*

Additional blood samples (10 ml) were collected in these 77 participants at the time of planned venepuncture for the main Hypo-COMPASS study following

informed consent. 5 ml of the blood sample was centrifuged and stored at -20 for serum ACE level analysis. The remaining 5 mls was transferred to two EDTA tubes and transported to Institute of Metabolic Science, Cambridge for the genetic analysis.

3.4.2.1 **Serum ACE analysis**

Serum ACE activity was measured using a rapid spectrophotometric method (Trinity Biotech Plc Bray, Co. Wicklow, Ireland, intra assay CV 5% [based on a mean value of 61 U/L] and inter assay CV 2 % [based on a mean value of 59 U/l]).

3.4.2.2 **Detection of ACE gene Polymorphism :**

DNA was extracted from frozen whole blood. The insertion / deletion polymorphism of the ACE gene was identified by polymerase chain reaction (PCR) using two primers flanking the site of insertion. Fragments of 191 bp (D allele) and 479 bp (I allele) were separated on a 2 % agarose gel and stained with SYBR Safe DNA Gel Stain. Visualisation was then done on a UV transilluminator. To prevent mistyping of ID to DD by preferential amplification, additional PCR amplification protocol was used utilising ACE 2 sense primer along with standard ACE anti-sense[269].

3.4.2.3 **Statistical Considerations**

The main HypoCOMPaSS study planned to recruit 100 participants based on a pilot study where intervention gave a Clarke score (an alternative to the Gold score for assessing hypoglycaemia awareness but ranked using a similar scale) of 2.6 $\pm$ 1.9 *vs* 4 $\pm$ 1.8 in control group. Although a post-hoc analysis, we

performed a power calculation in order to estimate our power for detecting a difference. For this ACE genotype sub study, if we assume that we might see similar differences in awareness scores between genotypes, a sample size of 22 in each group at 95% significance would give us 80% power.

Assuming that the I/D ACE genotype will show a Hardy–Weinberg distribution as in previous samples of T1D, we expected 25% of people to have high risk (DD), 25% to have II and 50% to be ID genotype.

We therefore aimed to approach all participants in HypoCOMPaSS. Assuming that there would be a high percentage participation of 90%, this gave likely samples of $\geq$23 in each target genotype.

Baseline clinical characteristics and demographics between genotypes were examined using One-Way ANOVA except for gender analysis where Chi square test was used for categorical data. SH, biochemical hypoglycaemia and Gold scores were analysed using one way ANOVA.

In the analyses below, where numbers are small, I have compared the DD “risk group” with combined “non risk group (ID and II groups). When analysed as risk ACE genotype ‘DD’ Vs. non risk ACE genotype ‘Non DD’ (ID/II), an independent sample T test was used to compare the genotypes. Chi Square test and Fisher exact Test was used to examine Responder (Improvement in Gold score) and Resolution (Improvement in Gold score below threshold value of 4) respectively.

SH episodes were also analysed for those who were not on Angiotensin Converting Enzyme Inhibitor (ACEI) therapy. Further, SH episodes during the study were examined exclusively for participants who had 1 or more baseline SH episodes before being enrolled in the study.

SH episodes in ACE genotypes were examined in post RCT follow up period.

Data were analysed using IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp) and a p value less than 0.05 was considered statistically significant.

All data are presented as mean with SEM.

3.5 Results –ACE genotype sub study

Data were analysed for 77 of the total 96 HypoCOMPaSS participants who took part in this sub study.

3.5.1 *Baseline characteristics of participants-ACE genotype sub study*

Table 3-1 shows the distribution of ACE genotype in participants. The distribution was broadly as expected with just half the participants being in the ID group. There was no differences between ACE genotypes in terms of gender, age, HbA1c or duration of diabetes.

Baseline diabetic retinopathy status was similar across genotypes as shown in Table 3-1. Hypertension data across genotypes could not be analysed as baseline Blood Pressure was not consistently recorded. Different antihypertensive tablets were recorded but clinical indications of these antihypertensive agents could not be established (i.e. hypertension, cardiovascular prevention or microalbuminuric diabetic nephropathy etc.).

There were no differences between ACE genotypes in the baseline (pre-RCT) rates of severe hypoglycaemia (DD 9.4±3.3, ID 8.2±1.8, II 8.3±3.8

episodes/person/year,p 0.940, N= 77) as shown in Table 3-1 and Figure 3-2 . The differences remained similar when analysed as risk vs non-risk ACE genotype [(DD 9.4±3.0, Non DD 8.2±1.6 episodes/person/year, p 0.72, n =727) (Figure 3-3)].

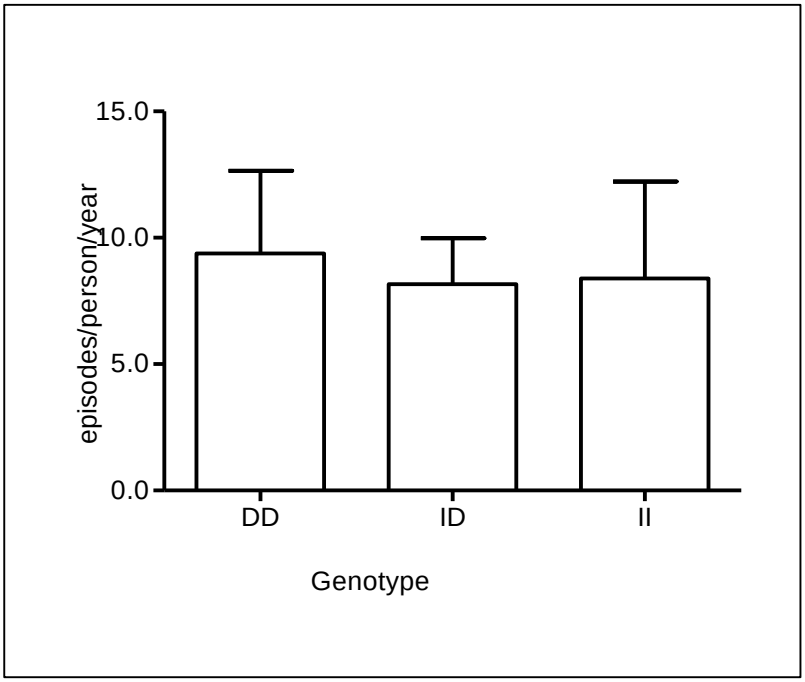
Study Intervention groups in the main HypoCOMPaSS Multiple Daily Injection (MDI), Continuous Subcutaneous Insulin Infusion (CSII), MDI with Real Time CGM (RT) and CSII with Real Time CGM (RT) were similar across the ACE genotypes (Table 3-2).

Table 3-1: Baseline characteristics of participants based on different ACE genotypes

	All n=77	DD n= 21	ID n=43	II n=13	p value*
Female Sex (n=49)	49	11	27	11	0.162 [a]
Age (years(n=77)	48.6 ± 1.3	47.1±2.9	49.8±1.7	47.0±3.2	0.650
Duration of diabetes (years) n=77	29.3±1.4	29.9±3.0	29.2±1.9	29.1±3.0	0.976
$HbA_{1c\ (\%)}$ baseline (n=77)	8.2±0.1	8.2±0.1	8.1±0.1	8.7±0.3	0.223
Severe Hypoglycaemia at baseline (n=77) (episodes/person/year)	8.5±1.4	9.4±3.2	8.2±1.8	8.3±3.8	0.940
Gold Score at baseline (n=77)	5.3±0.1	5.4±0.2	5.4±0.1	5.0±0.25	0.279
Diabetic Retinopathy (n=76)	46	13	24	9	0.172 [a]

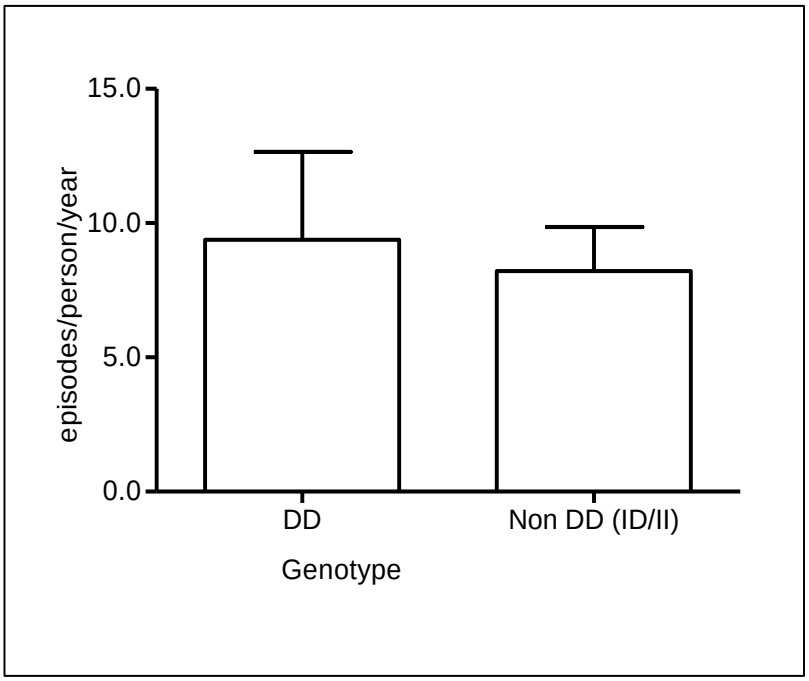
Data shown are mean ±SEM
* One way Anova
a Chi Square Test

Figure 3-2 Baseline Severe Hypoglycaemia across ACE genotypes



Data shown are mean ±SEM

Figure 3-3: Baseline Severe Hypoglycaemia - at risk ‘DD’ Vs ‘Non DD’ (ID/II) ACE genotypes



Data shown are mean ±SEM

Table 3-2 : Study Interventions based on ACE genotype distributions

	All n=77	DD n= 21	ID n=43	II n=13	p value
MDI(n)	19	4	13	2	0.77 [a]
CSII(n)	18	6	8	4	
MDI with RT (n)	22	6	11	5	
CSII with RT(n)	18	5	11	2	

a: Chi Square test

3.5.2 *Serum ACE levels and Glycaemic control of participants at the end of 6 months RCT period -ACE genotype sub study*

There were no differences between ACE genotypes in terms of HbA1c at the end of the 6 months RCT study period.

As anticipated (and in keeping with previous literature), serum ACE activity level was significantly higher in the DD ACE genotype. The difference persisted even when those treated with ACEI were excluded (Table 3-3).

Table 3-3 : Glycaemic control and serum ACE levels of participants at the end of 6 months RCT period

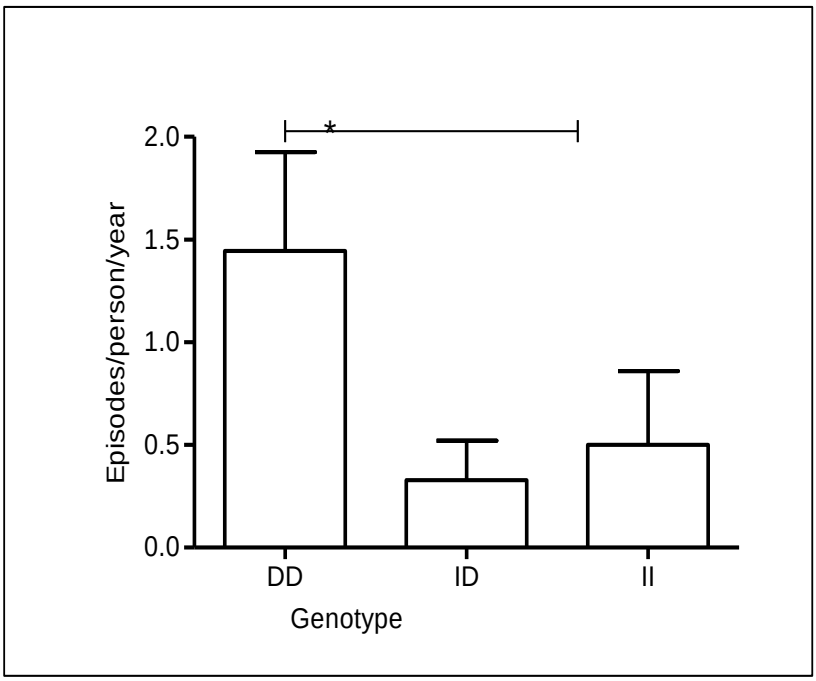
	All **n=77**	**DD** **n= 21**	**ID** **n=43**	**II** **n=13**	**p value***
HbA1c (%)	8.1±0.1 (n=76)	7.8±0.1 (n=21)	8.2±0.1 (n=42)	8.2±0.2 (n=13)	0.368
HbA1c(mmol)	65.2±1.1 (n=76)	62.6±1.7 (n=21)	66.2±1.7 (n=42)	66.1±2.4 (n=13)	0.368
ACE activity(U/l)	39.3±3.6 (n=68)	58.1±8.6 (n=18)	37.4±4.0 (n=39)	15.36±2.1 (n=11)	<0.001****
ACE activity (U/l) (without ACEI treated) (n=46)	51.2±4.2 (n=46)	76.7±6.5 (n=13)	45.3±4.5 (n=28)	18.2±2.7 (n=5)	<0.001****
ACE activity (U/l) (without ACEI or ARB treated)	50.8±4.9 (n=37)	78±7.0 (n=12)	42.7±5.2 (n=20)	18.2±2.7 (n=5)	<0.001****

Data shown are mean ±SEM
* One way Anova

3.5.3 *Severe Hypoglycaemia during RCT study period-ACE genotype sub study*

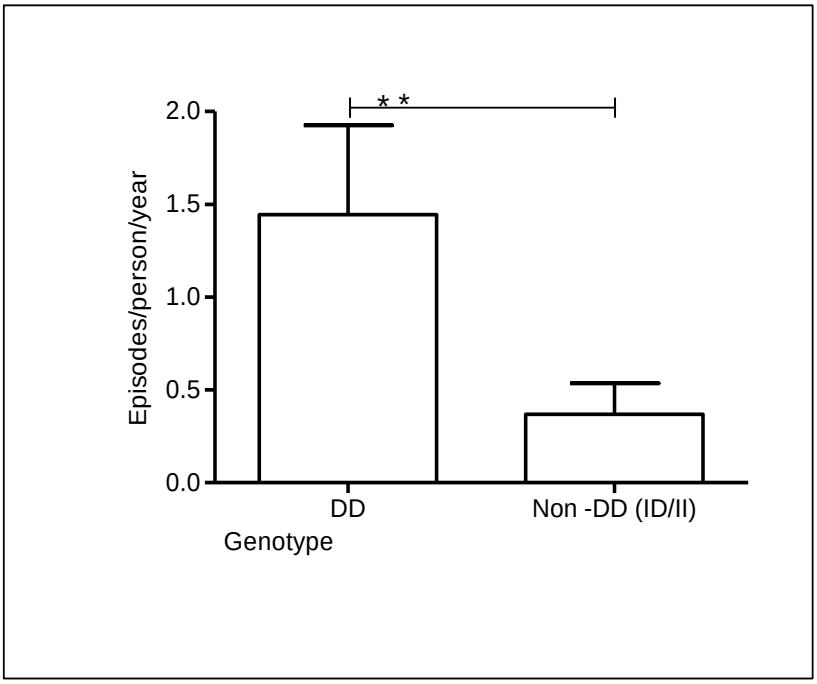
Despite no differences in baseline SH (as seen in Figure 3-2 and Figure 3-3 above), SH episodes in DD ACE genotype were markedly and significantly higher during the 6 months RCT study period (DD 1.44±0.48, ID 0.33±0.19, II 0.50±0.36 episodes/person/year, p 0.032 (Figure 3-4). This difference was also apparent when analysed as risk vs. non-risk ACE genotype, [(DD 1.44±0.48, Non DD 0.37±0.17 episodes/person/year, p 0.009, n =77) (Figure 3-5)]. The differences remained significant when those treated with ACEI therapy were excluded [(DD 1.59±0.62, ID 0.22±0.12, II 0.62±0.62 episodes/person/year, p 0.02, n =52) (Figure 3-6)]. As above, the differences persisted in this analysis of those not on ACEI when we compared risk vs non-risk ACE genotype [(DD 1.59±0.62, Non DD 0.30±0.15 episodes/person/year, p 0.006, n =52) (Figure 3-7)]. When those treated with either ACEI and/or ARB were excluded, differences were not statistically significant, likely because of reduced power [(DD 1.3±0.6, ID 0.2±0.14, II 0.72±0.72 episodes/person/year, p 0.097, n =40)]. Finally, I performed a further specific analysis of SH during the study including only the participants who had one or more episode of SH in the preceding 12 months prior to the study. SH was significantly higher in the DD group in this sub group analysis as well (DD 1.6±0.52, Non DD 0.41±0.19 episodes/person/year, p 0.009).

Figure 3-4: Severe Hypoglycaemia during 6 months RCT study period across ACE genotypes



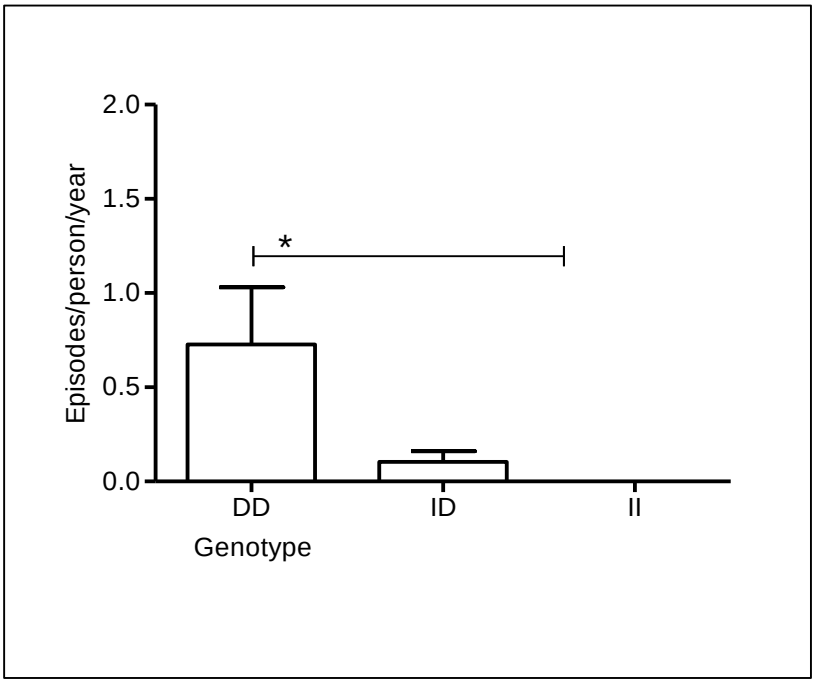
Data shown are mean ±SEM

Figure 3-5 : Severe Hypoglycaemia during 6 months RCT study - at risk ‘DD’ Vs ‘Non DD’ (ID/II) ACE genotypes



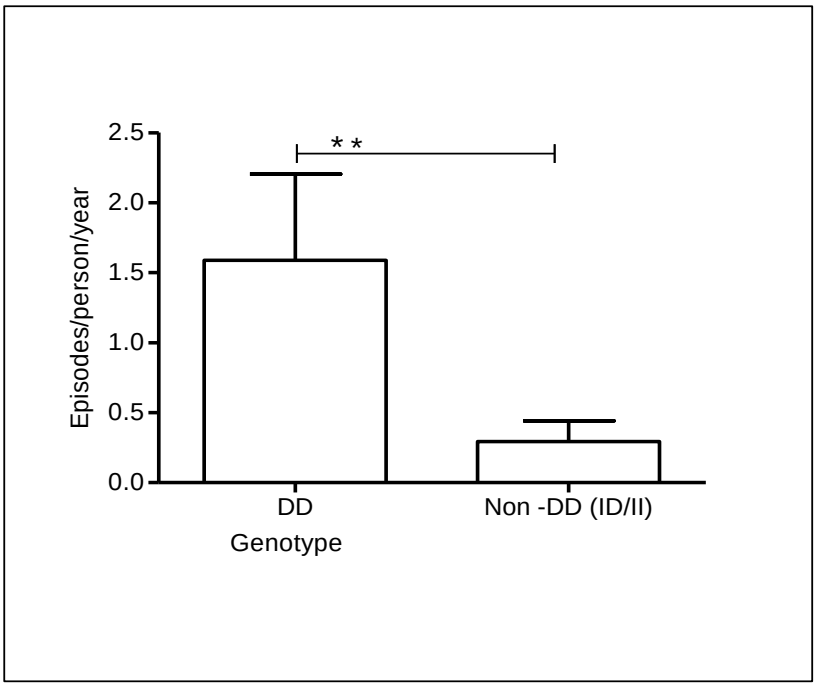
Data shown are mean ±SEM

Figure 3-6 : Severe Hypoglycaemia during 6 months RCT study period across ACE genotypes excluding those on ACEI therapy



Data shown are mean ±SEM

Figure 3-7 : Severe Hypoglycaemia during 6 months RCT study - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes excluding those on ACEI therapy



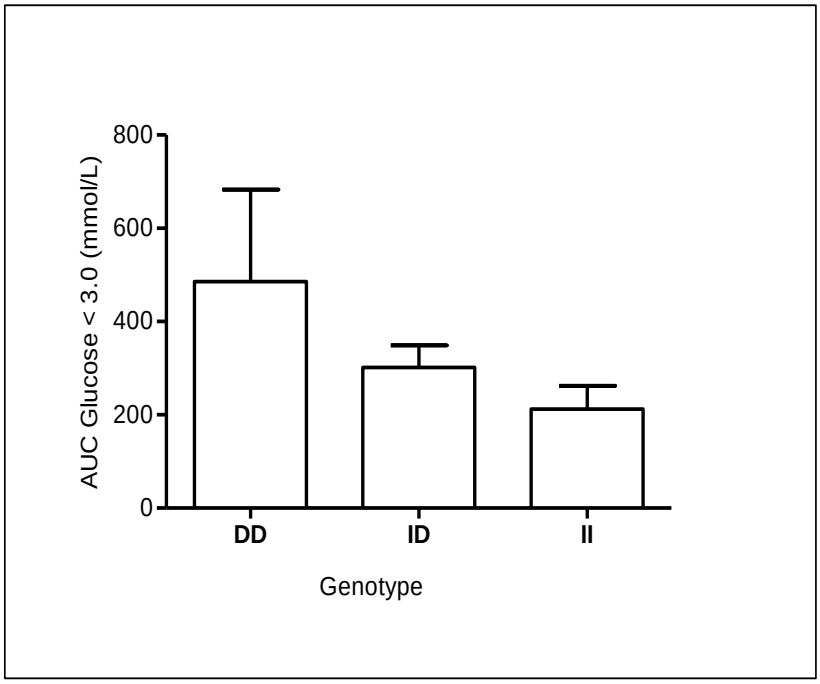
Data shown are mean ±SEM

3.5.4 *Biochemical Hypoglycaemia during the 6 months RCT study period -ACE genotype sub study*

There was no effect of the ACE genotype on the magnitude and duration of "biochemical hypoglycaemia" (glucose < 3.0mmol/L) during 6 month RCT period measured using monthly blinded CGM [(AUC DD 485±198, ID 302±47, II 212±50, p 0.27, n =75**)** (Figure 3-8)]. This effect remained non-significant when examined as risk vs non-risk ACE genotype [(AUC DD 485±198, Non DD 281±38, p 0.130, n =75). (Figure 3-9)].

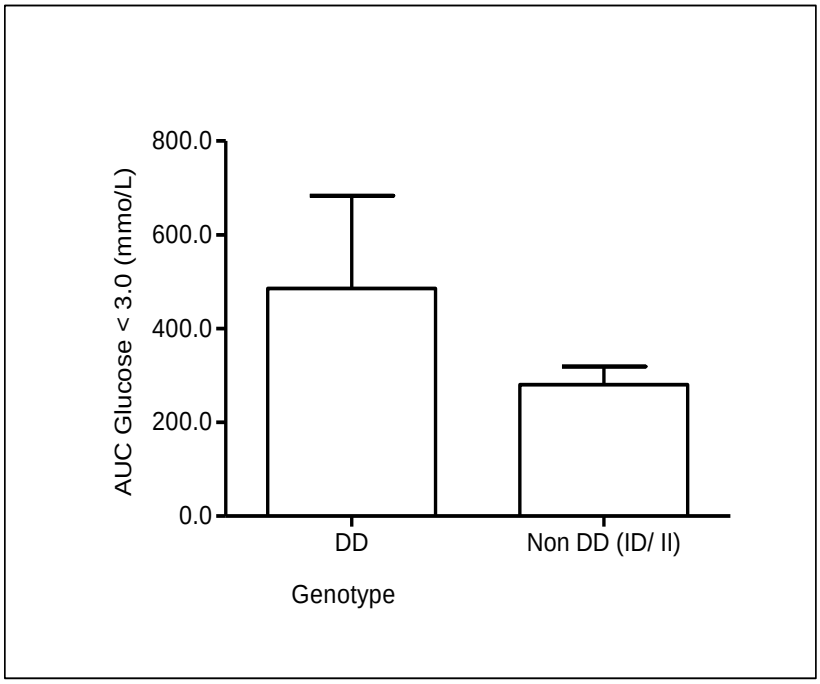
There was no difference between genotypes when lower threshold of hypoglycaemia glucose (≤2.5mmol/L) was used (AUC DD 126±26, Non DD 134±24, p 0.86)].

Figure 3-8: Biochemical hypoglycaemia (Glucose < 3.0 mmol/L) during 6 months RCT study period across ACE genotypes



Data shown are mean ±SEM

Figure 3-9: Biochemical hypoglycaemia (Glucose < 3.0 mmol/L) during RCT study period - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes



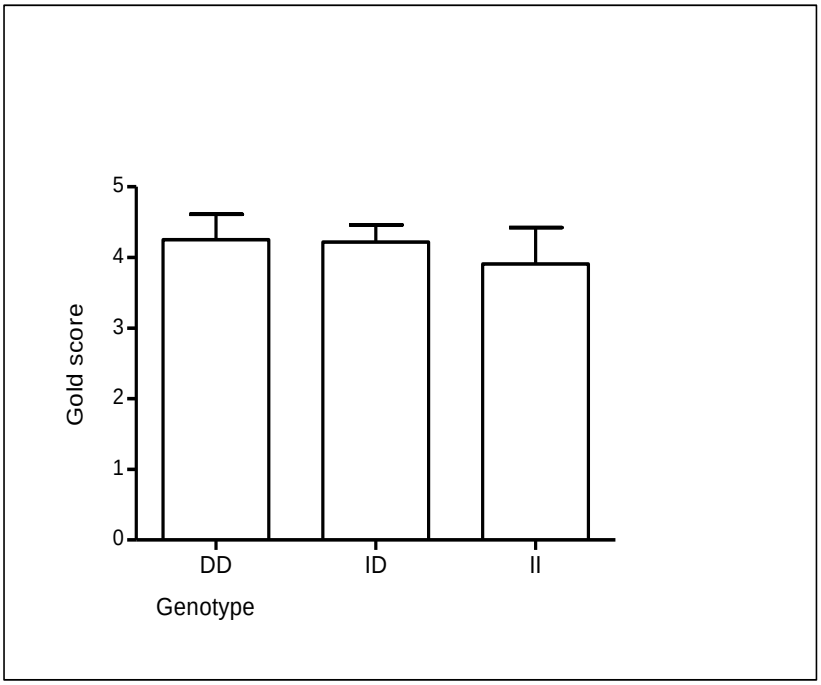
Data shown are mean ±SEM

3.5.5 *Awareness of Hypoglycaemia assessed at the end of 6 months RCT study period -ACE genotype sub study*

Awareness of Hypoglycaemia assessed at the end of 6 months RCT study period did not show ACE genotype difference in mean Gold score Questionnaire [(DD 4.25±0.36, ID 4.22±0.24, II 3.91±0.51 , p 0.82,n=72) (Figure 3-10)]. As expected, this effect remained non-significant when examined as risk vs non risk ACE genotype. [(DD 4.25±0.36, Non DD 4.15±0.22, p 0.82, n=72) (Figure 3-11)] Furthermore, at the end of the 6 months RCT study period, there were no ACE genotype differences in the proportion of participants who either "responded" i.e. improvement in Gold score Questionnaire or "resolved" (resolution of IAH), i.e.

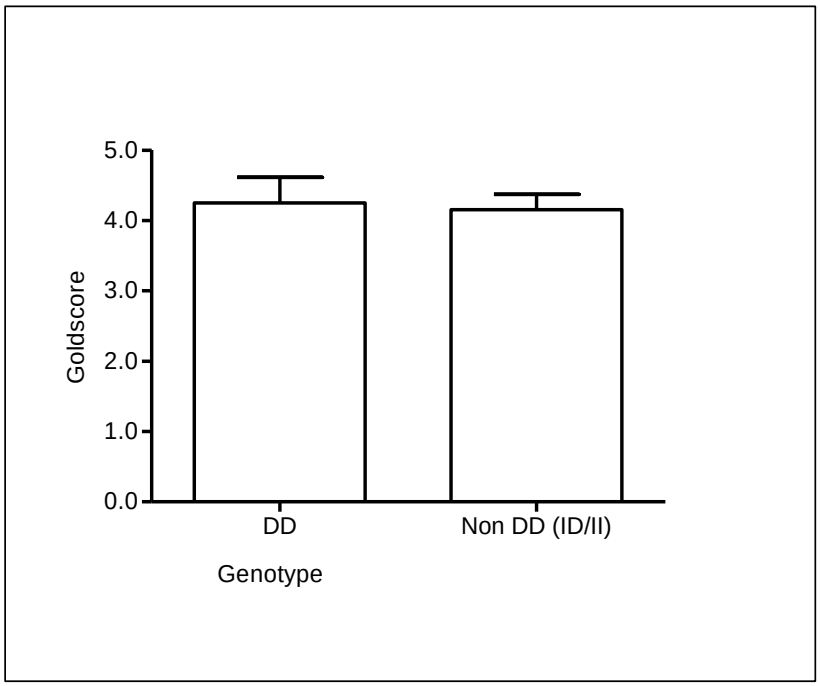
improvement in Gold score Questionnaire below a threshold value of 4 [(p 0.56 & 0.96 respectively) (Figure 3-12 & Figure 3-13)]. This effect remained non-significant when examined as risk vs non-risk ACE genotype [(p 0.41 & 0.79 respectively) Figure 3-14 & Figure 3-15].

Figure 3-10 : Awareness of Hypoglycaemia assessed by Gold score at the end of 6 months RCT study period across ACE genotypes



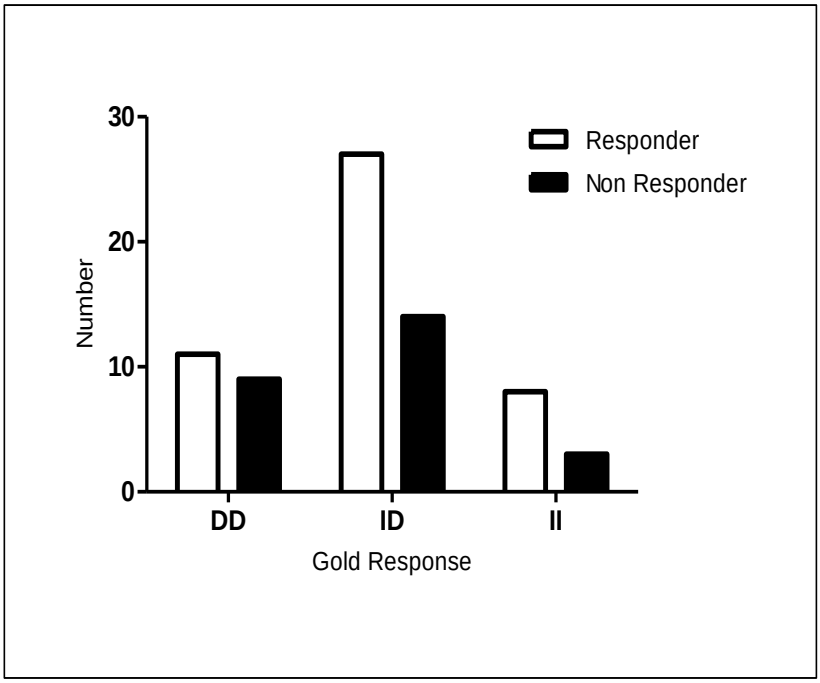
Data shown are mean ±SEM

Figure 3-11 : Awareness of Hypoglycaemia assessed by Gold score at the end of 6 months RCT study period - at risk ‘DD’ Vs ‘Non DD’ (ID/II) ACE genotypes



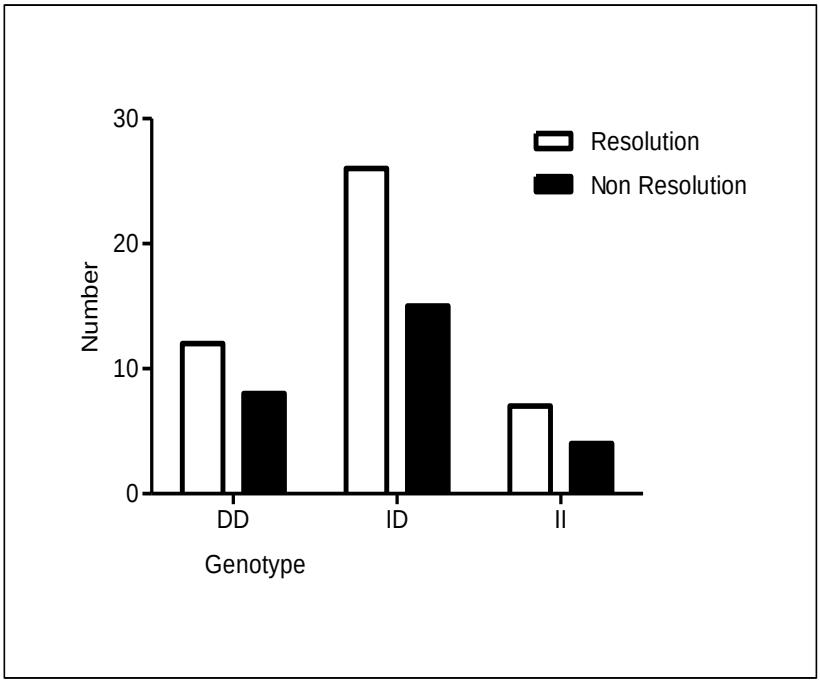
Data shown are mean ±SEM

Figure 3-12 :Proportion of participants whose Awareness of Hypoglycaemia improved as assessed by Gold score at the end of 6 months RCT study period across ACE genotypes



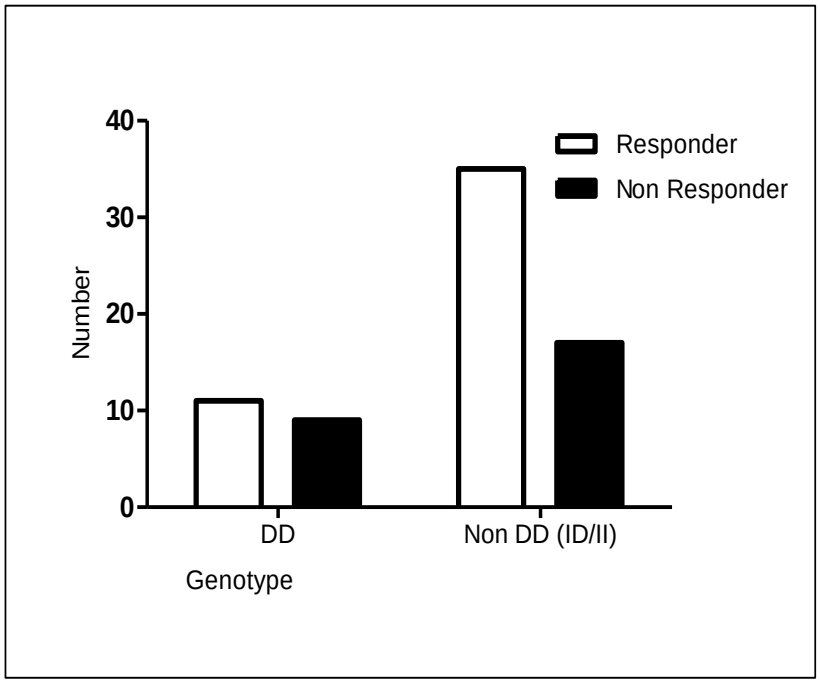
Chi -Square test

Figure 3-13 : Proportion of participants whose Awareness of Hypoglycaemia improved below a threshold value of 4 as assessed by Gold score at the end of 6 months RCT study period across ACE genotypes



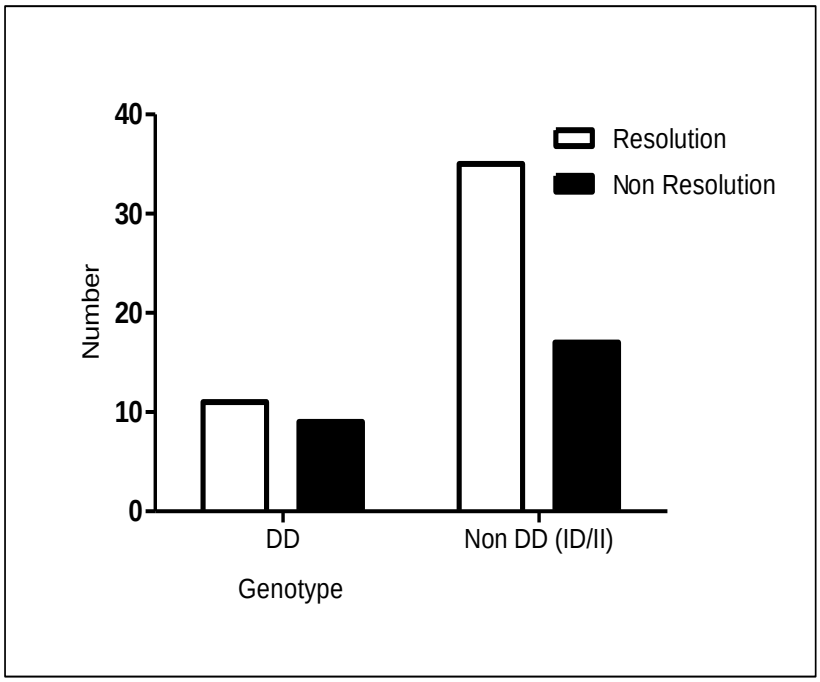
Chi -Square test

Figure 3-14 : Proportion of participants whose Awareness of Hypoglycaemia improved as assessed by Gold score at the end of 6 months RCT study period - at risk ‘DD’ Vs ‘Non DD’ (ID/II) ACE genotypes



Fisher exact test

Figure 3-15: Proportion of participants whose Awareness of Hypoglycaemia responded (i.e. improved below a threshold value of 4) as assessed by Gold score at the end of 6 months RCT study period - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes



Fisher exact test

3.5.6 *Clamped hypoglycaemia at the end of 6 months RCT study -ACE genotype sub study*

In the main HypoCOMPaSS study, 23 participants successfully completed the hyperinsulinemic hypoglycaemic clamp procedure at end of the 6 months RCT study. 21 of those participants took part in ACE genotype sub study. 7 of these 21 participants were on ACEI therapy at the time of clamped hypoglycaemia.

To explore the possible underlying mechanism of increased risk of severe hypoglycaemia in DD ACE genotype, I examined end-of-RCT clamped

hypoglycaemia results in these 14 participants who were not on ACEI therapy. Clamp procedure is explained in the appendix 5.

Symptoms scores and cognitive function were assessed during stepped hypoglycaemia along with counter-regulatory hormones measurement in chapter 2. In this subset, due to relatively small numbers participating in clamp studies in each group, I have analysed the data comparing risk ‘DD’ vs ‘Non-DD’ (ID and II) ACE genotypes.

Demographic details of these 14 participants who were not on ACEI therapy is shown in the table below (Table 3-4).

Table 3-4: Baseline characteristics of participants who took part in clamp procedure at end of the 6 months RCT and were not on ACEI therapy

	All n = 14	DD n= 7	Non DD (ID/II) n=7	p value*
Female Sex (n=14)	14	10	4	0.28 [a]
Age (years)	47.6±2.5	44±4.3	51.1±2.3	0.17
Duration of diabetes (years)	33.9±2.9	28.7±4.3	39.1±3.0	0.07
$HbA_{1c\ (\%)}$baseline	7.9±0.2	7.8±0.3	7.9±0.4	0.78
$HbA_{1c\ (mmol/mol)}$ baseline	62.9±2.7	62.1±3.3	63.6±4.5	0.78
Severe Hypoglycaemia at baseline (episodes/person/year)	9.9±3.9	10.1±6.8	9.7±4.3	0.95
Gold Score at baseline	5.4±0.2	5.7±0.2	5.1±0.4	0.22

Data shown are mean ±SEM
* Independent sample T-Test
a Fisher Exact test

Plasma glucose and dextrose infusion rate (DIR) for these 14 participants who took part in clamp procedure at end of the 6 months RCT and not on ACEI therapy is shown in Figure 3-16 and Figure 3-17 respectively.

Figure 3-16: Plasma glucose for 14 participants who took part in clamp procedure at end of the 6 months RCT and were not on ACEI therapy

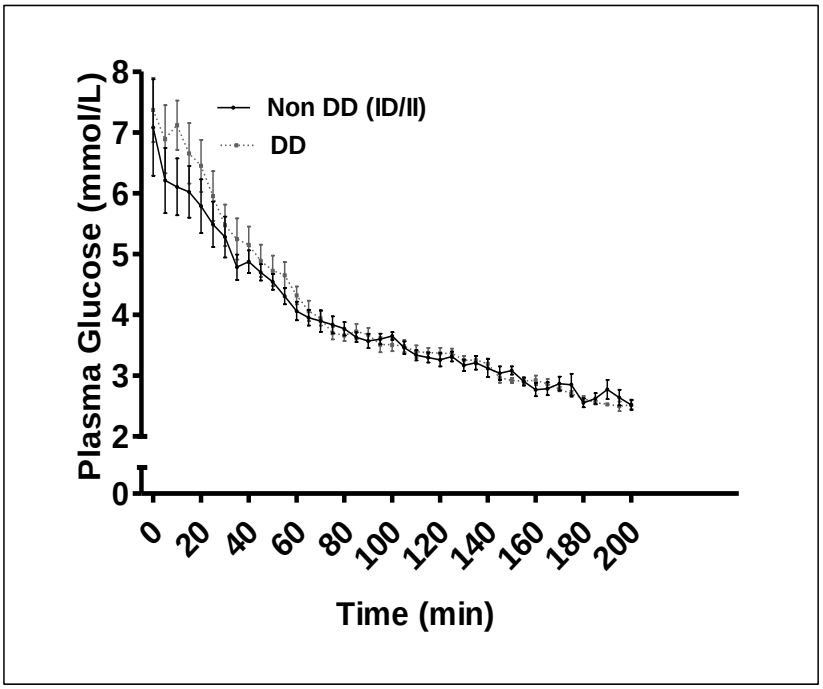
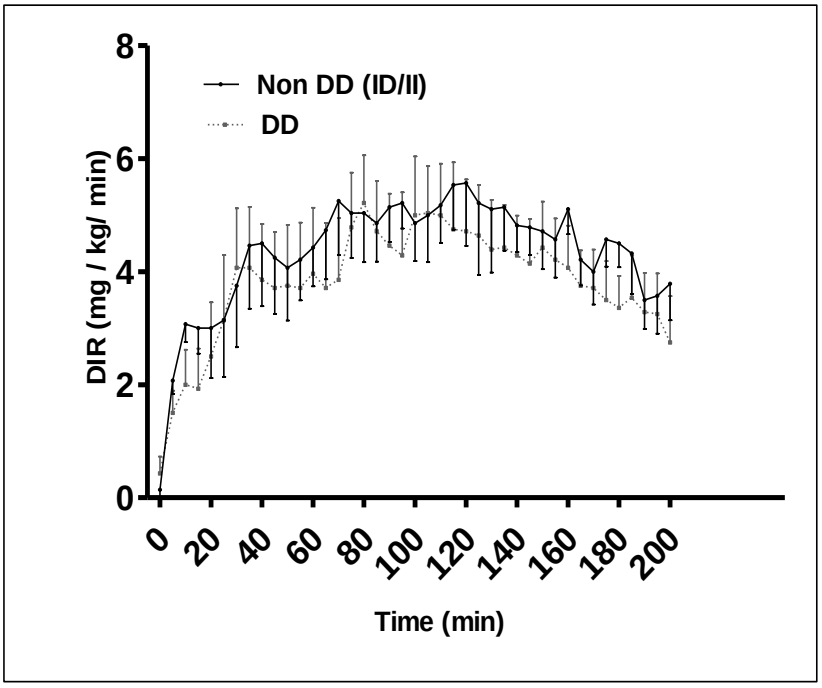


Figure 3-17: Dextrose Infusion Rate (DIR) for 14 participants who took part in clamp procedure at end of the 6 months RCT and were not on ACEI therapy



3.5.6.1 **Symptoms and hormonal responses in End RCT clamped hypoglycaemia-ACE genotype sub study**

In keeping with the Gold score reported in main RCT, the plasma glucose levels at which participants first felt low was comparable in DD ACE genotype compared to Non DD (ID/II) ACE genotypes (answer to the question ‘‘do you feel hypoglycaemic?’’ during stepped hypoglycaemic clamp) in participants not on ACEI therapy. Total AUC values for autonomic symptoms, neuroglycopenic symptoms and total symptoms were also comparable in both DD and Non DD ACE genotypes. Similarly, total AUC values for plasma metanephrine were also comparable in both DD and Non DD ACE genotypes (Table 3-5).

Given the prior hypothesis about ACE genotype affecting the sensitivity of brain to fuel deprivation, I also examined a specific neuroglycopenic symptom subset comprising scores for confusion, difficulty in speaking and clumsiness. These have been suggested to be more indicative of cognitive dysfunction[270]. Total 'cognitive dysfunction' scores were high in DD genotype, but this did not reach statistical significance (Table 3-5).

Table 3-5: Symptoms and hormonal responses at end RCT clamped hypoglycaemia in those who were not on ACEI - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes

	DD N=7	Non DD (II/ID) N= 7	*p value
Glucose levels subjects felt low (mmol/l)	2.5(2.2,3.4)	3.2(2.6,4.4)	0.16
Symptoms AUC			
Total	920.0±180.6	865.7±200	0.84
Autonomic	514.3±144.6	357.1±109.3	0.40
Neuroglycopenic	405.7±108.4	508.6±116.4	0.53
Total Cognitive Dysfunction Symptoms §	6.5±3.0	1.7±0.9	0.17
Hormones AUC			
Metanephrines	54165.7±9153.6	51028.6±10189.1	0.82

Data shown are mean ±SEM or Median (IQR)
* Independent sample T-Test or Mann Whitney U Test
§ Confusion, Difficulty in speaking, Clumsiness

3.5.6.2 **Cognitive Function Tests in End RCT clamped hypoglycaemia-ACE genotype sub study**

The plasma glucose at which cognitive function deteriorated as measured by Stroop Colour Word was significantly higher in 'DD' ACE genotype compared to 'Non DD' (ID/II) ACE genotypes [(3.4(2.8,3.9) vs 2.9(2.4, 3.0),P 0.038] in those not on ACEI therapy as shown in Table 3-6 & Figure 3-18 (mean values).

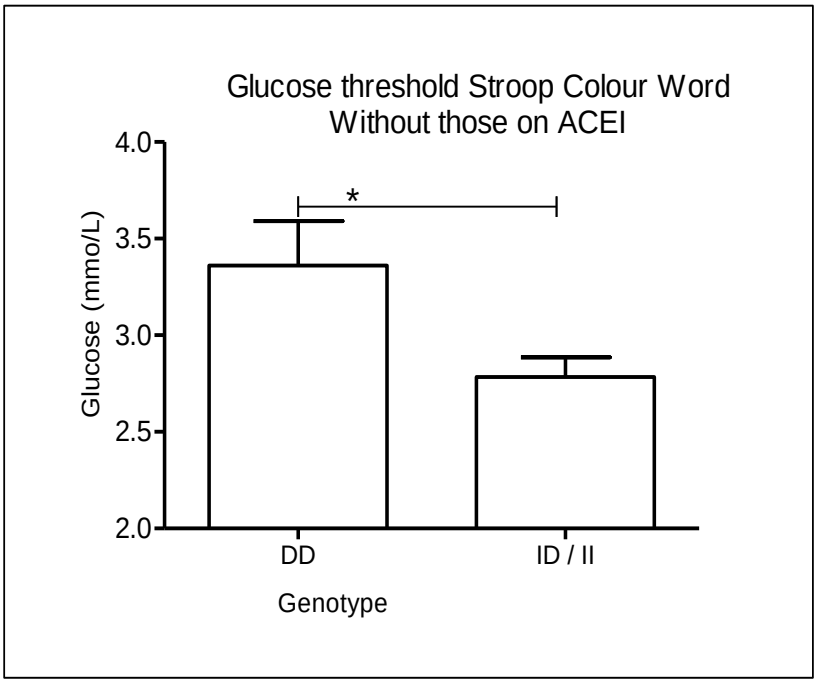
The plasma glucose threshold for deterioration of cognitive function (n=13 as one subject had no data collected for this parameter) as measured by four – choice reaction also appeared to be higher in the risk 'DD' ACE genotype but was statistically not significant (Table 3-6).

Table 3-6: Glucose (mmol/l) threshold at which cognitive function deteriorated in End RCT clamped hypoglycaemia in participants who took part in ACE genotype sub study and were not on ACEI therapy

	DD	Non DD (ID/II)	*p value
Stroop Colour word	3.4(2.8,3.9) N=7	2.9(2.4,3.0) N= 7	0.038
Four choice reaction time	3.3(2.6,3.6) N=7	2.9(2.6,3.1) N=6	0.29

Data shown are Median (IQR)
* Mann Whitney U Test

Figure 3-18:Plasma glucose threshold at which Stroop Colour Word deteriorated in End RCT clamped hypoglycaemia in those who were not on ACEI- at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes



Data shown are mean ±SEM

The total AUC values for cognitive function tests as assessed by Four choice reaction time and Stroop tests (Black & White reading, Colour reading and Colour Word reading) during stepped clamped hypoglycaemia were comparable in both DD and Non DD ACE genotypes (Table 3-7).

Table 3-7 : Cognitive Function Tests in End RCT clamped hypoglycaemia in those who were not on ACEI - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes

	DD N=7	Non DD (II/ID) N=7	*p value
Cognitive Function AUC			
Four Choice Reaction Time	5590.9±769.0	6287.2±1381.7	0.67
Stroop Black & White	9637.1±312.4	9626.2±402.2	0.98
Stroop Colour	14828.6±1068.9	13605.0±600.4	0.32
Stroop Colour Word	20694.3±1115.13	18665.0±2145.1	0.14

Data shown are mean ±SEM
* Independent sample T-Test

I have further explored to see if participant's cognitive function deteriorated during experimental blinded hypoglycaemia before the point at which subjects felt low (answer to the question ''do you feel hypoglycaemic?''). I found that in the DD ACE genotype group, cognitive function deteriorated significantly at earlier glucose values before the subjects developed hypoglycaemic symptoms compared to Non DD (ID/II) ACE genotypes when measured by Stroop Colour Word. On the contrary, participants with Non DD (ID/II) ACE genotypes felt low before decline in cognitive function as measured Stroop Colour Word (Table 3-8).

Although a similar trend was observed in at risk DD ACE genotype when measured by four choice reaction, this did not reach statistical significance (Table 3-8).

Table 3-8: Glucose (mmol/l) threshold at which cognitive function deteriorated minus glucose levels subjects felt low (mmol/l) in End RCT clamped hypoglycaemia in participants who took part in ACE genotype sub study and were not on ACEI therapy

	DD	Non DD (ID/II)	*p value
Glucose difference for Stroop Colour word	0.5±0.2 N=7	-0.8±0.4 N= 7	0.023
Glucose difference for Four choice reaction time	0.2±0.2 N=7	-0.7±0.4 N=6	0.055

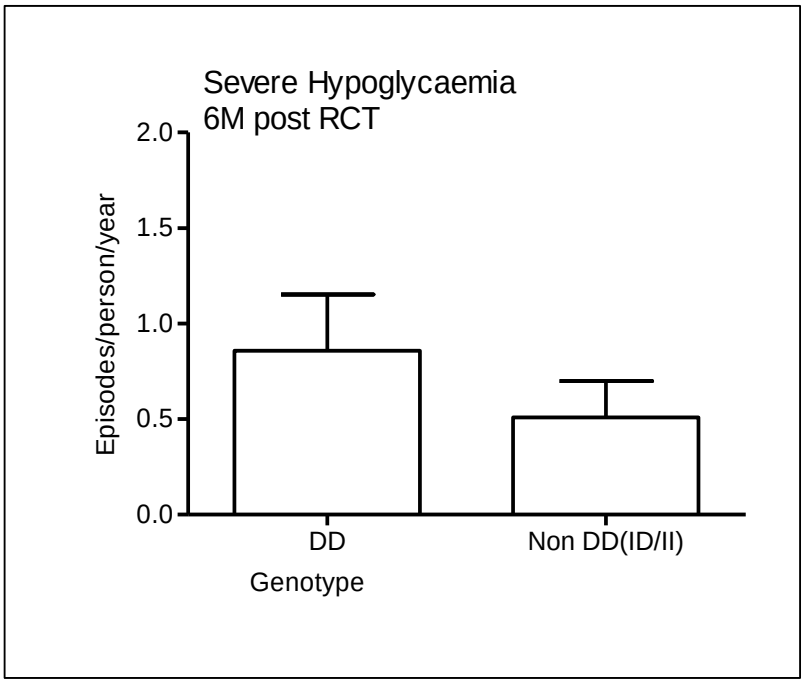
Data shown are mean ±SEM
* Independent sample T-Test

3.5.7 *Post-Randomised Controlled Trial follow-up period (post RCT) - ACE genotype sub study*

All participants of the main HypoCOMPaSS attended for further follow up visits at 6, 12 and 18 months after completion of RCT period. Given that SH is a relative infrequent event that clusters in time, even in those at greatest risk, I examined data collected over the whole 2 year period to see if there was an association with ACE genotype. In summary, there was no statistical difference

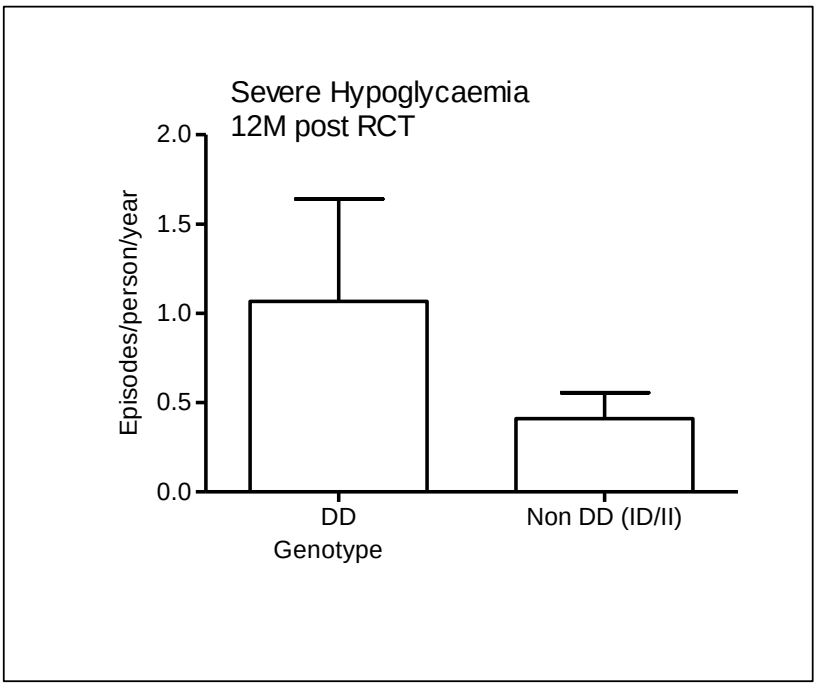
in SH between ACE genotypes in follow up period (cumulative SH at each follow up period) although SH (episodes/person/year) was numerically higher in at risk DD ACE genotype. [(6months DD 0.86±0.30, Non DD 0.50±0.20, p 0.33, n =77, (Figure 3-19), [(12 months DD 1.06±0.57, Non DD 0.41±0.14, p 0.11, n=76, (Figure 3-20)], [(18 months DD 0.82±0.43, Non DD 0.43±0.15, p 0.27, n=76, (Figure 3-21)].

Figure 3-19 : Severe Hypoglycaemia 6 months post RCT - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes



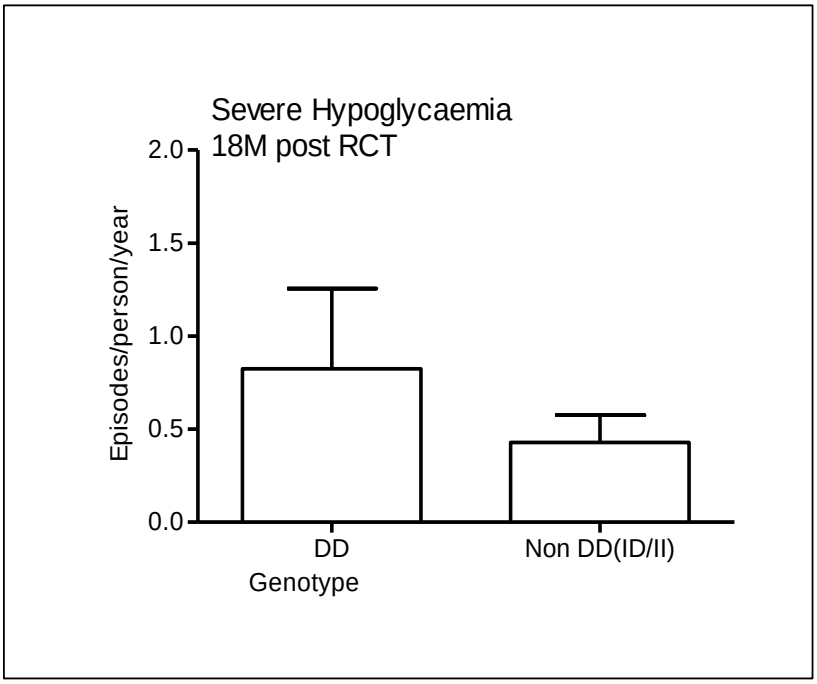
Data shown are mean ±SEM

Figure 3-20: Severe Hypoglycaemia 12 months post RCT - at risk ‘DD’ Vs ‘Non DD’ (ID/II) ACE genotypes



Data shown are mean ±SEM

Figure 3-21: Severe Hypoglycaemia 18 months post RCT - at risk ‘DD’ Vs ‘Non DD’ (ID/II) ACE genotypes



Data shown are mean ±SEM

Discussion

In line with main HypoCOMaSS study, the majority of the participants in this sub study were female and they had almost 3 decades of diabetes, with sub-optimal glycaemic control. There were no differences between ACE genotypes in terms of gender, age, HbA1c or duration of diabetes. Study Interventions (allocated by chance randomisation) were similarly distributed across the genotypes. In keeping with previous studies, serum ACE level was significantly higher in DD ACE genotype with or without ACEI therapy also validating the genotype results.

There were no differences between ACE genotypes in the baseline rates of severe hypoglycaemia (DD 9.4±3.0, Non DD 8.2±1.6 episodes/person/year). The main finding was that, following clinical optimisation during the RCT period, episodes of severe hypoglycaemia were reduced both in risk DD and Non DD (ID/II) ACE genotypes but residual SH was significantly high in DD ACE genotype [(DD 1.44±0.48, Non DD 0.37±0.17 episodes/person/year, p0.009)] and remained significant even when examined without ACEI therapy.

Many non-biologic or 'environmental factors' including diabetes education, self-management skills, hyperglycaemia avoiding behaviour, psychosocial issues, behavioral factors[107, 271] could have potentially contributed to SH at baseline in the participants at HypoCOMPaSS study. When these non-biologic factors were minimized during the RCT period, this might have enriched the underlying genetic factors contributing to SH in these participants. This could explain why we saw higher rates of SH in at risk DD ACE genotype compared with other genotypes during the RCT but not at baseline (where overall rates were higher and likely that many contributors to this). When SH episodes were further examined at 6 months, 12 months and 18 months post RCT follow up period, the difference was lost although the SH rates remained numerically higher in DD ACE genotype. One obvious explanation is that that the difference was lost during the follow up period when environmental and behavioural factors were again more prevalent following the end of the intensive RCT support period. This reinforces the concept that environmental factors largely contribute to SH episodes in people with T1D, but biological factors may also contribute to SH in some.

I also tried to examine what the potential mechanism for an effect of ACE genotype to alter SH risk might be. There was no effect of the genotype on magnitude and duration of biochemical hypoglycaemia measured using blinded CGM. Furthermore, there was no effect of genotype on impaired awareness of hypoglycaemia status as the proportion of participants whose IAH status improved or awareness to hypoglycaemia restored were similar in both DD and non DD ACE genotype. So, my findings show that DD ACE genotype is associated with residual risk of severe hypoglycaemia during study period without significant difference in improvement in IAH or indeed exposure to hypoglycaemia as compared to non-risk group.

I therefore examined the clamp data to try to understand mechanistically why this might be the case.

Counter-regulatory responses in the DD ACE genotype were no less as indicated by metanephrine responses which were similar in both genotypes (and if anything, the response was on the higher side in DD group). Similarly, there were no differences in the autonomic or total neuroglycopenic symptom scores. However, cognitive function as assessed by the most cognitively demanding of the Stroop tests, the Stroop Colour Word, deteriorated earlier during stepped clamped hypoglycaemia. I also found a trend towards a greater deterioration in 4 CRT and a trend towards greater symptomatic scores of cognitive dysfunctions in the DD group. Although this did not reach statistical significance, these are clinically significant.

I also found that in DD ACE genotype cognitive function deteriorated at earlier glucose values when measured by Stroop Colour Word before the subjects

developed hypoglycaemic symptoms. In contrast, participants with the “non-risk “genotype felt low before their cognitive function started to decline.

A limitation of the “end of RCT” clamp sub group analysis is the fact that this may not be sufficiently powered to show differences due to the smaller number of participants in each category. Nevertheless, this may be a significant signal which merits replication in larger studies in future.

Although Gold score is a validated tool it does not characterise IAH. Furthermore, it does not detect change in IAH. [122, 272]. So, my findings may have been limited by this. Recently a new questionnaire has been designed to address this issue[272].

My findings are consistent with the previous Scandinavian studies in the UK cohort, showing that the DD ACE genotype is associated with increased risk of severe hypoglycaemia[226, 228]. Furthermore, my data suggest that this might be mediated via greater cognitive dysfunction during hypoglycaemia in DD group and less in the presence of at least one copy of the I allele. This would be consistent with the data showing that the I allele seems to be associated with a greater tolerance of reduced substrate delivery e.g. in endurance athletes. The DD genotype neither increased the risk of biochemical hypoglycaemia nor awareness of hypoglycaemia but affected people’s ability to take action during mild hypoglycaemia episode, thus putting them at risk of severe episode (SH). One possibility is that preserved cognitive function in II/ ID genotypes allows somebody with T1D the opportunity to take judicious action once symptoms alert to prevent a further fall into deeper hypoglycaemia resulting in a severe episode. By contrast, an earlier deterioration in cognitive function in DD genotype during

hypoglycaemia (and thus judgement / decision making) might result in a greater propensity for a falling glucose to result in severe hypoglycaemia.

The findings of the study also raise the possibility that altering brain RAS activity therapeutically might even protect cognitive function and reduce risk of severe hypoglycaemia in those with T1D at risk of exposure to low blood glucose. I speculate that altering ACE activity therapeutically by brain-penetrant ACEI therapy in a selected at risk T1D people might protect against severe hypoglycaemia [270]. Some observational “population” data appear contradictory to this finding with some showing increased hypoglycaemia in people treated with ACEI perhaps attributable to a prescribing bias (e.g. avoiding other anti-hypertensives such as beta-blockers in those with problematic hypoglycaemia) and/or only certain ACEI being centrally active[254, 273]. For example, Ramipril, Trandolapril, Captopril, Fosinopril and Lisinopril cross the blood-brain barrier[274]. Other potential compounding factors like insulin effect and behavioural aspects of the people were overlooked in these retrospective studies [273, 275, 276]. Essentially these population studies had confounding environmental factors contributing to the SH. This study has minimised these factors and has studied residual SH risk.

In summary, my findings support the broad hypothesis that certain people may be at greater risk of hypoglycaemia due to their underlying biology, an important point for clinicians aiming at intensifying glucose control in diabetes.

I speculate that future systemic treatment with brain-penetrant ACEI in T1D people with DD ACE genotype and high serum ACE might reduce brain RAS signalling and thus protect against cognitive dysfunction during hypoglycaemia.

One of the challenges of performing trials studying ACEI and severe hypoglycaemia is that majority of people with T1D are likely to be on ACEI therapy for primary or secondary prevention of cardiovascular disease.

4 Accuracy & Use of a novel Continuous Implanted Glucose Sensor

4.1 Background

This study extends my interest in looking for methods to improve hypoglycaemia burden in people with type 1 diabetes. As a treatment modality, CGM has been shown to improve glycaemia and/or reduce time spent in hypoglycaemia and is approved by NICE for T1D people with disabling hypoglycaemia [62, 182]. However, the benefits of these traditional CGM systems are dependent on adherence with the system as discussed in previous chapters [70, 80, 277]. Some factors including adhesive issues, skin reactions and the need to change / reinsert the sensor every few days can limit use and nullify potential benefits[278].

The Senseonics Continuous Glucose Monitoring System CGM [(Senseonics CGM System), now called Eversense® CGM System (Senseonics, Inc., Germantown, MD, USA] is a novel implantable glucose monitoring device [279, 280]. The Eversense® CGM system as commercially available consists of a glucose sensor implanted in the upper arm, a transmitter which is worn over the sensor attached using adhesive and the application (app) in a mobile platform which displays glucose continuously.

The sensor of the system is a micro-fluorometer that is encased in a translucent biocompatible polymer capsule. It consists of glucose-indicating fluorescent hydrogel, light emitting diode and antenna. Glucose concentration is measured by means of fluorescence from the glucose-indicating hydrogel, which is

polymerized onto the capsule surface over the optical cavity. The light emitting diode serves as the excitation source for the hydrogel. The antenna receives both power and communication from externally worn transmitter. The final information is transmitted to smart phone via bluetooth [279, 280]. In contrast to other traditional CGM systems which utilize electrochemical enzyme-based glucose sensors, in this system no chemical compounds are consumed or formed, and the glucose-indicating hydrogel is not subject to the instability characteristics of enzymes[279-282].

This implantable CGM is indicated for continually measuring interstitial fluid glucose levels in adults with diabetes for period lasting between 3 to 6 months (latter in Europe).

A key part of the evidence allowing the licensing of the Eversense® system was the pivotal European PRECISE 1 study (and the subsequent US PRECISE II study). Cambridge was a study site within PRECISE1 study and I was the research fellow involved and responsible for all aspects of the study in Cambridge. As described below, there were some minor differences in the hardware of the system trialled compared with the final commercial product. The transmitter size was smaller in the trial. By design, this was not a study looking to see whether using the system improved glycaemic outcomes such as reduced hypoglycaemia but rather a study to examine efficacy and safety of this novel CGM system. In this chapter, I present data below from the Cambridge cohort and these form part of the (now published) PRECISE I publication [283].

4.2 Study Objective

The primary efficacy end point of the study was the accuracy of the Senseonics CGM System compared with a standard reference measurement (YSI glucose analyzer) as mean absolute relative difference (MARD) This was assessed during in-clinic visits.

The primary safety objective was to assess device related serious adverse events of this system.

Secondary end points included Error Grid Analysis and alarm performance. I also explored quality of life and a device-related questionnaire.

4.3 Study Design and Methods

This was a prospective, single-arm, multi-centre investigation enrolling 81 adults with diabetes mellitus across 6 sites in Europe subjects. Five of the 81 subjects were used for platform and procedure evaluation and five were designated for site training. The intent-to-treat analyses of the primary efficacy outcome thus included 71 participants. Below, I have presented data from the Cambridge cohort within this trial that I was personally responsible for studying.

4.3.1 *Participants*

Between July 3, 2014 and March 5, 2015, 10 adults with T1D were enrolled from the Wolfson Diabetes Endocrine clinic in Cambridge University Hospitals NHS Trust (Addenbrooke's Hospital), Cambridge as a part of multicentre study.

Inclusion criteria for the study were:

1. A clinical diagnosis of Type 1 diabetes more than 1 year from diagnosis

2. age 18 years or above
3. on insulin therapy
4. willing to comply to study with protocol requirements

Exclusion criteria for the study were:

5. history of severe hypoglycaemia as defined by American Diabetes Association[110] in preceding 6 months
6. diabetic ketoacidosis in the past 6 months
7. females who were lactating or pregnant or intending to become pregnant during the course of the investigation
8. any condition preventing or complicating the placement, operation or removal of the sensor
9. any skin condition that may affect sensor insertion or transmitter placement
10. any medical condition or illness that in the judgment of the investigator might interfere with the procedures, results or compliance during the course of this investigation, including anaemia, symptomatic coronary artery disease, unstable angina, myocardial infarction or stroke within 6 months of screening, uncontrolled hypertension (defined as systolic pressure >160 and diastolic pressure >100), congestive heart failure, any seizure disorder, renal failure defined as any prior dialysis or an estimated glomerular filtration rate (eGFR) below 60 mL/min per 1.73 m2 using CKD-EPI formula[284], history of cardiac arrhythmia
11. currently receiving any of the following therapies, or likely to need such treatment during the follow-up period of this study a) immunosuppressant

therapy b) chemotherapy for any form of cancer c) anti-coagulant therapy d) chronic systemic glucocorticoids (excluding topical, optical or nasal, but including inhaled e) antibiotic treatment for chronic infection (e.g. osteomyelitis)

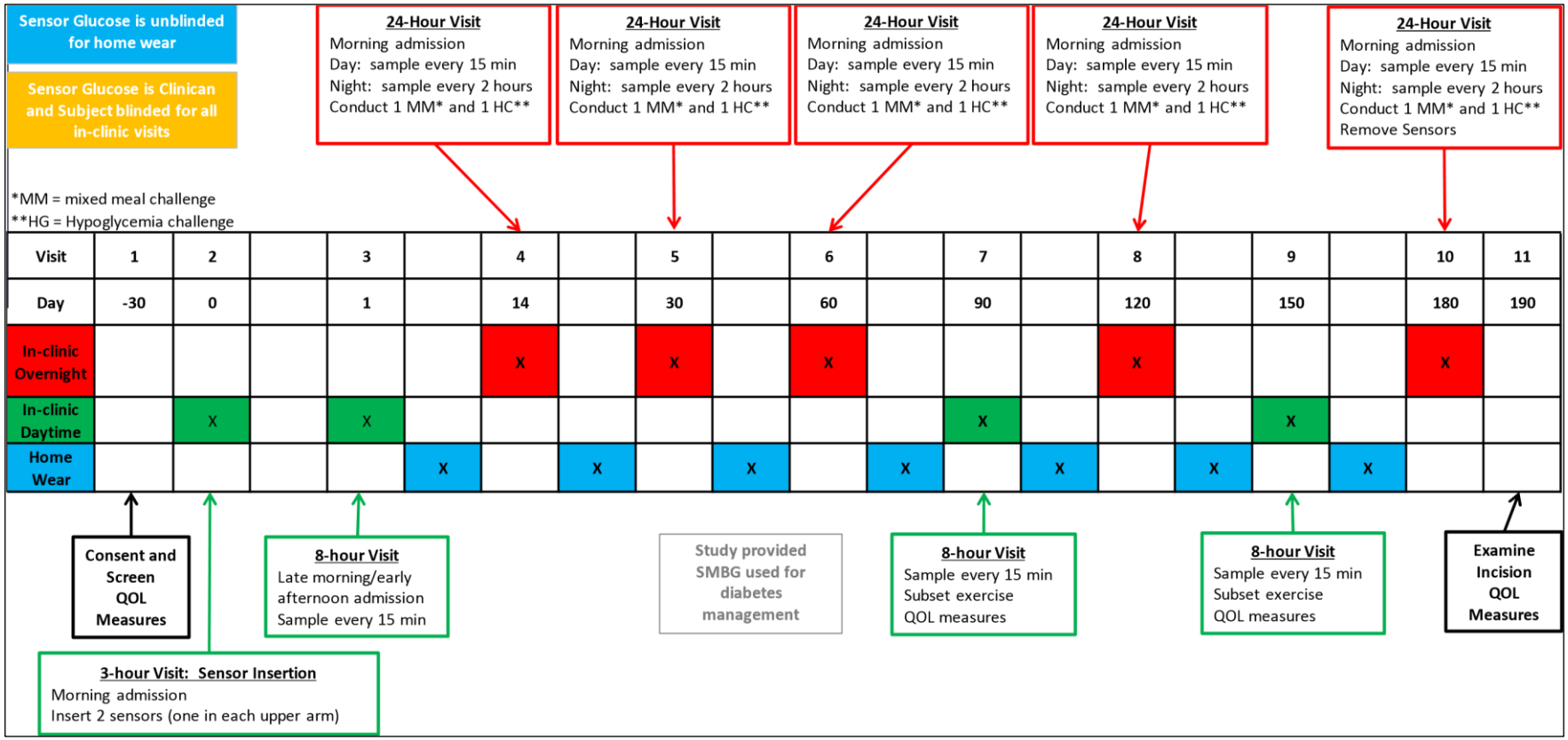
12. presence of any other CGM system with a transmitter located in either upper arm
13. any condition requiring or likely to require the use of magnetic resonance imaging (MRI)

4.3.2 *Study design and procedures*

This was a prospective, single-arm, multi-centre investigation.

Details of the study design have been summarised in subsequent sub section in this chapter and is also shown below.

Figure 4-1: Study Design & Procedure



The study protocol, participant information sheets and consent forms were approved by an independent Research Ethics Committee (NRES Committee Yorkshire & The Humber – Sheffield) and the study was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from each participant. The investigation included 11 in-clinic visits to the Wellcome Trust Clinical Research Facility (WTCRF) as described later.

4.3.3 *Senseonics CGM system*

Senseonics, Inc. (formerly Sensors for Medicine and Science, Inc. or "SMSI"), a medical device manufacturer headquartered in Germantown, Maryland, USA, developed a currently novel CGM system intended for measuring interstitial fluid glucose levels in adults with diabetes mellitus. The Senseonics CGM system measures interstitial fluid glucose levels continuously. Other commercially available transcutaneous continuous glucose monitoring devices currently have shorter operating lives of up to 7 days. The Senseonics sensor is intended to be inserted subcutaneously with no sensor part protruding from the skin and the operating life is intended to be up to 6 months or until the end of life indicator is reached.

The experimental Senseonics CGM System that I studied consisted of:

1. A small Sensor, (approximately 3.3 mm [0.130"] diameter x 15.7 mm [0.620"] length) which has a ring that elutes the steroid dexamethasone (Figure 4-2).
2. A battery-powered external Transmitter ("Transmitter") (Figure 4-3)

3. A mobile Medical Application for display of glucose information that runs on a Handheld Device [(HHD- Apple Inc. (iPod)](Figure 4-4).

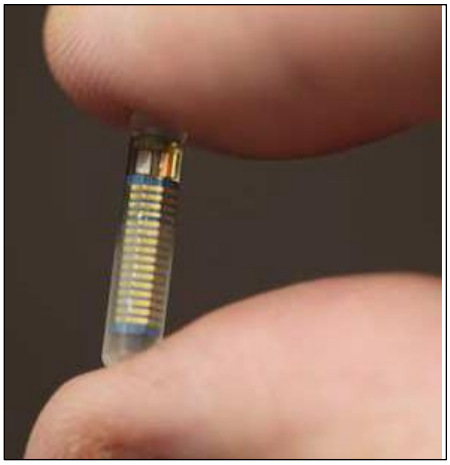


Figure 4-2 Sensor

Figure 4-3 Transmitter

Figure 4-4 HHD

Accessories to the system include

1. A blunt dissector (Figure 4-5) for creating a pocket under the skin.
2. An insertion tool (Figure 4-6) used to place the sensor into the pocket.
3. A transmitter, worn externally over the sensor insertion site (Figure 4-7), which powers the sensor and receives signals from the sensor across the skin.

Figure 4-5 Dissector

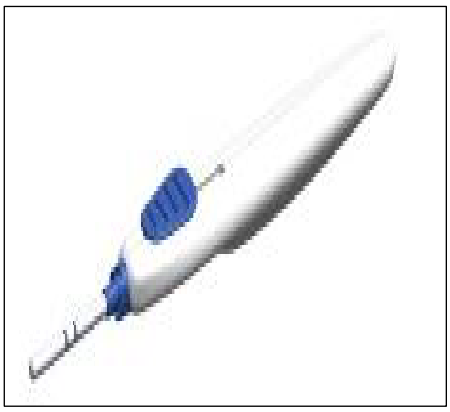


Figure 4-6 Insertion Tool

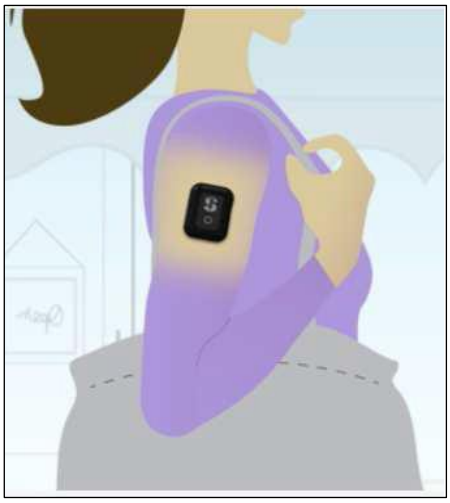


Figure 4-7 Transmitter worn over sensor

The sensor does not contain a battery or other stored power source; instead it is powered discretely, as needed, by a simple inductive magnetic link between the two components. Between readings, the sensor remains electrically dormant and fully powered down. At each query (set to a default of approximately every five minutes with a duration of 60 milliseconds) the transmitter first sends the power (via magnetic link) to activate the sensor, then uses this same magnetic link to capture the reading. This operational mode is commonly referred to as “speak when spoken to”[285].

The transmitter calculates the measured glucose value, the rate of change in glucose and all alarms that need to be alerted to the user. This information is then transmitted via bluetooth low energy with AES-CCM encryption to the Mobile Medical Application. The Mobile Medical Application displays glucose values, trends, and graphs as well as alerts. In addition, the wearer can be alerted where necessary through activation of the vibratory motor within the transmitter worn on the skin. The transmitter also contains digital storage media that enable extended data and profile information to be retrieved, downloaded to a computer or other electronic device, and reviewed by the physician or subject[285].

The limited efficiency of the inductive magnetic link limits the range for both power and signal “telemetry” to less than 2 centimetres. This intrinsic limitation of physics benefits the design by reducing the potential for crosstalk and interference between it and unrelated devices[285].

4.3.4 *Visit Schedules*

On a screening visit, following history, general physical examination and laboratory evaluation, the subject's eligibility was determined. Eligible subjects were then recruited for the study and invited for sensor insertion. Each subject had 2 sensors inserted on Visit 2 (Day 0) in upper arms with one sensor designated as primary sensor. Subjects were asked prior to insertion which arm they preferred as the primary sensor: self-reported dominant arm or self-reported non-dominant arm. The suggested primary sensor was in the non-dominant arm. Subjects then attended the Wellcome Trust Clinical Research Facility (WTCRF) for 8 further in-clinic visits, consisting of three 8-hour daytime study visits (Days 1, 90, and 150) and five 24-hour overnight visits (Days 14, 30, 60, 120, and 180) to evaluate sensor performance. Sensors were removed at the completion of Visit 10 (on Day 180) or earlier if the life of sensor was prior to day 180. Subjects then attended for a final follow up visit (Day 190) to evaluate healing of sensor removal sites. Between in-clinic visits, subjects wore the transmitter over the sensor at home except during transmitter charging, bathing or any other water activity.

Subjects and clinicians were display-blinded to all Senseonics CGM system glucose values during the in-clinic visits. The glucose alert/alarm features of the Senseonics CGM Mobile Medical Application were unavailable while the subjects were display-blinded. Of note, subjects were given access to the glucose data collected on the transmitter including glucose alerts/alarms, during home use i.e. for the majority of the study period. Of note, this was an important difference in PRECISE I compared with US-based PRECISE II study where data were blinded[283].

4.3.5 *24-Hour Overnight Visits*

Each overnight visit was approximately 24 hours in duration. Changes in the subject's concomitant medications, medical conditions, and the occurrence of medical adverse events since the last visit were reviewed. The subject's total diabetes and medication history was reviewed at each visit. An intravenous cannula was placed for blood sampling. In addition, blood was drawn for a Full Blood Count and dexamethasone measurement Full blood Count was done to check anaemia status as a safety management. The Full Blood Count was analysed in the clinical haematology laboratory at Addenbrooke's Hospital. Dexamethasone was measured to ensure there was no systemic absorption of the dexamethasone from the sensor. The samples for dexamethasone were stored at minus 80 degree for later measurement. Both transmitters were placed over the sensors at least 30 minutes prior to the first blood draw and sensor readings were taken throughout the visit. The secondary sensor was used and calibrated in 8hr visits only. Here I present primary sensor data.

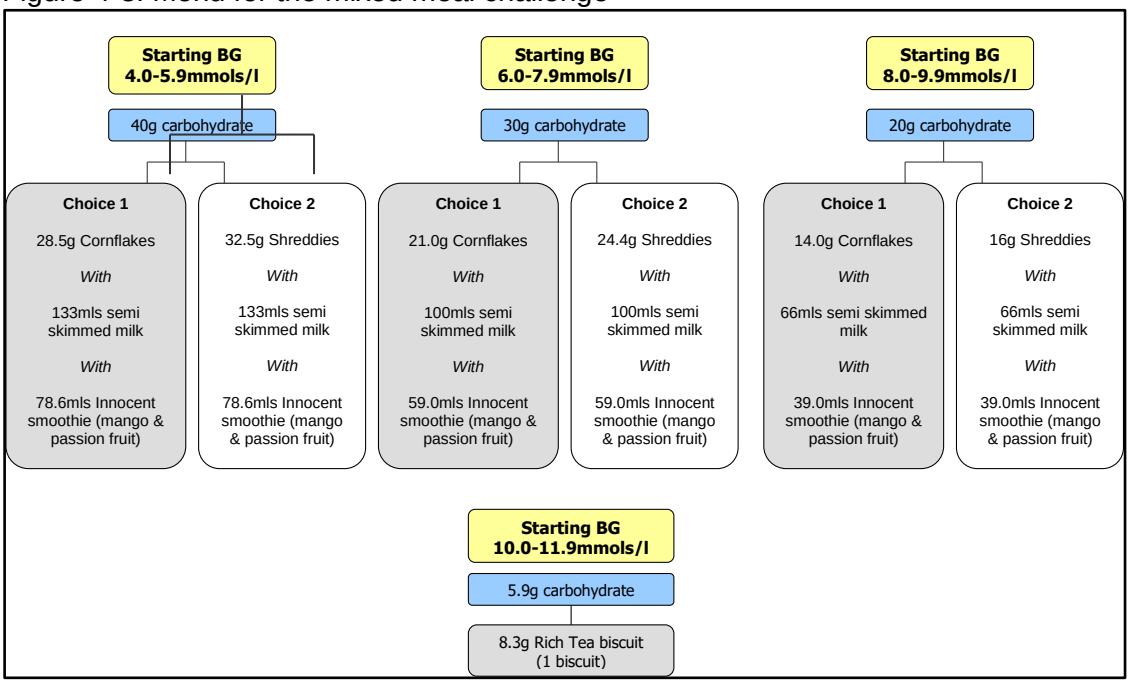
Venous blood samplings were performed for plasma glucose determinations using YSI in WTCRF. Blood sampling for plasma glucose were performed at 15-minute intervals during the daytime (7:00 – 23:00) and at 2-hour intervals during the night-time (23:00 – 7:00) for a total of approximately 24 hours. More frequent sampling was done at glucose values ≤4.44mmol/L to ensure subject safety. This sampling was no more frequent than every 5 minutes and did not last for longer than a consecutive duration of approximately 1 hour at glucose values ≤4.44mmol/L.

Subjects were required to perform at least two Capillary Blood Glucose testing (CBGT) for calibration. CBGT in the clinic were done concurrent with or prior to (within 5 minutes) blood draws taken for plasma glucose determinations.

A subset of subjects was exposed to a mixed meal challenge with the expectation of raising the glucose level to >11.10 mmol/L for >30 minutes followed by the induction of hypoglycaemia with an insulin bolus at ~4 hours after the start of the mixed meal challenge to reach a glucose level of ~3.06 mmol/L with subsequent carbohydrate rescue. The sequence of the mixed meal and hypoglycaemic challenge was determined based on clinical feasibility on the day of each visits.

Menu for the mixed meal challenge was designed after discussion with the dietitians and is attached in the Figure 4-8.

Figure 4-8: Menu for the mixed meal challenge



The Insulin doses for hypoglycaemia induction was cautiously decided based on participant's insulin: carbohydrate ratios; glucose correction factor and precedent physical activity as well. Participants used their own brand of bolus insulin. Some individuals needed more than one bolus insulin dose to achieve target glucose level of 2.9mmol/L or less. Investigators were encouraged to perform these challenges to allow a range of glucose values to be created but these were not mandatory. Subjects without defined insulin: carbohydrate ratios and glucose correction factors, as well as subjects and those at high risk for complications due to hypoglycaemia or hyperglycaemia did not undergo the mixed meal or hypoglycaemia challenges.

Free choice of meals and snacks was served meals to the subjects at other times of the day. Subject used their usual home insulin regimen for meal insulin coverage. In fact, mixed meal or hypoglycaemia challenges were not performed after the first few months of the study as the emerging experience was that sufficient glucose values in hypoglycaemia and hyperglycaemia range were experienced without participants needing to be subjected to experimental manipulation. Approximately 75 blood samples were obtained at each 24-hour overnight visit.

4.3.6 *8-Hour Daytime Visits*

Each daytime visit was approximately 8 hours in duration. The study procedure was similar to the 24 hours visit with the exception that venous sampling for determination of plasma glucose sampling period was for 8 hours during the day and subjects did not stay overnight. A free choice of meals and snacks was served, and the subjects used their own home insulin dosing parameters to cover the meals. Subjects did not undergo mixed meal or hypoglycaemia

challenges during 8-hour visit. Approximately 33 blood samples were obtained at each 8-hour visit.

4.3.7 ***Home Use Days***

Subjects maintained their normal daily activities during home use days and used the study-supplied glucometer to self-manage their diabetes. Participants were advised to check their home blood glucose profile at least 4 times a day using study supplied CE-marked ACCU-CHEK® Aviva self-monitoring blood glucose (SMBG) meter (Roche Diagnostics GmbH, Germany) using ACCU-CHEK Aviva test strips (distributed by Roche Diagnostics Ltd, West Sussex, UK). Subjects were provided with a transmitter to wear over the sensor at all times (except while charging, bathing or during any other water activities) from the end of insertion visit until the end of visit of the study. Subjects were provided with a handheld device [(HHD- Apple Inc. (iPod)] capable of running the Mobile Medical Application. Subjects were prompted by MMA for calibration by SMBG measurements (a minimum of 2 each day, approximately 12 hours apart and pre-meals) using the study glucometer. Subjects were trained in proper sensor calibration on the day of sensor insertion. Subjects were asked to charge the transmitter each day for 15 mins according to verbal and written instructions. Subjects were allowed to observe the glucose values displayed from the primary transmitter while successfully calibrated between in-clinic visits. Glucose alert and alarm features of the primary transmitter were functional. The study glucometer meter was used to document the time of each CBGT and incidents of hypo- and hyperglycaemia.

Incidents of hospitalization due to hypoglycaemia, hyperglycaemia and ketoacidosis were documented at the subsequent in-clinic visit.

4.3.8 *Sensor Calibration*

Briefly, the system required calibration of the sensor from the readings from their Accu-chek Aviva meter. The sensor calibration automatically moved through three phases: warm up, initialization, and daily calibration. The warm up duration was the first 24 hours after insertion and glucose values were not displayed. Initialization was performed a minimum of 24 hours after sensor insertion. Following the warm up phase, entering four successful calibration SMBG readings within 24 hours were required for successful completion of sensor initialization phase. Glucose values were displayed after the second SMBG reading in the initialization phase was successfully entered. Daily calibration updates required entering 2 calibration SMBG readings.

If the subject failed to wear the transmitter for more than 24 hours or was unable to enter a successful calibration SMBG measurement, then glucose was not displayed, and the patient would have to re-enter the initialization phase.

4.3.9 *Safety Management*

Baseline safety laboratory tests conducted at screening visits were: Full Blood Count, Coagulation profile, Electrolytes, Liver Function tests, Dexamethasone & Iron level. In each subsequent in- clinic visits, blood sample were taken for dexamethasone determination.

During in clinic visits, standard protocols were followed in the event of hyperglycaemia, hypoglycaemia and ketosis. Briefly, glucose levels <3.33 mmol/L were treated with carbohydrate and glucose levels >16.67 mmol/L were treated with correction insulin as appropriate per clinic standard-of-care.

In each subsequent visit, blood sample were taken for dexamethasone determination. Blood dexamethasone concentration was measured to ensure the dexamethasone from sensor was not absorbed systemically.

4.3.10 *Other Laboratory Tests*

HbA1c was measured at visit 3 (day 90) and Visit 10 (day 180) or earlier if the designated time for sensor removal was reached.

4.3.11 *Quality of Life Measures*

Quality of Life was assessed using validated health questionnaires at screening visit, visit 3 (day 90) and Visit 10 (day 180) or earlier if the designated time for sensor removal was reached. However, the CGM Impact Scale was not done at baseline but at the end of 3 months or earlier if the designated time for sensor removal was reached.

Subjects completed the following questionnaires:

4.3.11.1 **Beck Depression Inventory II (BDI-II)**

This contained 21 questions that were scored on a scale value of 0 to 3. Higher total scores indicate more severe depressive systems[286].

4.3.11.2 **Diabetes Quality of Life Measure (DQOL)**

This contained 46 questions that were scored on a scale of 1 to 5. Higher scores indicate higher quality of life[287].

4.3.11.3 **CGM Impact Scale & Device Acceptance questionnaires**

This is a scale containing 16 items [288] intended to gauge impact of CGM on various aspects including behavioural, cognitive and emotional domain.

Furthermore, 33 customised questionnaires were also developed with a view to assess user acceptance to this CGM system[289]. This was assessed at the end of the 3 months of the study or earlier if the designated time for sensor removal was reached.

4.3.12 *Sensor Insertion Procedure*

Two sensors were inserted in each subject's upper arms (Day 0) with the primary sensor inserted in the participant's self-reported non dominant hand unless participants preferred to use the dominant arm. All the sensors in Cambridge were inserted by me under local anaesthesia following aseptic precautions as per the hospital protocol policy. Study supplied insertion tools were used for the procedure. The sensor site was marked and documented.

4.3.13 *Sensor removal Procedure*

Sensors were removed on Visit 10 (Day 180) or earlier when the designated time for sensor life was reached. Sensor removal was done by me under local anaesthesia (except one- as described below) following full aseptic procedure as per the hospital protocol.

4.3.14 *Analytical methods:*

Arterialized plasma glucose was measured using a Yellow Springs analyser (YSI STAT Plus, Farnborough, UK, intra- assay coefficient of variation (CV) 1.5 % and inter assay CV 2.8 %).

HbA1c was measured with ion exchange high-performance liquid chromatography (G8 HPLC Analyzer, Tosoh Bioscience, CA, USA; inter-assay coefficients of variation 1.3% at 31.2mmol/mol, 0.8% at 80.5mmol/mol).

Dexamethasone was measured using a highly sensitive liquid chromatography –tandem mass spectrometry with a lower limit of detection of 2ng/mL[290].

4.3.14.1 Statistical analyses

Numerical and clinical accuracy outcomes were calculated using Sigma Plot version 9.0 (Systat Software, Inc., San Jose California USA) and statistical analyses were conducted using SPSS version 21(IBM Software, Portsmouth, United Kingdom). Data were compared using the paired t-test. Results are presented as mean±SEM. All p-values less than 0.05 were considered statistically significant.

Senseonics CGM System sensor accuracy was evaluated using data collected during in clinic use. Numerical accuracy was assessed by overall mean of absolute relative deviation (MARD) where ARD is the absolute relative difference of paired (within 5 minutes) Senseonics CGM system and YSI readings divided by the YSI reading for YSI readings. Main measured outcomes included mean ARD for overall pairs in all glucose range, pairs in the euglycaemic range (4.0–10.0mmol/l), hypoglycaemic range (≤3.9mmol/l) and hyperglycaemic range (>10.0mmol/l) stratified according to reference plasma (YSI) glucose measurements.

MARD was hierarchically calculated at successive monthly intervals up to 6 months.

The % of data points of the Clarke Error Grid was used to evaluate clinical accuracy. Clinical accuracy was assessed using the Clarke Error Grid Analysis (EGA) [291]. The EGA takes into account not only the difference between the system-generated and reference blood glucose values but also the clinical significance of this difference. The grid breaks down a scatterplot of a reference glucose meter and an evaluated glucose meter into five regions:

1. Region A contains values within 20% of the reference Sensor

2. Region B contains values that are outside of 20% but would not lead to inappropriate treatment

3. Region C contains values leading to unnecessary overcorrection in treatment

4. Region D contains values indicating a potentially dangerous failure to detect hypoglycaemia or hyperglycaemia

5. Region E contains values that could lead to errors in treatment of hypoglycemia for hyperglycaemia and vice-versa

Percent of measures in the clinical accuracy Zone A was estimated along with 95% confidence interval.

A Bland-Altman analysis plot was used to compare the limits of agreement between Senseonics continuous glucose sensor and YSI glucose values. This plot compared the difference in readings between the Sensor and YSI to the average of the two readings across the accuracy evaluation period.

4.3.14.2 **Alarm Performance (Low/High Alert/Alarm):**

An important usability and acceptability aspect of all CGM systems is whether the alarms function effectively and accurately, allowing detection of a blood

glucose that is out of range (particularly hypoglycaemia) without an unacceptable number of false alarms.

The glucose alert and alarm performance of the Senseonics CGM System was evaluated retrospectively on sensor data collected. For the alarm performance evaluation, the following definitions were used:

1. ***Hypoglycaemic event*****:** Two or more successive YSI measurements below the alarm threshold or one YSI measurement 0.33 mmol/L below the alarm threshold.
2. ***Hyperglycaemic event*****:** Two or more successive YSI measurements above the alarm threshold or one YSI measurement 6% above the alarm threshold.
3. ***True Threshold Alarm***: A threshold alarm that occurred ±30 minutes from the start of a hypoglycaemic or hyperglycaemic event.
4. ***True Alarm Rate****:* The percentage of time the glucose level was beyond the threshold and an alarm was activated.

   True alarm rate = events detected by true threshold alarms / total events x 100
5. ***Missed Alarm Rate****:*

   The percentage of time the glucose level was beyond the threshold and an alarm was not activated.

   Missed alarm rate = events not detected by true threshold alarms / total events x 100
6. ***False Threshold Alarm****:*

   A threshold alarm that occurred when YSI measurement within ±30 minutes was not beyond the threshold setting.

7. ***False Alarm Rate***: The percentage of time an alarm that occurred when the glucose level was not beyond the threshold setting.

   False alarm rate = false threshold alarms /total threshold events x 100.

Projected alarms occurred when the glucose value and the rate of change of glucose predicted the crossing of an alarm threshold at a future time determined by the sensitivity setting – the low sensitivity was 10 minutes, the medium sensitivity was 20 minutes, and the high sensitivity was 30 minutes. Projected alarms were designed to alert the patient to the possibility of a future high or low glucose event. The above performance parameters were estimated for low glucose (≤3.9 mmol/L) as well as high glucose (>10 mmol/).

4.4 Results

One Cambridge participant was withdrawn from the study, two weeks after the insertion of sensor, due to a medical reason unrelated to the study procedure or devices (suspected chronic fatigue syndrome). This participant's CGM data prior to being withdrawn were used for analysis of accuracy of CGM.

In Cambridge, the mean life of all sensors was 100±16 days (mean±SEM). Life of sensor reached 6 months in 2 participants. The average number of clinical sessions was 5.56±1.88 and number of days use was 105.8± 50.23. Duration of home use was 101.68±48.85 days (mean ±SEM). Data from all 10 participants were analysed (age 37.6±3.6 years, HbA1c 8.1±0.2% [65±2.6mmol/mol] mean±SEM).

Baseline demographics of Cambridge participants are outlined below. (Table 4-1)

Table 4-1 Baseline characteristics

	Adults (n=10)
Age (years)	37.6±3.2
Gender (male/female)	4 / 6
Insulin regime (CSII/MDI)	8/1
Body mass index (kg/m^2)	28.3±1.2
Haemoglobin A1c (%)	8.1±0.2
HaemoglobinA1c (mmol/mol)	65.0±2.6
Duration of diabetes (years)	17.4±2.8

Data shown are mean±SEM

4.4.1 *Sensor numerical accuracy*

The total number of days that participants spent between sensor insertion and sensor removal (or earlier if designated time of sensor reached) in Cambridge was 1044 days. The total duration of sensor use for the whole Cambridge cohort was 21,880 hours. This was equivalent to 96% of total study duration.

A total 313,448 sensor data points were collected during inpatient clinical visits. A total of 2431 sensor–YSI glucose pairs (within 5 minutes) were available for the analysis. The overall numerical sensor accuracy expressed as mean ARD was 11.8±0.2 % (Table 4-2). The mean ARD in the euglycaemia (4-10 mmol/l range) was 12.6±0.3 % (N = 1,434 pairs). Senseonics CGM System sensor accuracy as measured by mean ARD was lowest in the hyperglycaemic range [(>10.0 mmol/l) (9.8±0.3%, N = 935 pairs)]. The mean ARD was highest in the hypoglycaemic range [(≤3.9 mmol/l) (mean ARD 26.0±3.8%, N= 62 pairs)] & [(≤3.0 mmol/l) (mean ARD 59.4±3.8%, N= 6 pairs)]. However mean AD in the hypoglycaemic range was 0.9±0.1 mmol/l (≤3.9 mmol/l) & 1.6 ±0.8 (<3.0 mmol/l) Of note, sensor accuracy was significantly reduced in the 6th month of use, $p < 0.05$ (Table 4-3).

Table 4-2 Sensor accuracy for Cambridge participants for the whole glucose range and stratified according to reference glucose (YSI) during the clinic visits

	Whole range (1.1-27.8 mmol/l)	Euglycaemia (4.0 – 10.0 mmol/l)	Hyperglycaemia (>10.0 mmol/l)	Hypoglycaemia (≤3.9 mmol/l)	Hypoglycaemia <3.0 mmol/l)
N of glucose sensor - YSI pairs	2,431	1,434	935	62	6
Mean YSI glucose (mmol/l)	9.3±0.1	7.5±0.0	12.4±0.1	3.5±0.0	2.8±0.1
Mean AD±SEM (mmol/l)	1.0±1.0	0.7±0.0	0.5±0.0	0.9±0.1	1.6±0.8
Mean ARD±SEM (%)	11.8±0.2	12.6±0.3	9.8±0.3	26.0±3.8	59.4± 29.9

Data are mean± SEM

Table 4-3 Sensor accuracy for Cambridge participants for the whole glucose range and stratified according to month since transplant during the clinic visits.

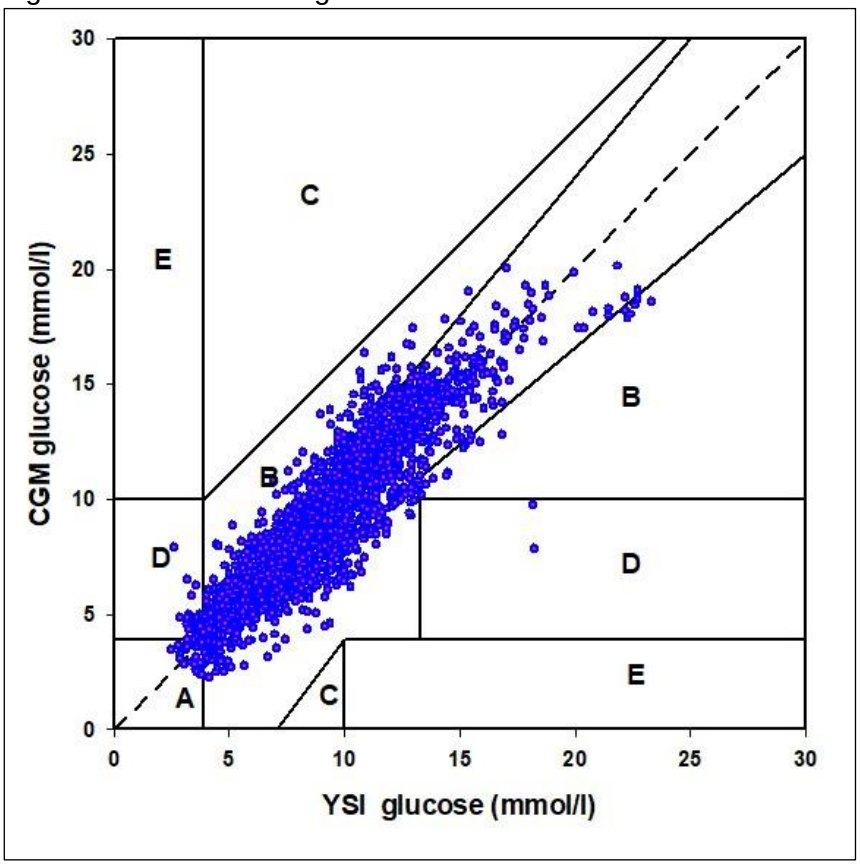
Month (s) since implant	Number*	ARD
1	10	10.6±0.3
2	9	11.1±0.4
3	5	11.5±0.5
4	4	12.8±0.6
5	2	9.5±0.9
6	2	21.2±0.4

Data are mean± SEM
*Number of sensors that were still functioning and had not reached end of life.

4.4.2 *Sensor clinical accuracy*

In Cambridge participants, the Senseonics CGM System sensor had 99.3% of measurements in CEG zones A + B [(A: 81.71%, B: 17.6%, C: 0.0%, D: 0.7%, E: 0.0 %) as shown in Figure 4-9.

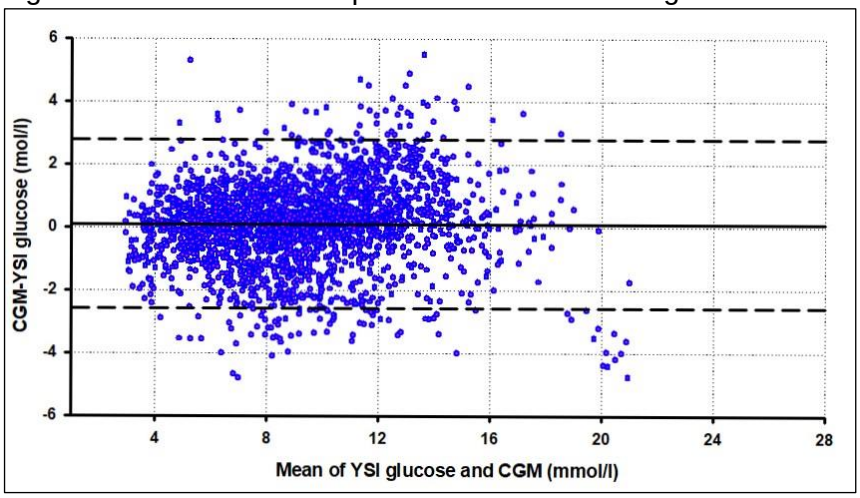
Figure 4-9. Clarke error grid of sensor and YSI



Solid black line represents the mean difference between the sensor and capillary glucose values; dashed lines indicate 1.96 x SD of the difference

Information related to sensor accuracy over the range of glucose values defined is shown in Bland –Altman in Figure 4-10

Figure 4-10: Bland-Altman plots of sensor and YSI glucose



Solid black line represents the mean difference between the sensor and capillary glucose values; dashed lines indicate 1.96 x SD of the difference

4.4.3 *Glycaemic control*

The study was not designed / powered to study changes in HbA1c. There were no significant changes in HbA1c during the study period. (Table 4-4).

Table 4-4. Comparison of change in HbA1c and QOL Health questionnaires at the end of study compared to baseline values

	Baseline	3 months	6 months/End of Study
HbA1c- mmol/l	8.2±0.3(N=10)	8.0±0.2(N=8)	8.2±0.3(N=6)
HbA1c -%(N=8)	65±3(N=10)	52±8(N=8)	65±3(N=6)

Data shown are mean±SEM

4.4.4 ***Hypoglycaemia***

Time spent in hypoglycaemia (<3.0 mmol/l) during the study period (home use) based on sensor glucose data decreased in the second month (0.8%) compared to the first month (1.1%). This was sustained until 3rd month (0.8 %) but rose in subsequent months (Table 4-5).

Performance of CGM in clinic alarm for hypoglycaemia threshold (glucose values ≤3.9 mmol/L) showed a True Detection Rate of 100 % with a false alarm rate of 43 % (Table 4-6). Alarm performance for glucose values below 3.0 mmol/l could not be analysed as alarm threshold was already set for higher glucose level of ≤3.9 mmol/L as a safety precaution.

Table 4-5. Percentage of time spent on hypoglycaemia over time based on Sensor glucose data during study duration (home and clinic use)

Months	Time spent on Hypoglycaemia (Glucose (<3.0 mmol/l)
1	1.1%
2	0.8%
3	0.8%
4	1.4%
5	1.8%
6	2.4%

Table 4-6 Hypoglycaemic alarms performance of CGM system during in clinic visit

Low Alarm Setting	True Detection Rate	Missed Detection Rate	False Alarm Rate
	% (n/N)	% (n/N)	% (n/N)
3.9 mmol/l	100 (6/6)	0(6/6)	43(15/35)

4.4.5 *QOL Health questionnaire*

QOL Health questionnaires at baseline, 3 months and 6 months / end of the study is shown in Table 4-7.

Table 4-7. Comparison of change in QOL Health questionnaires at the end of study compared to baseline values

	Baseline	3 months	6 months/ End of study
BDII	5±2.5	2.3±1.1	2.7±1.6
		N=9	N=6
DQOL	64±8	54±5	64±7
		N=9	N=6

Data shown are mean±SEM

Overall QOL score did not change significantly at 3 or 6 months compared with baseline (Table 4-7). This is perhaps not unexpected as this is a broad scale examining generic quality of life.

However, CGM had much positive impact on their perception of diabetes self-management. 67 % felt that they were more motivated to manage their diabetes. CGM had brought element of safety among participants with 83.3 % reporting feeling safer during exercise and 50 % during sleep. 67 % reported less fear of hypoglycaemia and same proportion were confident of avoiding serious hypoglycaemia with 50 % feeling safe whilst driving (Table 4-8).

Table 4-8 CGM Impact Scale at 3 months

		Things are much better	Things are slightly better	Neutral	Things are slightly worse	Things are much worse
1. Do you now feel more confident or less confident that you can control your diabetes?	2.00±0.26	16.7%	66.7%	16.7%	0.0%	0.0%
2. Do you now feel more or in control or less in control of your life and your diabetes?	2.33±0.21	0.0%	66.7%	33.3%	0.0%	0.0%
3. Do you now feel more hopeful or less hopeful that you can avoid long-term complications?	2.50±0.22	0.0%	50.0%	50.0%	0.0%	0.0%
4. Do you now feel more motivated or less motivated to keep up with your diabetes management?	2.17±0.31	16.7%	50.0%	33.3%	0.0%	0.0%
5. Is it now harder or is it easier to adjust your insulin doses correctly?	2.50±0.34	16.7%	16.7%	66.7%	0.0%	0.0%
6. Have your blood glucoses become more or become less of a “roller coaster”?	2.50±0.34	16.7%	16.7%	66.7%	0.0%	0.0%
7. Has your A1C improved or has it worsened?	2.50±0.34	16.7%	16.7%	66.7%	0.0%	0.0%
8. Do you now feel more free or less free to do the things in your life you really want to do?	2.67±0.33	16.7%	0.0%	83.3%	0.0%	0.0%
9. Do you now feel more safe or less safe when exercising?	1.83±0.31	33.3%	50.0%	16.7%	0.0%	0.0%
10. Do you now feel more safe or less safe about sleeping?	2.33±0.33	16.7%	33.3%	50.0%	0.0%	0.0%
11. Do you now feel more fearful or less fearful about hypoglycemia?	2.00±0.37	33.3%	33.3%	33.3%	0.0%	0.0%
12. Do you now feel more confident or less confident that you can avoid serious hypoglycemia?	2.00±0.37	33.3%	33.3%	33.3%	0.0%	0.0%
13. Do you now feel more safe or less safe while driving?	2.17±0.40	33.3%	16.7%	50.0%	0.0%	0.0%
14. Are your relationships with your family and friends now better or worse?	3.00±0.00	0.0%	0.0%	100.0%	0.0%	0.0%
15. Is your partner now worrying less or worrying more about sleeping at night?	2.33±0.42	33.3%	0.0%	66.7%	0.0%	0.0%
16. Are your friends and family now bothering you less or bothering you more about your diabetes?	2.50±0.34	16.7%	16.7%	66.7%	0.0%	0.0%

4.4.5.1 **Free Text Responses**

These were the free text responses from the participants about their view on this CGM system:

1. ‘‘I was very impressed with CGM system’’
2. ‘‘I liked it. It was useful, easy to use, easy to wear’’
3. ‘‘Thought it was excellent. Found it much more accurate than other CGM’’
4. ‘‘Very positive, Brilliant’’
5. ‘‘Good for wearability and being able to walk away from the iPod without losing data but inaccurate and failed early’’
6. ‘‘Very good product’’
7. ‘‘Some alarms need to be worked on as low sensor temperature happened quite a lot and wouldn't display glucose for a very long time. Other than that, loved it’’
8. ‘‘Calibration was awkward especially when the ‘low temperature’ meant it would not read BGs & therefore calibration’’
9. ‘‘Really liked the accuracy and the graphics and ease of current BG & trend’’
10. ‘‘Ipod/transmitter issue was frustrating and placing the transmitter was a bit fiddly’’
11. ‘‘It was awkward to analyse more than few hours of data, the way it calibrates low temperature & calibration issue’’
12. ‘‘High ambient light even with black arm band’’
13. ‘‘Brilliant once you get into routine of charging extra devices’’
14. ‘‘Alarms low sensor temperature would happen quite a lot’’
15. ‘‘Sometimes transmitter would get knocked on my arm. If the transmitter could be reduced in size, then the CGM system would be more appealing to me’’
16. ‘‘Mildly annoying – just the warning high light and cold’’
17. ‘‘Dislike having to scroll data rather than see days of data altogether’’
18. ‘‘Sensor too cold alarms, transmitter falling off during exercise’’

4.4.6 *Safety*

The CGM system was safe with no device related events. There was 1 report of mild inflammation post-extraction, but this did not result in a clinically significant infection. Dexamethasone level remained below level of detection in all the participants for all the visits. This confirmed that there was no systemic steroid absorption from the sensor.

One participant required surgical removal of sensor by surgeons as I failed to remove the sensor. This was done in WTCRF under aseptic precautions.

4.5 Discussion

The objective of the study was to assess the efficacy and safety of this novel implantable continuous glucose monitoring sensor.

The sensor had an accuracy similar to other current CGM systems with a MARD of 11.8 % for overall glucose values. MARD was higher in the hypoglycaemic range (26%) which is similar to other standard CGM[70, 72]. However mean AD was satisfactory in hypoglycaemic range at 0.9mmol/l. In our subjects, sensor accuracy was significantly worse during month 6. These data from the Cambridge cohort are comparable with the overall PRECISE I study findings

[MARD 11.6 % for overall glucose values and 21.7 % in hypoglycaemia range (defined as ≤4.2mmol/L in the PRECISE I paper)].

Importantly the sensor performed well on the clinically-weighted accuracy analysis. Specifically, there were no glucose values in Clarke Error Grid Analysis in Zone C (zone indicating risk of over-correction leading to hypoglycaemia). Further, there were no values in Region E (zone indicating potential errors in treatment of hypoglycaemia for hyperglycaemia and vice-versa).

Time spent in hypoglycaemia (below ≤3.0 mmol/l) was reduced within a month into the study period but this was not sustained beyond 3 months.

The accuracy of CGM systems in general have much improved in the last decade or so with latest CGM's MARD around 10 % as opposed to near 20 % MARD for earlier devices[72, 90]. For example, the first commercially available MiniMed CGM had MARD of almost 20 %. [60, 63, 70, 71]. The FDA recently approved a CGM device for non-adjunctive use (i.e. without the need to confirm this from SMBG) for insulin dosing with a reported MARD of less than 10 % [87]. However, there is an ongoing debate about whether this cut of value of 10 % MARD for a CGM device to be considered for non-adjunctive use is appropriate. Shapiro and colleagues have suggested that this is a flawed approach [69, 88]. However, Kovatchev et al. suggested that if a CGM device has MARD of 10 % or less, it would be feasible to use the device for insulin dosing based on a mathematical modelling study. The group went on further to conclude that increase of sensor accuracy below the 10% MARD threshold did not contribute substantively to better glycaemic outcomes. This however was based on assumption that the sensor produces reliable data without signal interruption or

loss of sensitivity[86]. It would be ambitious to believe that future CGM would completely eliminate errors in glucose measurement based on the way CGM measures interstitial glucose.

The CGM system appeared to have a positive impact in their self-perception of diabetes with more than 4/5th reporting that they felt much safer during exercise and 2/3rd had less fear of hypoglycaemia and were confident to avoid serious hypoglycaemia having been on CGM for 3 months. The CGM in clinic alarm performed very well with True Detection Rate of 100 % for hypoglycaemia (blood glucose below 4 mmol/L).

The CGM system was used by the participants 96% of the time over the study period. This is highly encouraging, confirming acceptability of device by the users especially when compared with previous studies. For example CGM was used by 70% of the time only by participants in HypoCOMPaSS study.[225] Previous studies have shown that the benefit of CGM is highest compared to self-monitored glucose monitoring using finger prick method if used with a positive approach and if used most of the time [71, 80, 278].

Further, there was no serious procedure related adverse reaction except for minor skin inflammation which settled without intervention. This is important as previous implantable sensors have failed to reach the commercial market due to various physical factors – acceptability issues, procedure related problems [64, 281, 292-296].

An advantage of implantable CGM is that it does not require weekly self-insertion of a sensor compared to currently available transcutaneous sensor

system. This also has the added advantage of not requiring users to remove the sensor as detaching transmitter alone was enough when not using the system, allowing greater flexibility for users. This also minimises skin and adhesive issues faced by people using transcutaneous CGM [278].

Transcutaneous Real-time CGM systems such as Dexcom G4, MiniMed Sof-Sensor, Medtronic Enlite and also Flash FreeStyle® Libre (Abbott Diabetes Care, Inc., Alameda, CA, USA) that rely on electrochemical sensors are susceptible to varying degrees of interference from several medications including acetaminophen, salicylates, and ascorbic acid[75, 76]. This interference is dependent on the voltage applied to the sensor electrode and also on the sensor membrane[75].

Eversense® CGM sensor uses non–enzyme based fluorescent polymer to measure glucose and does not contain an electrode[279]. In a study, substances such as acetaminophen (paracetamol) and ascorbic acid that were frequently reported to interfere with enzymatic, electrochemical-based transcutaneous CGM systems did not affect Eversense® sensor readings. However, other less common drugs mannitol & tetracycline still did interfere with the Eversense® sensor readings [282]. The latest generation Dexcom G6 sensor was also shown to have non-significant interference with paracetamol[76].

There are drawbacks though. Firstly, and probably most importantly, this approach requires invasive technique to insert and remove the sensor. With commercialisation, this requires separate training for the clinicians performing

this. Also, of note, a specialist surgeon may be required to remove the sensor if fibrosis/scarring and/or sensors inserted too deeply.

There is also the concern about repeated use of sites which may scar. It is possible that other insertion sites might be tested and become licenced in the future with the development of a smaller transmitter in the current commercial model compared with the one used in these studies [278].

Other frustrations for participants were those who experienced frequent ‘low temperature’ alarm and connectivity issue when the transmitter failed to connect with the display. Improved sensor life and accuracy up to or beyond 12 months would likely change the cost- benefit ratio for many more people who might be prepared to undergo procedures.

Clarke Error Grid (CEG) has some drawbacks and have not been widely embraced[297]. The zone boundaries are not connected sequentially therefore a small change in glucose concentration reported by a sensor can move a result from the correct value zone A to the critical zone D, or vice versa[298]. So more refined Consensus Error Grid has been developed. This grid is based on a consensus of 206 international clinicians whose responses were analysed and processed into a risk error grid. Compared to Clarke Error Grid, Consensus Error Grid is continuous, and each point of the grid has its own risk value. This minimises the drawbacks of Clarke Error Grid [89, 297, 299, 300].

In summary, the Cambridge cohort data were consistent with those seen at other PRECISE I sites, showing that the Senseonics CGM system appears safe and shows accuracy comparable with current transcutaneous CGM system [71, 79]). Participants reported high device use satisfaction and were more confident of avoiding severe hypoglycaemia at the time of exercise and driving.

5 Exhaled Breath Volatile Organic Compounds during Hypoglycaemia in Type 1 Diabetes

5.1 Background

The rationale for this study was to extend my interest in looking for novel ways to detect hypoglycaemia to improve overall hypoglycaemia burden in people with T1D. Hypoglycaemia and/or fear of hypoglycaemia limit the ability to achieve intensive glucose lowering in diabetes, desirable to reduce complication risk [105].

It is desirable for people with T1D to be managed on intensive insulin therapy to lower average glycaemia and minimise/ prevent long term complications but also avoid severe hypoglycaemia at the same time. Hence, people with T1D (with support from their clinical teams / carers / families etc.) have the difficult task of achieving and sustaining a high level of self-management skills to maintain this balance.

In order to guide insulin therapy, people with T1D are advised and expected to monitor their capillary blood using a finger prick test several times a day as per the standard recommendation[231]. Unfortunately, these Capillary Blood Glucose testing (CBGT) are painful and inconvenient [232, 233, 301]. People with diabetes find these CBGT tests more painful than insulin injections[302]. Even children with T1D are expected to do these painful tests several times a day and so this may contribute to poor compliance to insulin therapy in this age group [303-305].

Despite decades of research exploring alternative to obtaining glucose values using finger prick test, currently people with diabetes (particularly those with T1D) are still required to monitor their glucose on a daily basis using these painful methods of capillary glucose measurement.

5.1.1 *Alternatives to Capillary Blood Glucose Testing (CBGT)*

Efforts have been made over the last few decades to develop a non-invasive alternative to CBGT. These methods measure glucose in various body fluids for example sweat, tears, urine, or across skin. Methods such as infra-red, Raman, photo acoustic and thermal spectroscopy, polarimetry, glucose-sensing contact lenses and electrical impedance have all been tried. Some of these are optical whereas others are non-optical based [306, 307].

Some of these technologies worked well *in vitro* but struggled when faced with an *in vivo* system. As an example, in 2002, the US Food and Drug Administration approved a reverse iontophoresis-based device (‘Gluco Watch’) as an adjunct to CBGT. However, there were major limitations of the device (costs, lag time, procedural hassle, local skin irritation and poor accuracy) and it was withdrawn from the market[308]. Pendragon Medical Ltd. (Zurich, Switzerland) developed a wrist-band-based glucose monitor called “Pendra in 2003 using bio impedance spectroscopy which was also withdrawn from the market because of poor reliability[309]. There has been some success with minimally invasive continuous glucose monitoring (CGM) devices which are self-implanted under the skin. However, some CGM still has significant drawbacks - it requires self-implantation of sensors under the skin, cost, the requirement for the patient to possess a degree of technological ability and, for most devices, the continued

need for calibration using CBGT [79, 185, 310, 311]. Despite having some supportive NICE guidance [62, 182, 312], current penetrance is very patchy across the UK [313]. Abbotts' recent commercially available Flash Glucose Monitoring FreeStyle 'Libre' and latest generation Dexcom CGM system have eliminated the need to calibrate by CBGT (and other systems in near future will probably also reduce this burden) but it is still painful system [95] and may perform less well at hypoglycaemia[191]. Endeavours to find suitable noninvasive methods /devices are still ongoing [306, 314].

Over the last few decades, breath Volatile Organic Compounds (VOCs) have been investigated as a tool for diagnosing and monitoring various disorders in human beings. VOCs in human may arise from both endogenous (e.g. respiratory surfaces and peripheral tissues from the metabolic by-products of various physiological and metabolic pathways[236]) and exogenous sources (breathed in environmental air). Some inhaled VOCs, once absorbed into the bloodstream, may undergo complete or partial enzymatic metabolism, altering the ratio between inhaled and exhaled concentrations [236, 315]. For example, 80% of ethyl benzene is metabolized by hepatic enzymes, leading to exhaled concentrations that are substantially lower than inhaled air [236, 315] . Acute metabolic changes may also modify this inhaled/exhaled ratio linking the exhaled levels to specific metabolic events. As an example, a reported study observed a 2-fold increase in exhalation of aromatic compounds (e.g. ethyl benzene) during experimental hyperglycaemia. This could have been explained by an increase in glucose load and blood flow to the liver inhibiting enzymatic catabolism of several VOCs, resulting in greater fractions of these compounds being exhaled [235, 315]. The internal lung surface has also been reported to

modify or generate some of the VOCs which in turn affect the exhaled gas mixtures [236, 316].

5.1.1.1 **VOCs and diabetes**

To date, there has been some limited research interest in some of the VOCs shown to be associated with diabetes. Pulmonary vasculature and lung function are thought to be impaired in people with diabetes due to thickening of epithelial and endothelial capillary basement membrane [317, 318]. Furthermore, the binding of advanced glycation end products to their receptors (RAGE), which occurs in the lung in healthy subjects and significantly increases in diabetes, further increases reactive oxygen species production, and promotes fibrotic growth factors [318-320]. These factors could potentially interfere with quantification and interpretation of various VOC in diabetes.

A small number of VOCs that could potentially be used as non-invasive diagnostic tools in diabetes, have been reported in the literature [235, 316, 321-326]. Various studies have suggested a correlation between some specific VOCs and blood glucose. This however has not been convincingly and consistently replicated in other similar studies.

Key VOCs reported to be associated with diabetes in the above mentioned studies are acetone, isoprene, ethanol, methyl nitrate, ethyl benzene and propane. I have briefly discussed each of these below.

A. *Ethanol*: Ethanol is a product of **glucose fermentation by gut flora** and is affected by blood glucose level. A study by Kulikov et

al. found that light hydrocarbons like ethanol were raised in the exhaled breath of women who had risk factors for T2D [324, 327].

B. *Ethyl Benzene*: This is an aromatic hydrocarbon, **exogenous in origin** and is partly metabolized by hepatic enzymes. Hence, exhaled ethyl benzene could conceivably be affected by liver function and blood flow change during hyperglycaemia [235, 236, 324].

C. *Methyl Nitrate*: This is an alkyl nitrate is commonly found in **atmosphere**. Analysis of exhaled breath in children with T1D during euglycaemia or “spontaneous hyperglycaemia” revealed a strong correlation between the kinetic profiles of plasma glucose and exhaled methyl nitrate [328]. Exhaled methyl nitrate has been suggested to be a marker of oxidative stress [325, 329]. Methyl nitrate may be a reflection of metabolic changes due to hyperglycaemia, rather than hyperglycaemia *per se* in people with T1D [328].

D. *Acetone:* Ketone bodies (*b-hydroxybutyrate, acetoacetate)* are **produced by the liver** during fatty-acid metabolism, and are used as an energy source when glucose is not immediately available. Acetone is derived by the decarboxylation of acetoacetate and the dehydrogenation of isopropanol[330]. In a study, intake of glucose in healthy people resulted in reduction of breath acetone[234]. Linear correlation of exhaled acetone with the plasma glucose in people with T1D has been reported but this correlation did not seem to be robust in T2D [236, 331, 332].

E. *Isoprene***:** It is one of the most commonly studied VOCs to understand and predict underlying metabolic activity because it is easily measurable and also found to be sensitive to underlying metabolic and physiological changes [333]. It is the most abundant biogenic hydrocarbon emitted by earth's vegetation i.e. **present in the atmosphere** and is also a major hydrocarbon **endogenously produced by mammals** [334]. It is an unsaturated hydrocarbon and chemically known as 2-methyl-1, 3-butadiene. This has a molar mass of 68.11g/mol and boiling point of 34.1 C [335]. Insight into origin of isoprene is based on *in vitro* studies from rodent liver [336, 337]. In human beings it has been suggested that liver may be the source of isoprene based on indirect evidence from statin studies[337]. In human beings the main source of isoprene has been reported to be derived from the cholesterol synthetic mevalonate pathway [337] in which acetyl-CoA is converted into mevalonate which then is transformed into dimethylallyl pyrophosphate (DMPP). *In vitro* study of rat hepatocytes has suggested that isoprene is derived from acidic decomposition of DMPP [336, 337].

The mevalonate pathway has also shown to be linked to cell proliferation & DNA replication further strengthening the potential benefit of exhaled isoprene to monitor various metabolic pathways [333, 338].

Whilst some groups report individual VOCs correlating with glucose in experimental setting, others report the need for more than one exhaled compound to generate a “fingerprint”. For example, the Galasseti group observed that glucose correlated significantly [correlation coefficient of 0.91 (range r = 0.70–0.98)] when ethyl benzene, methyl nitrate, ethanol and acetone when combined together in a prediction model in a glucose tolerance study [234].

There are anecdotal reports of behavioural patterns in dogs in response to glycaemia in human beings, with rescue dogs being trained to detect hypoglycaemia in their owners with T1D [339]. Dogs may be alerted in many ways, but at least in part in human may be by some of the exhaled VOCs in breath.

In summary, a niche exists for alternative non-invasive cheap and convenient methods for monitoring changes in blood glucose in diabetes. Any such noninvasive methods would be extremely beneficial in children and could be used by care-givers or parent in certain scenarios for example while children are asleep or when they are non-compliant.

5.2 Study Objectives

The primary objective of the study was to examine changes in VOCs under controlled experimental hyperglycaemia and hypoglycaemia. I hypothesized

that I would see changes in VOCs at different blood glucose levels, particularly hypoglycaemia.

5.3 Study Design

5.3.1 *Participants*

Adults aged 18 or above with a clinical diagnosis of T1D of at least 6 months duration were recruited from the Wolfson Diabetes Clinic at Cambridge University Hospitals Foundation Trust. Smokers or those with chronic lung conditions were excluded from the study. There were no other exclusion criteria. People with history of Diabetes Keto Acidosis, SH, IAH or those with poor glycaemia were able to take part in the study. An independent research ethics committee approved the study in advance and all subjects provided written informed consent.

5.3.1.1 **Insulin Clamp studies**

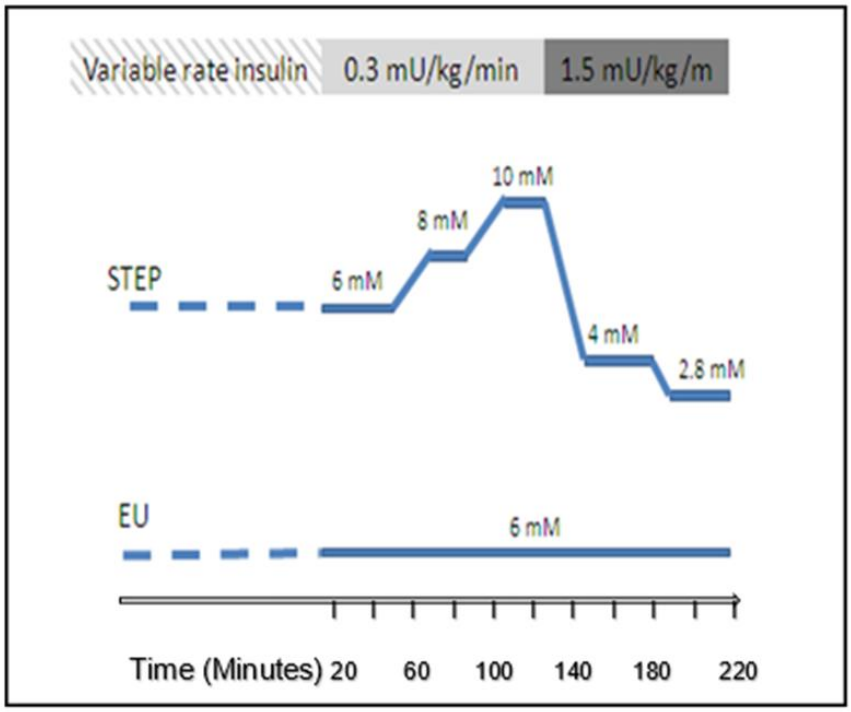
Participants were randomised to 2 clamp study days using a locally generated random-number computer code in this single blinded cross over study design. Participants were studied on 2 separate days at least a week apart.

Prior to each study day, I provided participants with clinical advice on insulin dosing to prevent hypoglycaemia during the 24 hours prior to study. Participants fasted from midnight and were admitted to the Wellcome Trust Clinical Research Facility at 7AM and received variable rate intravenous insulin to stabilize glucose before starting the clamp study at 10:30 AM.

For the clamp study, a primed continuous intravenous infusion of soluble human insulin (Actrapid, Novo Nordisk, Crawley UK) was run at 0.3 mU/Kg/min for 120 minutes then increased to 1.5 mU/kg/min for the final 140 minutes). In parallel, a 20% dextrose infusion was adjusted according to arterialized plasma glucose measured every 5 to 10 minutes (Yellow Springs Instrument 2300 STAT Plus™ Glucose & Lactate Analyzer), sampled from a retrograde cannula on the dorsum of the hand warmed to 50-60 °C. The clamp activity is summarized in the Figure 5-1.

On one study day (STEP), plasma glucose was raised sequentially in 40-minute steps for 120 minutes (targets 6.5, 8.5 and 10.5 mmol/L). After increasing insulin infusion to1.5 mU/kg/min, plasma glucose was then lowered to 4.5 and 2.8 mmol/L (60 minute and 40 minute step respectively). On control days (CON), procedures were identical, except that plasma glucose was maintained throughout at euglycaemia level with target glucose of 6mmol/L (Figure 5-1).

Figure 5-1: Clamp Study design - Insulin Infusion and target plasma blood glucose on both study days



Breath was sampled every 20 minutes in two 1.1 litre breath bags (Fischer Analysen Instrumente GmbH) as shown in Figure 5-2. To minimize dead space sampling, subjects held their breath for a count of 3 seconds and partially exhaled before breathing fully into the breath bags. Breath Bags were sealed immediately. Sealed breath bags were transported to Oxford Medical Diagnostics, Oxford on the day of each clamp study.

Breath samples collected in breath bags were sealed and analysed within 24 hours of each study days. From previous literature, we identified isoprene, acetone, methyl nitrate, ethanol, ethyl benzene and propane as candidates for measurement. We also measured oxygen and carbon dioxide. Samples of ambient air from the clinical research facility were also taken for analysis.

Plasma samples were taken every 20 min for the measurement of B-Hydroxybutyrate and every 40 min for measurement of glucagon and insulin. In addition, at every 20 mins interval point of care ketone (β-hydroxybutyrate) was measured using Abbott's FreeStyle Optium Ketone meter using FreeStyle Optium B –ketone test strips.

Figure 5-2: Breath Bag into which breath samples were collected

On each day, the total clamp study period was 220 minutes aiming to finish at 2PM when participants were given a meal and recommenced on regular subcutaneous insulin regimen. Participants contacted me a day after clamp

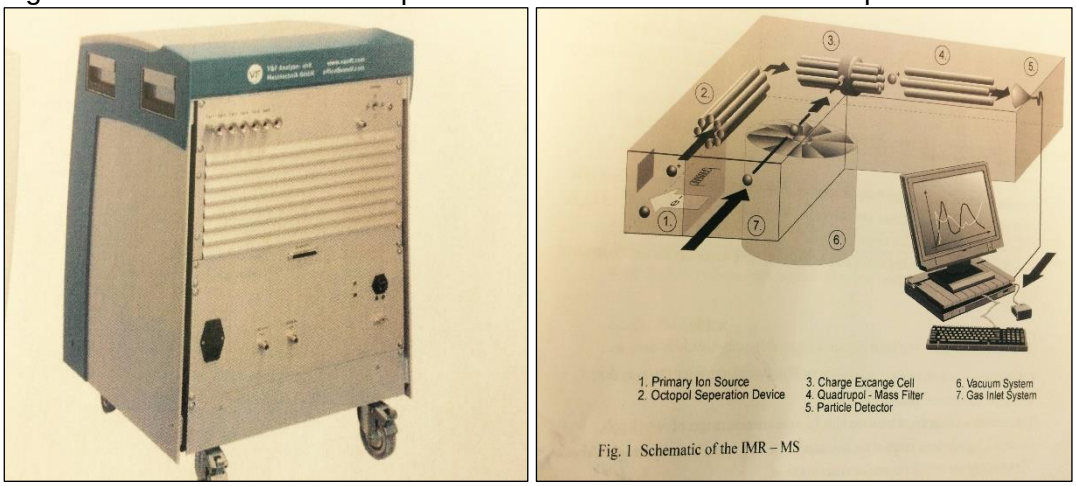
procedure and I ensured they had safe blood glucose and also provided with further advice regarding insulin adjustments if necessary.

5.4 Analytical Methods

5.4.1 *Breath samples*

Breath samples were analysed by collaborators at Oxford Medical Diagnostics within 24 hours of each clamp study. VOCs (isoprene, acetone, methyl nitrate, ethanol, ethyl benzene and propane) were measured using Soft-ionisation mass spectrometry (V&F Airsense Compact Ion-Molecule-Reaction Mass Spectrometer)[340] shown in Figure 5-3. This was measured by a researcher blinded as to clamp study order and glucose values. Mean of 2 values for VOCs obtained at each time point was used for the purpose of analysis.

Figure 5-3: V&F Airsense Compact Ion-Molecule-Reaction Mass Spectrometer



5.4.2 ***Blood Samples:***

Arterialized plasma glucose was measured in real time using Yellow Springs analyser (YSI STAT Plus, Farnborough, UK, intra- assay coefficient of variation (CV) 1.5 % and inter assay CV 2.8 %).

Insulin, Glucagon and b-OH Butyrate was measured at Core Biochemical Assay Laboratory (CBAL), Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK. All samples were measured in the same batch.

Plasma Insulin samples were assayed on a Diasorin Liaison XL automated immunoassay analyser using a one-step chemiluminescence immunoassay (Intra-assay CV 3.0% at 69.6 pmol/L, 2.0% at 649.8 pmol/L and Inter –assay CV 11% at 34 pmol/L, 7.0% at 135 pmol/L, 6.7% at 365 pmol/L & 5.9% at 1204 pmol/L).

Mercodia (Uppsala Sweden) Glucagon ELISA immunoassay was used for the quantification of glucagon (Intra-assay CV 5.1% at 10.5pg/ml; 3.6% at 18.2pg/ml; 3.3% at 76.7pg/ml and Inter -assay CV 9.5% at 70.3pg/ml, 9.5% at 202.7pg/ml).

Stanbio B-Hydroxybutyrate Liquicolour kit (Texas, USA) was used to measure Beta Hydroxybutyrate (Intra-assay CV 1.7% at 290 µmol/L, 1.4% at 1090 µmol/L, and Inter-assay CV 4.6% at 195µmol/L, 6.2% at 528µmol/L & 3.1% at 958 µmol/L).

Where glucagon and B-Hydroxybutyrate were below lower level of detection (4.5 pg/ml and 50 µmol/L respectively), lowest measurable values were used.

5.4.3 *Statistical Considerations*

Powering for isoprene from previous work from the host lab, assuming an SD of 280 ppb, a sample size of 8 gave 80% power at a significance level of 0.05 to detect a difference of 80 ppb between STEP and EU [326]. We aimed to complete 8 paired studies.

Raw VOC values were adjusted for exhaled CO_2 (to 5%) to correct for breath sample contamination with airways dead-space. To examine the effects of altering plasma glucose on breath, VOC values obtained on CON days were subtracted from those obtained at identical times on STEP days. To look specifically for a biomarker of low blood glucose, VOC during hypoglycaemia (2.8 mmol/l on step days) were compared with values from non-hypoglycaemia range. Two sample t test was used to compare the values.

Spearman correlation test was used to examine correlation between plasma glucose and VOCs (STEP – CON) across the range of experimental glucose values.

Data were analysed using IBM SPSS Statistics for Windows, Version 22.0. (Armonk, NY: IBM Corp) and p value less than 0.05 was considered statistically significant. All data are presented as mean with SEM.

5.5 Results

We recruited 9 participants. One participant (male) sadly died at home with a witnessed nocturnal severe hypoglycaemic episode (unrelated to the study) 4 weeks after the first arm and before completing the second clamp of the study. Data are shown for remaining 8 participants who completed both arms of the study.

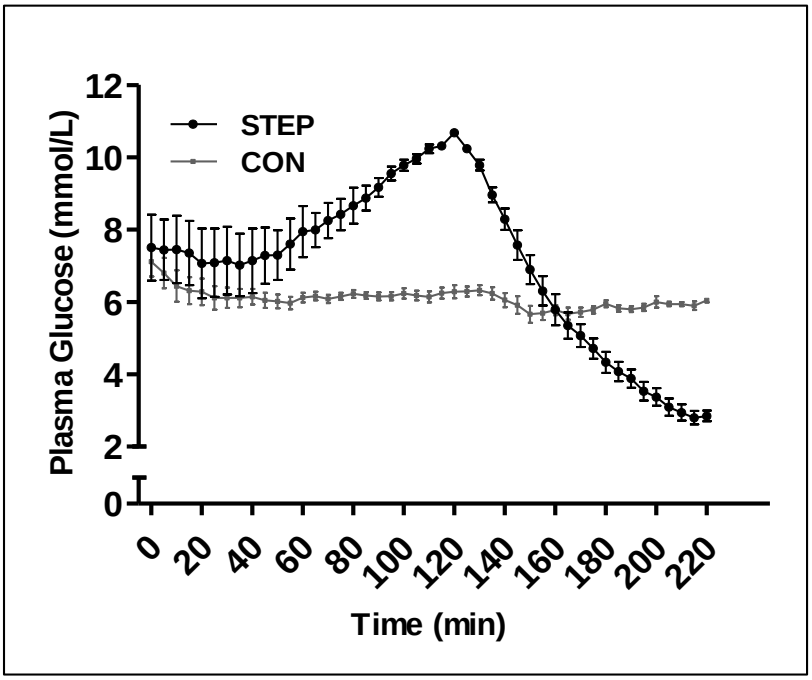
5.5.1 *Baseline characteristics:*

All were females, mean ±SEM (range) for age was 46.3± 4.8 years (19 to 60) years); duration of diabetes 22.9± 6.9(2 to 59 years); weight 72±4.9 kg (53.6 to 90 kg). All but 1 used insulin pump therapy.

5.5.2 *Plasma Glucose & Dextrose infusion Rates (DIR)*

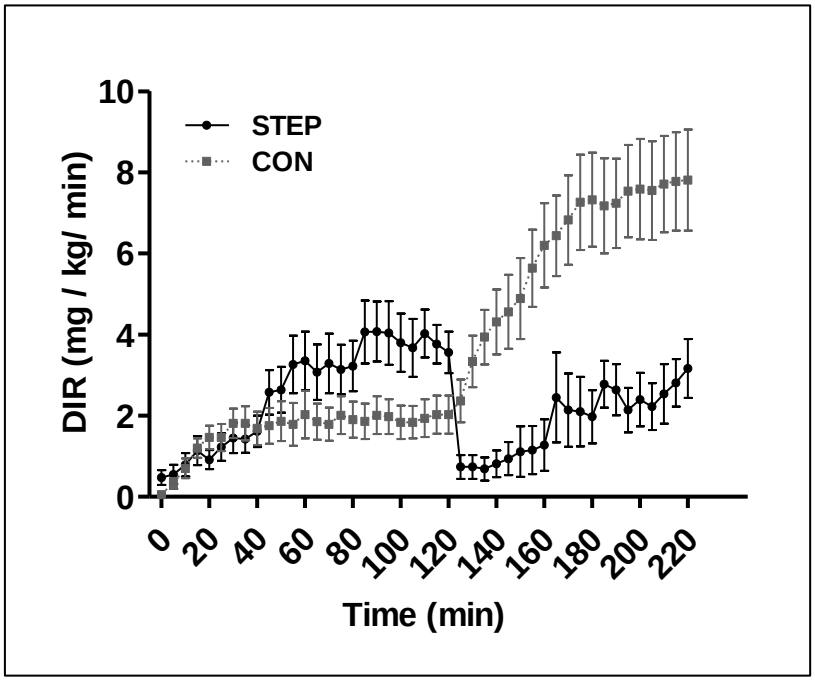
Plasma glucose was progressively altered on STEP days (7.1±0.8, 8.7±0.4, 10.7±0.1, 4.3±0.3 and 2.8±0.1 mmol/L) and maintained on CON at 6.2±0.1 mmol/L (Figure 5-4). As anticipated, more dextrose was needed during STEP studies during hyperglycaemic steps and less during hypoglycaemic phase (Figure 5-5). Plasma Insulin levels were similar on both study days (Figure 5-6).

Figure 5-4: Plasma Glucose during studies



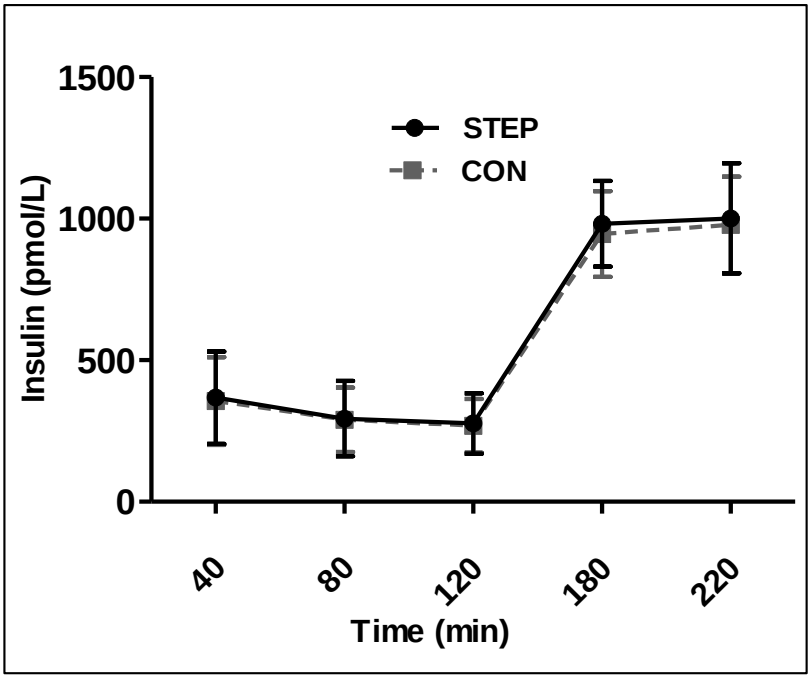
Data shown are mean ±SEM

Figure 5-5 : Dextrose Infusion Rate (DIR) during studies



Data shown are mean ±SEM

Figure 5-6 : Plasma Insulin levels during studies



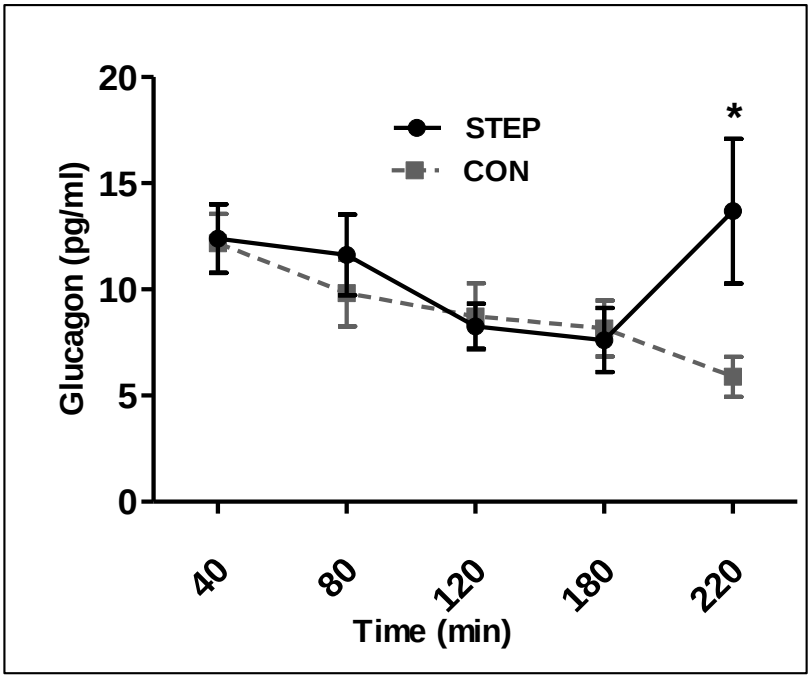
Data shown are mean ±SEM

5.5.3 *Plasma Glucagon & B-Hydroxybutyrate during studies*

5.5.3.1 **Plasma glucagon**

Plasma glucagon levels were similar on both study days at non-hypoglycaemia level. However, there was a small statistically significant (but probably clinically insignificant) rise in glucagon at hypoglycaemia (13.7 $\pm$ 3.4 *vs* 5.9$\pm$0.9 pg/ml, P 0.025) as shown in Figure 5-7.

Figure 5-7: Plasma Glucagon levels during studies

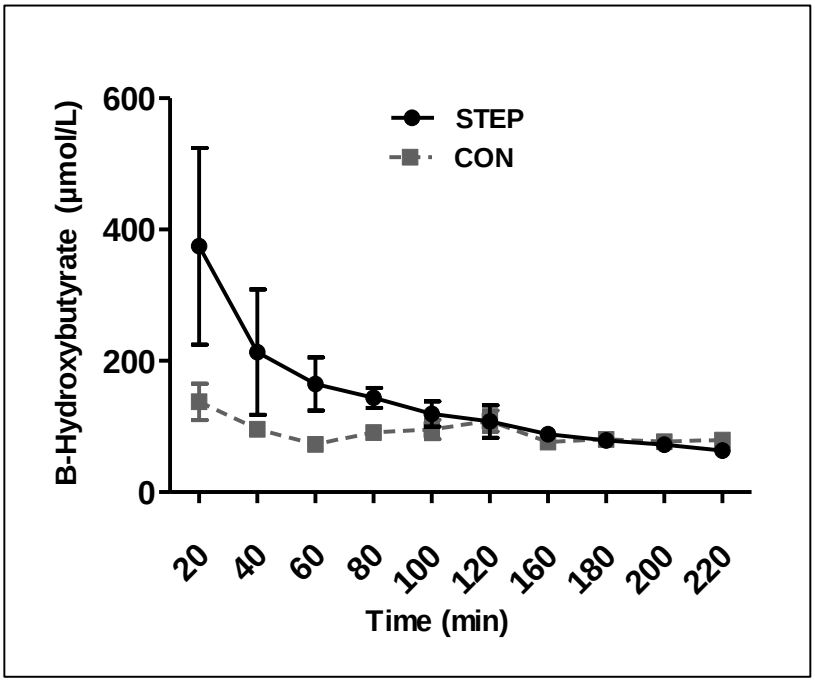


Data shown are mean ±SEM
*2 sample t test at 220 min STEP vs. CON measures

5.5.3.2 **Plasma B-Hydroxybutyrate**

Plasma B-Hydroxybutyrate levels were similar on both study days at all glucose values. As anticipated, B-Hydroxybutyrate gradually and inexorably fell during the insulin infusions as shown in Figure 5-8.

Figure 5-8 Plasma B-Hydroxybutyrate levels during studies



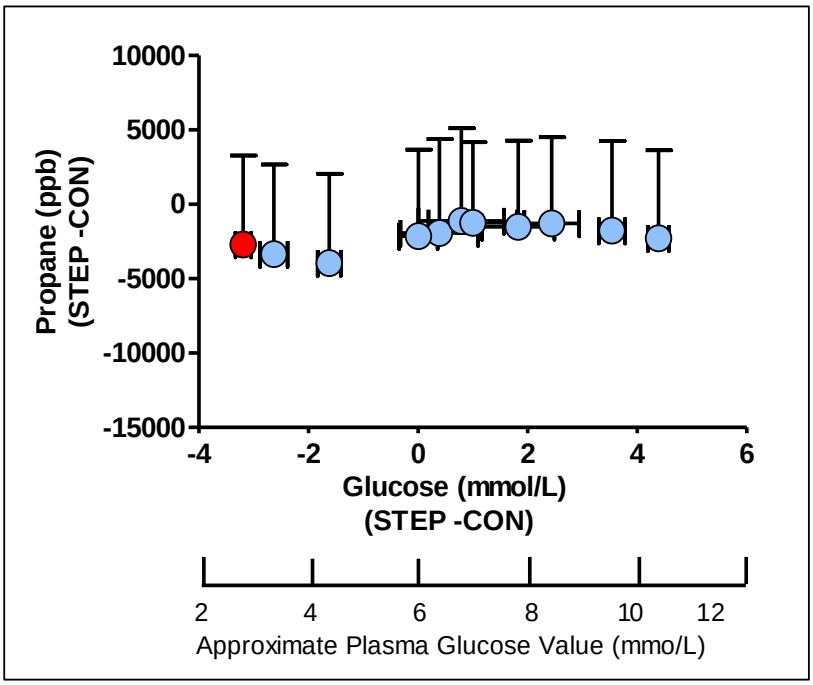
Data shown are mean ±SEM

5.5.4 *Exhaled VOCs during studies*

5.5.4.1 **Exhaled Acetone, ethanol , methyl nitrate, ethanol, ethyl benzene and propane**

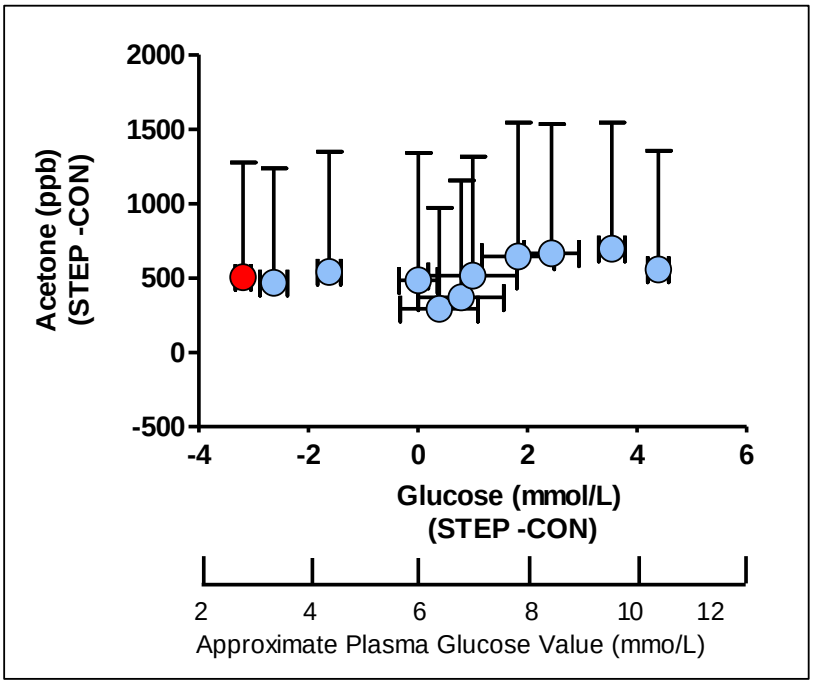
There was no obvious correlation between exhaled propane, acetone, methyl nitrate, ethanol & ethyl benzene and plasma glucose across the range of experimental plasma glucose values as shown in Figure 5-9 to Figure 5-13.

Figure 5-9: Exhaled Propane during studies



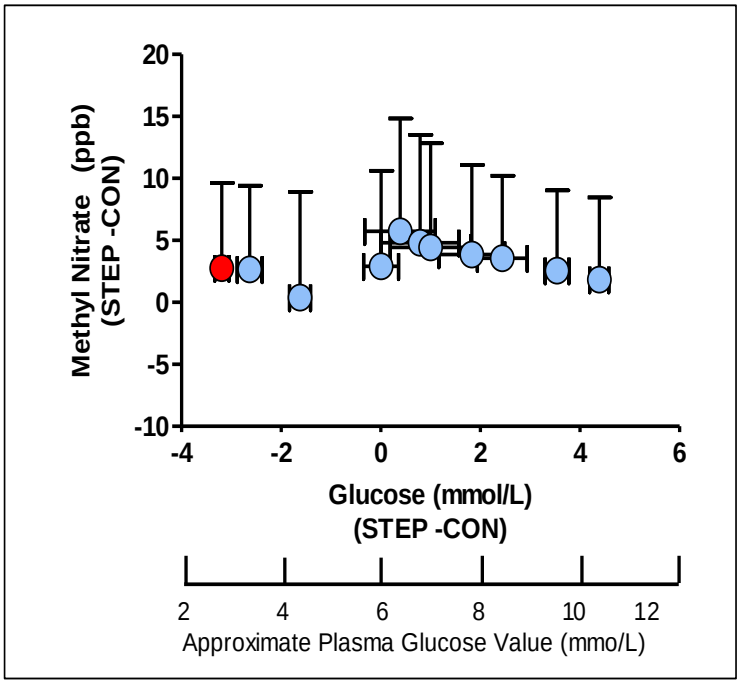
Data shown are mean ±SEM

Figure 5-10: Exhaled Acetone during studies



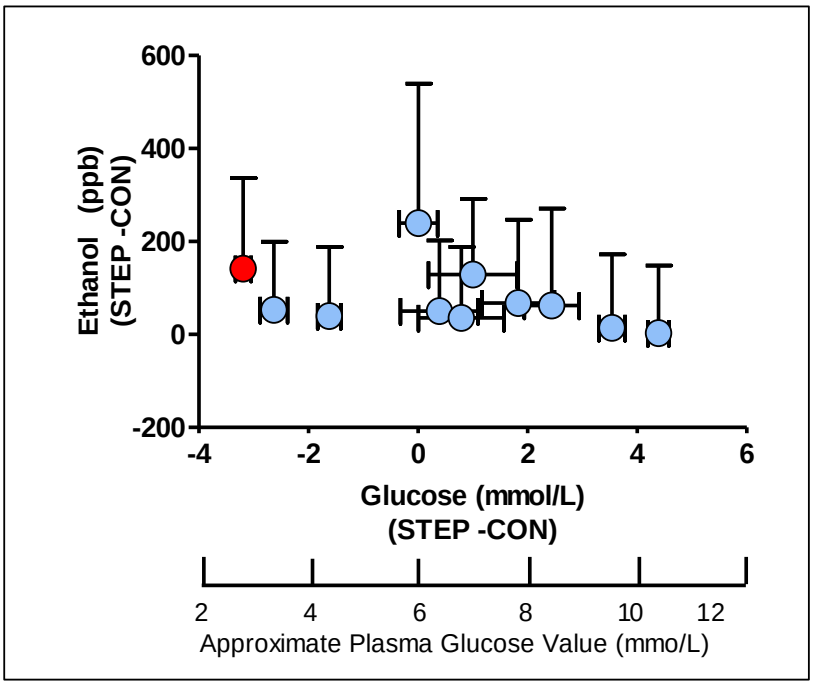
Data shown are mean ±SEM

Figure 5-11: Exhaled Methyl Nitrate during studies



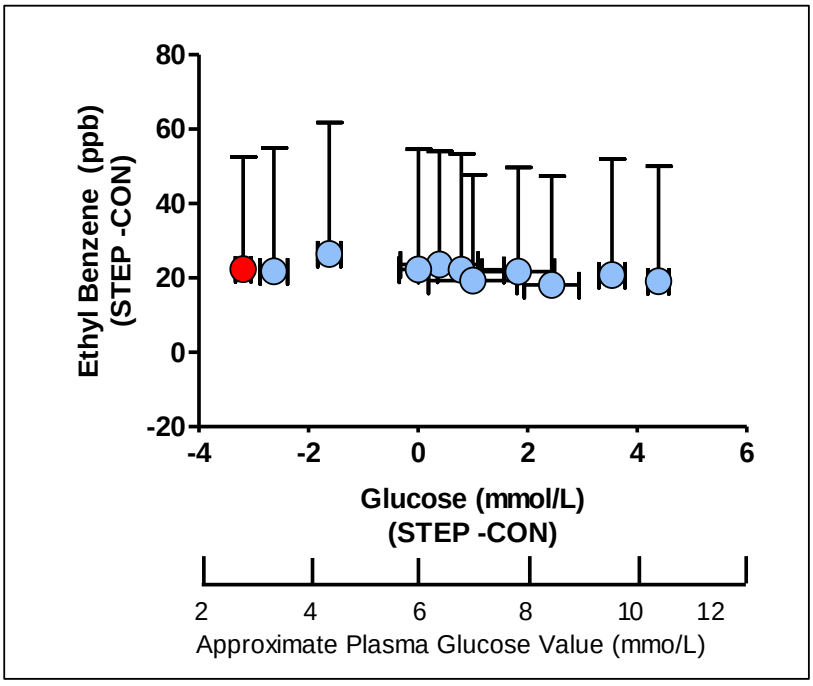
Data shown are mean ±SEM

Figure 5-12 Exhaled Ethanol during studies



Data shown are mean ±SEM

Figure 5-13: Exhaled Ethyl Benzene during studies

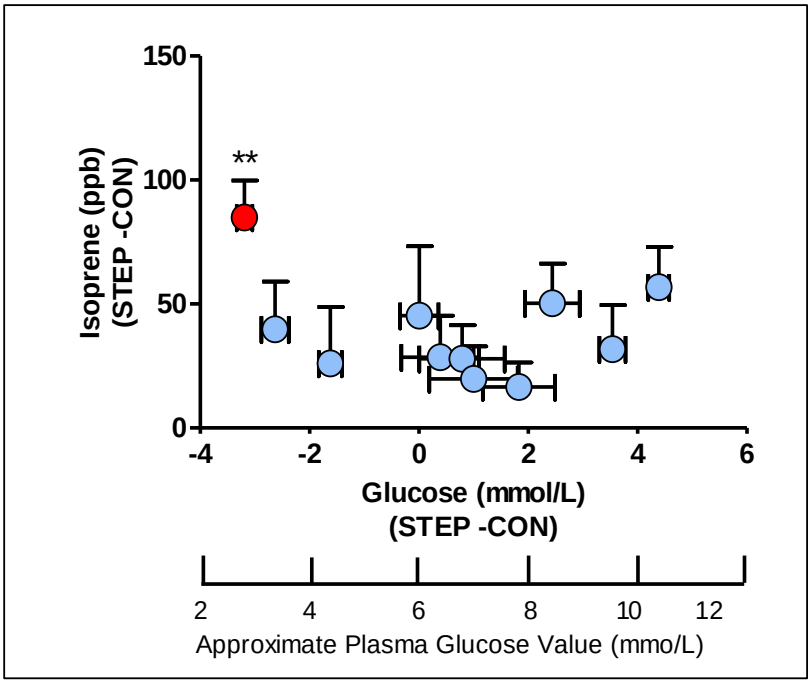


Data shown are mean ±SEM

5.5.4.2 **Exhaled isoprene during studies**

Exhaled isoprene rose significantly at experimental hypoglycaemia (220 minute values) compared to the averaged non-hypoglycaemia range (85 ± 15 vs. 34 ± 6 ppb, p 0.007). Outside hypoglycaemia, there was no correlation between exhaled isoprene and plasma glucose across the broader range of experimental plasma glucose values as shown in Figure 5-14.

Figure 5-14: Exhaled breath isoprene during studies



Data shown are mean ±SEM

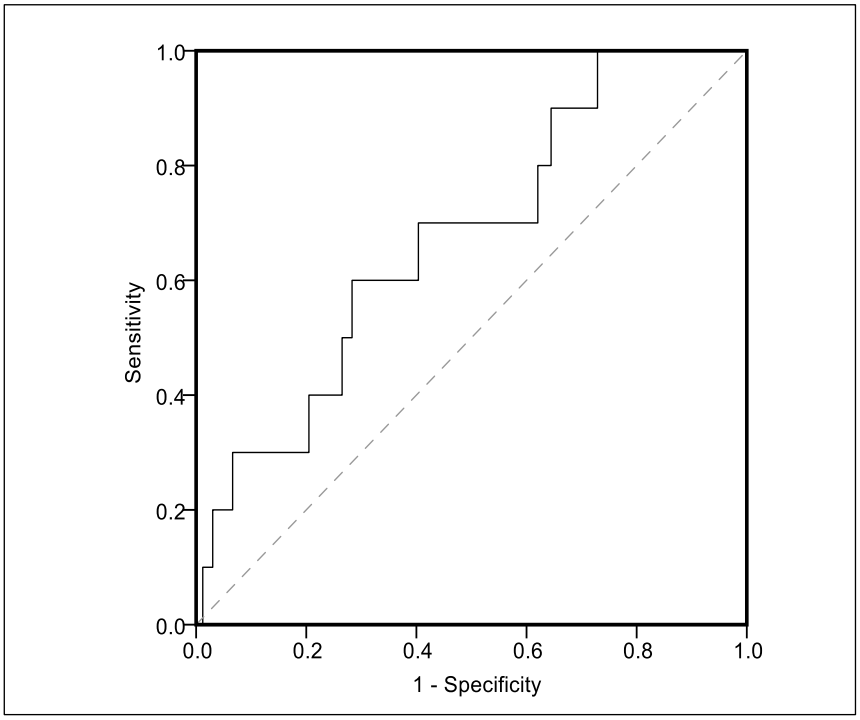
**2 sample t test at 220 min STEP compared to non-hypoglycaemia

Receiver operating characteristic (ROC) curve analysis of Isoprene

The Receiver Operating Characteristic (ROC) analysis of isoprene's accuracy in identifying hypoglycaemia are shown in Figure 5-15 & Figure 5-16.

Using a cut-off value 3 mmol/L to define hypoglycaemia, I found average predictive value of Isoprene with an area under the ROC curve (AUC) of 0.67(p 0.06). However, applying a cut off value of 2.8 mmol/L for hypoglycaemia showed better performance with an area under the ROC curve (AUC) of 0.84, p (0.021).

Figure 5-15: ROC of Isoprene in detecting hypoglycaemia with plasma glucose of ≤ 3 mmol/L

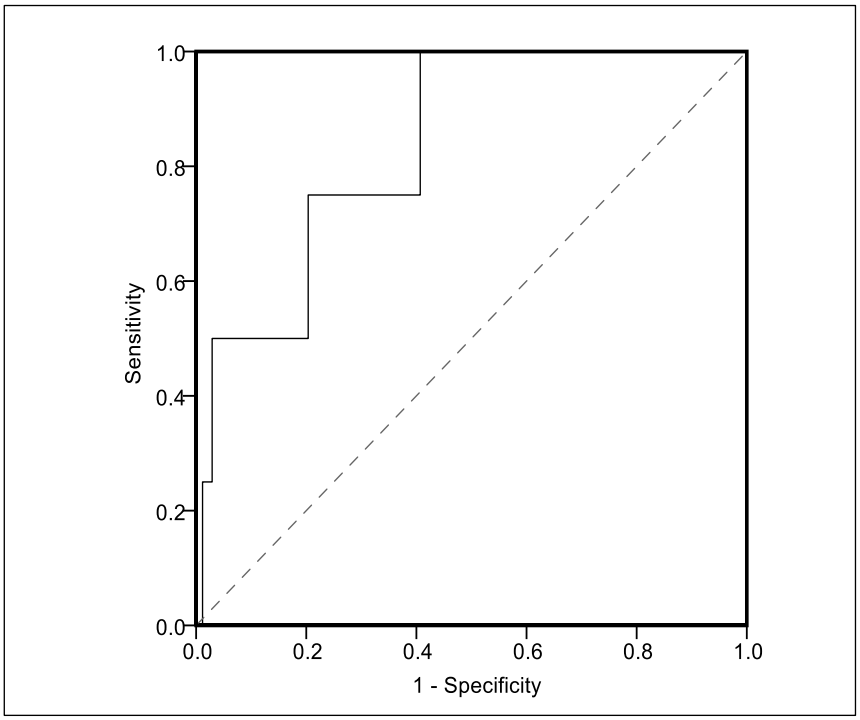


AUC: 0.67; SE: 0.08; CI: 0.51, 0.83; **p: 0.65**

Here Sensitivity (true positive rate) of exhaled Isoprene is plotted in function of false positive rate (1-Specificity) to predict hypoglycaemia using 3mmol/L of plasma glucose as

hypoglycaemia cut-off point. Each point on the curve represents a sensitivity/specificity pair. Ideal ROC curve with 100 % specificity & 100% sensitivity would pass through the upper left corner. Here area under the curve shows that exhaled Isoprene **cannot** distinguish hypoglycaemia (glucose <3mmol/L).

Figure 5-16: ROC of Isoprene in detecting hypoglycaemia with plasma glucose of ≤ 2.8 mmol/L



AUC: 0.84; SE: 0.08; CI: 0.67, 0.99; ***p: 0.021**

Here Sensitivity (true positive rate) of exhaled Isoprene is plotted in function of false positive rate (1-Specificity) to predict when plasma glucose of 2.8mmol/L of plasma glucose as hypoglycaemia cut-off point. Each point on the curve represents a sensitivity/specificity pair. Ideal ROC curve with 100 % specificity & 100% sensitivity would pass through the upper left corner. Here area under the curve shows that exhaled Isoprene can distinguish hypoglycaemia (glucose <2.8 mmol/L).

5.6 Discussion

In keeping with my hypothesis, breath isoprene increased significantly when plasma glucose was low under these experimental conditions. On the basis of this finding, I speculate that breath isoprene may be used as a potential biomarker target for detecting low glucose during hypoglycaemia. However, one of the major limitations of the potential use of the VOCs including isoprene in diagnosis and monitoring of therapy in diabetes is the fact that the source of endogenous isoprene remains undetermined and that there is not a great deal of understanding of metabolism of this VOC in diabetes.

Isoprene, an unsaturated hydrocarbon also known as 2-methyl-1,-3-butadiene, is one of the commonest human breath VOCs [334]. The source of endogenous isoprene remains undetermined although rodent studies suggest it may form during breakdown of dimethylallyl pyrophosphate in the cholesterol synthetic pathway[336]. In keeping with this, exhaled isoprene can be altered by statin therapy [337]. In this study, all but one participant was not on statin therapy. As participants were advised not to eat after 10 pm the night before the study, the participant on statin may have omitted statin the previous night of the study.

It is however unclear why a fall in glucose should result in increased breath isoprene. Glucose can alter hepatic lipid synthetic pathways, acting via carbohydrate response element binding protein (CHrEBP) to induce transcriptional changes [341]. However, known CHrEBP targets are in fatty acid

synthetic pathways (fatty acid synthase, acetyl CoA carboxylase and stearyl CoA desaturase) rather than cholesterol biosynthesis *per se*. Furthermore, transcriptional changes induced by a fall in glucose would be predicted to decrease flux into lipogenesis pathways [342]. Exhalation of endogenously-produced VOCs may be affected both by formation/-breakdown, delivery into pulmonary vasculature and ventilation. Given the speed in the rise of isoprene that we observed, another possible explanation for our findings is increased delivery of isoprene to pulmonary vasculature during hypoglycaemia [343]. Dynamic circulatory changes at low blood glucose include increased heart rate, systolic pressure and hepatic blood flow. Against this, we saw no changes in other VOCs which might have been altered in parallel by haemodynamic changes.

Of note, the small rise in glucagon at hypoglycaemia was largely accounted for by one participant with short duration T1D who mounted a glucagon response to hypoglycaemia.

One of the major strengths of this study is that the insulin clamp technique with control comparison study days allowed us to match carefully exposure to circulating insulin. This is important as insulin is a potential confounder in other studies as insulin is likely to have effects independently of changes in blood glucose. As an exemplar, a previous study also used insulin clamps in a T1D cohort, reporting that clusters of VOCs identified by multi-linear regression rather than an individual VOC correlated with plasma glucose [344] but failed to perform control studies as we have done. A second major strength of our

study is that the association between (experimental) hypoglycaemia and VOCs was examined, something which has not been published in literature. Thirdly we subjected participants to various blood glucose levels including hypoglycaemia mimicking the values experienced by people with T1D at home on a regular basis.

A limitation of our approach however is that we could only measure a discrete repertoire of candidate VOCs and it is possible that other unmeasured VOCs in breath might also change during hypoglycaemia, perhaps adding to the diagnostic power. The study design terminated the participants study at hypoglycaemia range, so we cannot predict isoprene curve during recovery when the glucose level was raised back to euglycaemia or hyperglycaemia range.

In summary, these data suggest that breath VOCs could offer a non-invasive alternative or adjunct to capillary or interstitial blood glucose testing for monitoring changes in blood glucose in diabetes, particularly for detection of hypoglycaemia.

6 In vitro stability of diluted insulin aspart in insulin pump (CSII)

6.1 Background to current study

The rationale for this study was to look into ways that would ultimately help minimise hypoglycaemia in different subgroup of people with T1D.

Infant, toddler, elderly people and some adults with diabetes may require low dose of insulin and sometimes this may be less than 5 units per day. So, the margin of error is small in these individuals to prevent both hypoglycaemia and hyperglycaemia. [220, 238, 345-347]. American Diabetes Association (ADA) has acknowledged the need of lower doses of insulin in young children who may require as low as 0.5-1U/kg/day and could be even lower during honeymoon phase[348]. Some people are sensitive to insulin due to co- morbid conditions such as Addison's' disease, hypopituitarism, chronic kidney diseases[237-240]. Those people who are sensitive to insulin may need less than 1 unit of insulin for each dose adjustment to prevent hypoglycaemia[238, 346, 349-351]. It has been speculated that high frequency of hypoglycaemia observed in children and adolescent could be due to errors in administration of small amount of insulin [346, 351].

A study by Keith et al. compared accuracy of low dose insulin administrations using insulin syringes, pen devices and an insulin pump device. In this study, pen and pump devices were found to more accurate than the syringes at the 1-unit and 2-unit doses of U100 (100 units/mL) insulin delivery with pump devices being the most precise of all. Unsurprisingly, all devices were reasonably

accurate and precise at higher dose at 5 units of insulin delivery. Worryingly, the syringes were highly inaccurate at doses lower than 5 units, overdosing by as much as 31% at the 1-unit target dose[345].

U20 (20 units/mL) insulin were withdrawn from the market when U100 (100 units/mL) insulin were standardized[351, 352]. This left a gap in in the market for insulin dosing below 1 unit. Insulin pens that deliver half unit insulin have been designed to help those people who are sensitive to insulin [351, 353-355]. At the moment accuracy of insulin below 0.5 unit is achieved through insulin pump only [351]. Currently, commercially available insulin pumps in the UK can deliver as low as 0.025 of bolus insulin[356]. Recently a study examined in vitro accuracy of bolus and basal insulin delivery of 10 different commercially available insulin pump devices. All insulin pump systems, with the exception of patch pump, delivered at least 95% of all 1U boluses within 15% of target. Precision of individual boluses was found to be higher with the larger boluses[357]. Large deviations were observed in the basal insulin delivery in the first 12 hours although variations were much lower in overall 72 hour period[357].

In our clinical practice as well, as discussed above, some of the people with T1D have required very low doses of insulin to control glycaemia in keeping with reported literature [237-240]. These people are prone to hypoglycaemia in spite of low dose insulin, in part because of the challenges of delivering small amounts of insulin accurately[351].

Short acting insulin analogues are nearly always used in CSII. Insulin aspart, insulin glulisine and insulin lispro are the analogue insulins currently used in CSII. A more rapid formulation of NovoRapid (Fiasp) was been approved in April

2017 in UK but sparsely used to date. In a systematic review, safety and clinical efficacy of these analogue insulin have been found to be comparable during CSII use in various in vitro and clinical studies [358].

One overnight study performed recently showed short term (<24h) stability and no effect of dilution on pharmacokinetics [359].

The rationale for the work described in this chapter is based on the overarching hypothesis that diluting the insulin analogue in people with T1D with very low insulin requirements would allow an increase in infusion rates and thus minimise glycaemic variability including hypoglycaemia in people with T1D.

There is only one product in the market available to be used in diluting analogue insulin. Novo Nordisk supplies diluting 'Diluting medium for NovoRapid® (insulin aspart) and Levemir® (insulin detemir). This Diluting medium is only recommended for diluting NovoRapid® and Levemir®. This has been recommended to be is used in the treatment of people with diabetes requiring extremely low doses of insulin. The medium contains Glycerol, Disodium phosphate dihydrate, Metacresol, Phenol, Sodium hydroxide, Hydrochloric acid and Water for Injections. NovoRapid® diluted with Insulin Diluting Medium for NovoRapid® and Levemir® in a sterile sealed glass vial may remain in patient use at ambient temperature (below 30 ^{0} C for up to maximum of 28 days[360].

The Novorapid vial contains insulin aspart, Glycerol, Phenol, Metacresol, Zinc chloride, Disodium phosphate dihydrate, Sodium chloride, Hydrochloric acid (for pH adjustment), Sodium hydroxide (for pH adjustment), and Water for injections. A NovoRapid®(insulin aspart) vial used in CSII , during use or when carried as spare can be stored for a maximum of 28 days [361].

Previous published work *In vitro* studies looking at the stability of analogue insulin in CSII were done in extreme lab settings [362, 363]. Similarly, clinical studies concentrated on cannula occlusion due to analogue insulin in CSII and the clinical outcome [358, 364] but did not look at stability and recovery of insulin analogue at the end of recommended 28 day in CSII users particularly when kept close to body temperature. Furthermore, there is no literature on the stability (once diluted in a vial as people might be expected to do) and use of diluted insulin aspart in CSII.

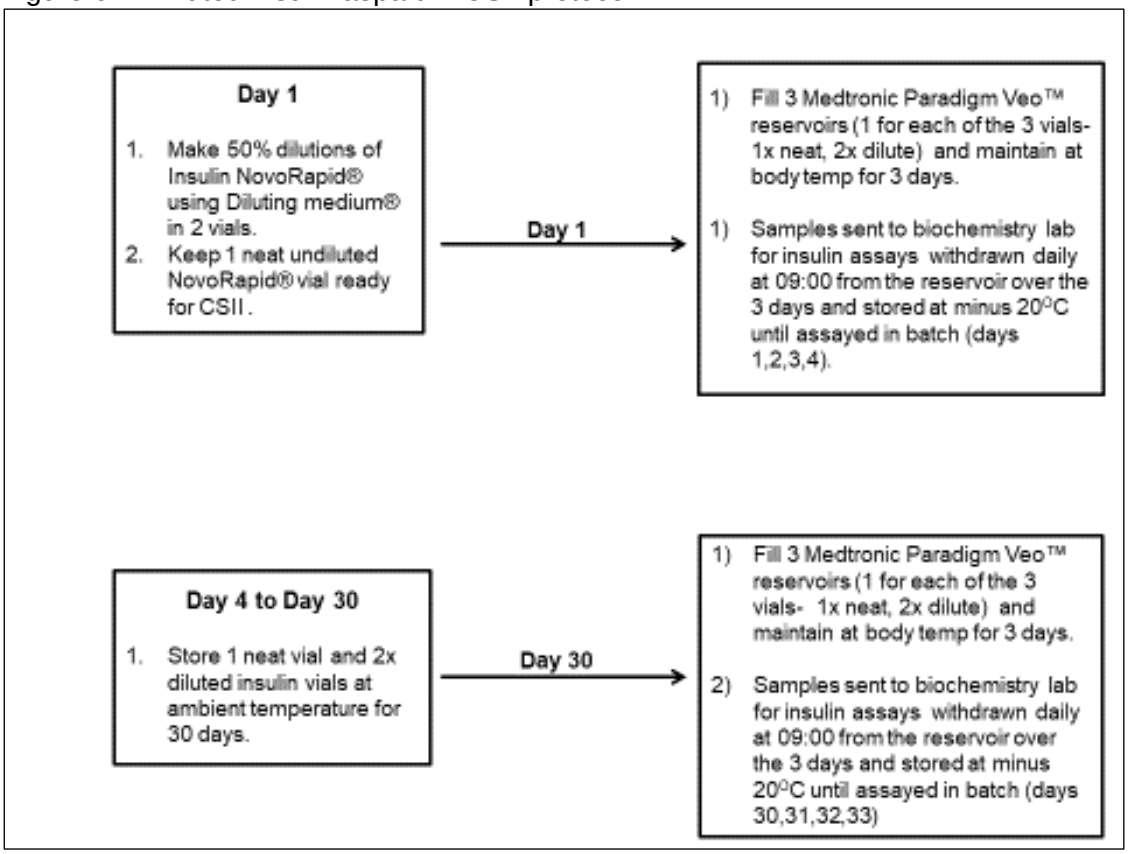
6.2 Study Objective

To study *in vitro* stability of diluted insulin aspart at and beyond 30 days in a simulated use via CSII

6.3 Study Design

Regular strength (100 U/ml) insulin aspart (NovoRapid®) was diluted at 1:1 by mixing with 'Insulin Diluting medium® for NovoRapid' (50 % dilution) by the researcher (myself). The following protocol was followed as shown in the diagram below (Figure 6-1)

Figure 6-1: Diluted insulin aspart in CSII protocol



6.3.1 ***Study Procedure***

Day 1

1) Make 50% dilutions of Insulin aspart (NovoRapid®) using 'Insulin Diluting medium® for NovoRapid') in 2 vials. The procedure was done under aseptic conditions and was kept ready to fill in plastic reservoirs for Medtronic Paradigm Veo™ pump.

2) Keep 1 neat undiluted insulin aspart (NovoRapid®) vial ready to fill in plastic reservoirs for Medtronic Paradigm Veo™ pump (Medtronic MiniMed, Inc.)

3) Fill three plastic reservoirs with neat and diluted insulin aspart (1x neat, 2x diluted insulin).

4) Place all three reservoirs filled with neat and diluted insulin aspart in three different Medtronic Paradigm Veo™ pumps.

5) Wear the three Medtronic Paradigm Veo™ at usual body sites to maintain the insulin close to body temperature simulating people with T1D on CSII.

6) Samples sent to biochemistry lab for insulin assays drawn at 0, 24, 48 and 72 hours post dilution from the reservoir over the 3 days (days 1, 2, 3, 4).

7) Store samples at minus 20^0C at biochemistry lab until assayed in batch.

Day 4 to Day 30

Store remaining insulin (1 neat insulin vial and 2x diluted insulin vials) at ambient temperature for 30 days.

Day 30

1) Fill three plastic reservoirs with stored neat and diluted insulin aspart (1x neat, 2x diluted insulin).
2) Place all three reservoirs filled with neat and diluted insulin aspart in three different Medtronic Paradigm Veo™ pumps (Medtronic MiniMed, Inc.).

3) Wear the three Medtronic Paradigm Veo™ at usual body sites to maintain the insulin close to body temperature simulating people with T1D on CSII.
4) Samples sent to biochemistry lab for insulin assays drawn at t 0, 24, 48 and 72 hours from the reservoir over the 3 days (days 30, 31, 32, 33, 34).
5) Store samples at minus 20^0C at biochemistry lab until assayed in batch.

I followed the above protocol and wore three CSII pump devices Medtronic Paradigm Veo™ (Medtronic MiniMed, Inc.) for 3 days on 2 occasions 30 days apart.

6.3.2 *Analytical Methods*

Insulin was assayed at Core Biochemical Assay Laboratory (CBAL), Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK on a singleton DELFIA automatic immunoassay analyser using a two-step time resolved fluorometric assay (intra- assay CV 2.4% at 33.9 umol/L; 1.8% at 82.2 umol/L; 1.7% at 181umol/L and inter-assay CV 3.1% at 29 umol/L , 2.1% at 79.4 umol/L, 1.9% at 277 umol/L & 2.0% at 705 umol/L).

The assay measures NovoRapid with an estimated recovery of 138% (based on a 1:2,000,000 dilution). Theoretical concentration for the diluted insulin was 300umol/l (Table 6-1).

Insulin aspart was also measured in unused NovoRapid® for control.

Table 6-1: Expected concentration of insulin in the diluted samples

Vial	Concentration	Dilution for assay	Theoretical Concentration
Normal vial	100U/ml	1:1,000,000	600umol/l
		1:2,000,000	300umol/l
Diluted (50%) vial	50U/ml	1:1,000,000	300umol/l

6.3.3 *Statistical Considerations*

Percentage of recovery of insulin aspart over the period of 33 days for diluted insulin is compared against the neat insulin value for the day. Mean and Coefficient of variation (CV) of the measured insulin was also analysed.

Data were analysed using IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY: IBM Corp). All data are presented as mean with SEM.

6.4 Results

There were 8 samples each for neat, diluted 1 and diluted 2 insulin and 1 sample for control. One neat sample at 24 hr (Day 2) could not be measured due to a technical error.

The neat samples all measured very close to the theoretical value of 600umol/l and all the 1:2 dilutions were very close to the expected with mean recovery of 46% against the neat insulin aspart (NovoRapid®) value for corresponding days.

The baseline dilution of 274 umol/L & 295 umol/L for diluted 1 and diluted 2 insulin aspart suggest that manual dilution at 1:1 was not perfect. However, concentration remained stable at 33 days with values of 282 umol/L & 276 umol/L for diluted 1 and diluted 2 insulin respectively. Mean values and Coefficient of variation (CV) of the measured insulins is shown in the Figure 6-2 & Table 6-2.

Figure 6-2: Stability of Insulin Aspart over 33 day's period with simulated use of neat and diluted insulin

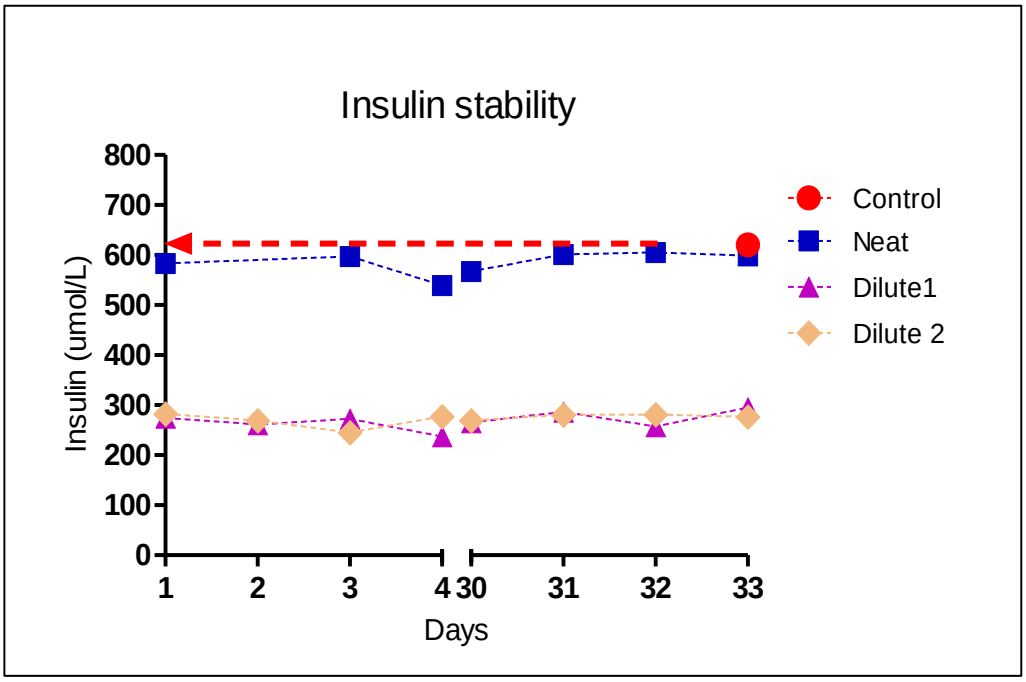


Table 6-2 : Stability of Insulin Aspart over 33 day's period with simulated use of neat and diluted insulin for 3 days a month apart

Insulin (umol/L)	Day 1	Day 2	Day 3	Day 4	Day 30	Day 31	Day 32	Day 33	Mean± SEM	CV (%)
Control	620									
Neat	583	-	597	539	567	601	605	598	584.3±9.0	4.08
Diluted 1 (50 %)	274	261	272	237	265	286	257	295	268.4±6.3	6.67
Diluted 2 (50 %)	282	269	245	277	269	280	281	276	272.4±4.3	4.46
Diluted 1 Recovery (%)	47.00	-	45.6	43.9	46.7	47.6	42.5	49.3	46.1±0.9.	4.99
Diluted 2 Recovery (%)	48.37	-	41.0	51.4	47.4	46.6	46.4	46.1	46.8±1.	6.63

6.5 Discussion

In vitro study showed that all the 1:2 dilutions were very close to the expected value with mean recovery of 46% against the neat insulin aspart (NovoRapid®) value for corresponding day. Furthermore, the concentration remained stable at 30 to 33 days. The coefficient of variation of the measured insulins was also

acceptable and satisfactory at 6.67% & 4.46%, well within experimental error taking into account the 1:1 million dilution involved in measuring the insulin concentration. It also shows that stability data can be replicated with similar results in both diluted insulin aspart samples.

The data also confirm the product literature recommendation that neat insulin aspart (NovoRapid®) is stable for up to 28 days once opened and kept in ambient temperature.

Overall my data confirm that diluted insulin aspart using recommended 'Insulin Diluting medium® for NovoRapid' use was stable beyond 28 day up to 33 days in vitro study during simulated insulin pump use.

The strength of my study is that, to my knowledge this is the first study examining stability of diluted insulin aspart in a simulated CSII use mimicking the body temperature and in real clinical situations as any individual with T1D on CSII therapy would have done. It is possible that unpublished work performed by insulin manufacturers exists. I have also examined the stability of insulin aspart using recommended diluting medium in CSII. My data also confirm that neat insulin aspart is also stable at room temperature for 28 days. Previous work examined the stability of neat insulin aspart during simulated use in insulin pump. In contrast to my study, previous work was in a lab setting with physical fibrillation of samples for 7 days, and not mimicking any effect of exposure to potential temperature effects of being worn close to the body [362]. To my knowledge this is also the first study where recovery of neat insulin aspart at the end of recommended 28 days period of use in CSII has been looked at.

6.5.1 *Pilot Data*

As a clinical post-script and following the encouraging finding of this in vitro study, I diluted insulin aspart in CSII for one individual with T1D who had been experiencing frequent mild hypoglycaemia (more than 5 times per week) in spite of very low basal insulin dose requirement (5.5 units per day). As described below, diluted insulin aspart was clinically effective in reducing time spent below 3.9 mmol/L and without worsening of glycaemic control. Patient also had a sustained improvement in overall glycaemic control even 15 months post dilution.

6.5.1.1 **Baseline characteristics**

Age: 68 year

Gender: Female

Weight: 52 .5 kg

Duration of diabetes: 23 years

Baseline HbA1c: 73 mmol/mol

Current medications: Novorapid (via CSII), Lisinopril 5mg

Total daily dose of insulin: 13.8 units (Basal 5.5 units, Bolus 8.3 units).

Carbohydrate ratio (g/U): 00:00 - 17.0

13:30 - 17.0

14:30 – 18.0

19:00 – 12.0

Insulin sensitivity (mmol per U):5.0

6.5.1.2 **Procedure followed:**

Patient was fitted with professional retrospective CGM (iPro®2, Medtronic MiniMed, Inc) for 7 days a week before using diluted insulin aspart in CSII.

Patient was then trained to prepare diluted insulin aspart by mixing NovoRapid® with 'Insulin Diluting medium® at 1:1 ratio (50 % dilution). Pump settings were adjusted to ensure she received same concentration of insulin for basal and bolus insulin post dilution i.e. insulin carb ratio was halved, basal insulin rate was doubled, and insulin sensitivity was halved.

On the first day of training, person was trained to dilute insulin aspart. Thereafter they filled the insulin reservoir with diluted insulin every 3 days at home. She then stored the diluted but unused stored insulin in ambient temperature in a cool and dry place. Patient prepared the diluted insulin aspart at least every 28 days.

HbA1c was done at baseline and 3 months. She was fitted with professional retrospective CGM (iPro®2, Medtronic MiniMed, Inc) for 7 days at baseline and 4 months post dilution.

6.5.1.3 **Retrospective CGM data**

Mean glucose was higher at 3 months post dilution compared to baseline (9.1±0.1 vs 8.3±0.1 mmol/L). However, time spent in glucose ≤ 3.9 mmol/L was reduced from 4 % at baseline to 1% at 3 months post dilution. Number of days blood glucose was ≤ 3.9 mmol/L, was reduced from 4 days at baseline to 1 day at 3 months. Details of CGM are shown in Table 6-3,Figure 6-3, Figure 6-4 & Figure 6-5.

Baseline HbA1c was 73 mmol/ mol (pre-dilution). Post -dilution HbA1c was 75 mmol/ mol, 65 mmol/ mol and 67 mmol/ and 68 mmol/ mol at 3, 6 and 9 and 15 months respectively.

Table 6-3: Comparison of retrospective (blinded) CGM data at baseline and 4months after insulin aspart dilution for the CSII (pre and post dilution)

CGM Glucose (mmol/L)	Pre -Dilution	Post - Dilution
Number of sensor values	1874	1875
Mean±SEM	8.3±0.1	9.1±0.1
High excursion(number)	21	24
Low excursion(number) ≤ 3.9 mmol/L glucose	6	2
		-

Figure 6-3: Time spent in various glucose levels as per the CGM sensor data at baseline before dilution of insulin aspart in CSII

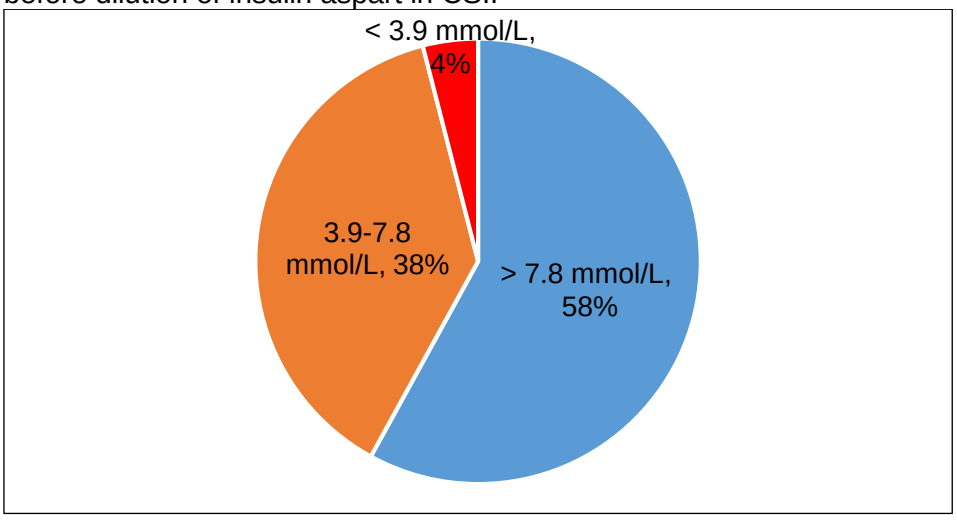


Figure 6-4: Time spent in various glucose levels as per the retrospective CGM sensor data 4months post dilution of insulin aspart in CSII

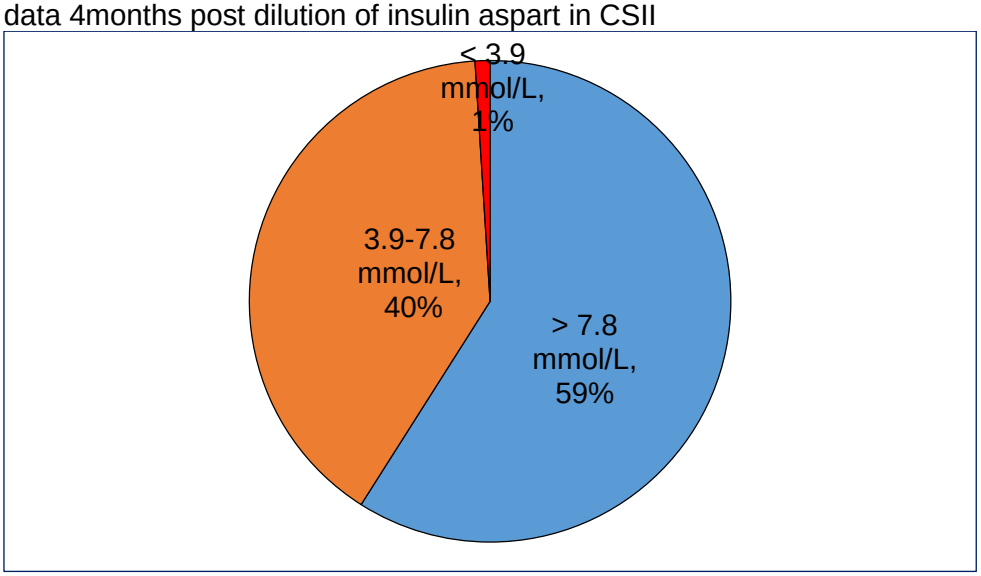
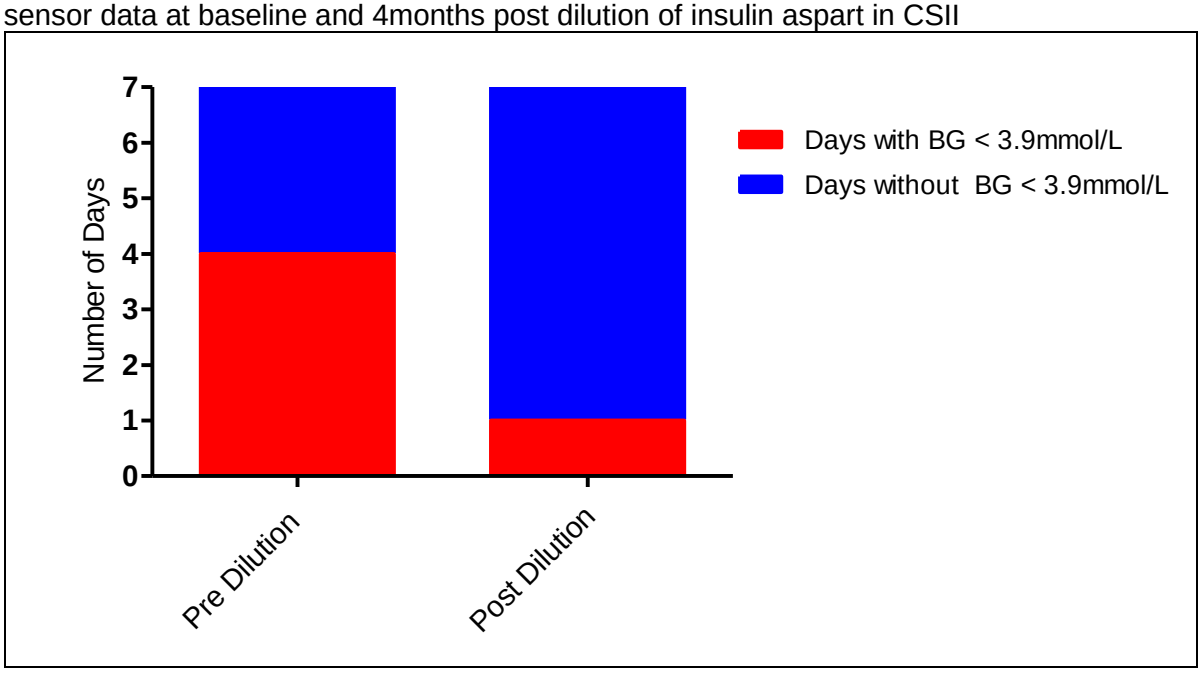


Figure 6-5: Number of days spent in glucose levels below 3.9 mmol/L as per the CGM sensor data at baseline and 4months post dilution of insulin aspart in CSII



6.5.1.4 **Discussion :**

Within the limitation of sample size (n=1), diluted insulin aspart was clinically effective in reducing time spent in glucose below 3.9mmol/ L and improve and maintain glycaemic control. The limitation of my approach in this pilot study is that this individual's hypoglycaemia frequency had started to improve already before insulin dilution possibly due to health care support. Therefore, the baseline CGM may not have captured the frequency of hypoglycaemia she usually experienced before being invited for this pilot study. I speculate that use of CGMS longer than 1 week could have captured more information both at baseline and after the dilution. Sustained improvement in glycaemic control could be due to combination of HCP support and improved accuracy of low dose insulin delivery by virtue of higher volume also observed by a recent report[357]. It would be interesting to see the results in large cohort of T1D.

7 Conclusions

Hypoglycaemia still remains a problem for some people with T1D despite improvement in insulin delivery, glucose monitoring and structured education and psychological support. Because there are likely many multifactorial contributors to hypoglycaemia risk (physiological, demographic, social, psychosocial/ behavioural), a single therapeutic approach or intervention to reduce hypoglycaemia burden is unlikely to work for all. We need to have a holistic approach to address the problem of hypoglycaemia in people with T1D. The work in my thesis involved investigation of three domains of clinical hypoglycaemia: prediction of hypoglycaemia, detection of hypoglycaemia (using a novel glucose monitoring system) and exploring better insulin delivery.

8.1 Prediction of Hypoglycaemia

I examined the HypoCOMPaSS clamp data looking for possible factors that could predict response to clinical interventions in those with a significant burden of hypoglycaemia. I could not find any obvious biomedical indicators that might potentially be targeted to reduce hypoglycaemia burden in this well phenotyped cohort. This apparently negative result however is encouraging for clinical practice as the absence of irreversible factors that could contribute to hypoglycaemia suggests that intensifying clinical strategies aimed to reduce hypoglycaemia burden could still work for anyone with problematic hypoglycaemia i.e. there is no particular group of T1D that is obviously resistant to clinical intervention(s).

Findings from the ACE genotype sub study showed that homozygous deletion (DD) in the polymorphism of DD ACE gene was associated with risk of SH in HypoCOMPaSS participants, thus confirming the suggestion from literature that some people with T1D are biologically prone to severe hypoglycaemia. On the basis of the clamp data, this association could perhaps be explained by a greater decline in those with the DD polymorphism in cognitive function during hypoglycaemia, thus making these individuals prone to severe episodes. I speculate that centrally acting ACE inhibitors may be beneficial in those with homozygous deletion of ACE gene to prevent severe hypoglycaemia. However, the findings need to be further explored. Importantly, there is a need for more mechanistic work targeting the ACE/RAS system directly in brain. Of note, my host laboratory for my thesis is currently exploring hypoglycaemia mechanisms in mice models including brain targeting approaches.

Measuring ACE level and identifying ACE gene polymorphism may detect some who are more prone to severe hypoglycaemia. The importance of my finding is that (i) this might allow clinical strategies to be targeted by genotype (e.g. reinforcing for some that they are more likely to experience cognitive dysfunction during hypoglycaemia) and (ii) perhaps future drug therapy with agents working via central ACE/RAS pathways might be another therapeutic approach to minimise severe hypoglycaemia in some people with T1D.

8.2 Detection of Hypoglycaemia

Benefits of traditional CGM systems for reducing hypoglycaemia are dependent on user compliance with the devices. Multiple factors including skin adhesive

issues, skin reactions and the requirement to change the sensor every week can play a part in the compliance with standard CGM system. The (currently novel) implantable glucose monitoring device developed by Eversense® CGM System can minimise these issues. My analysis of Cambridge participants showed that this system was safe, and participants found it easier to use. The efficacy was comparable to standard commercial CGM system. Furthermore, it was reliable in hypoglycaemia range level as well. This formed part of the evidence for the pivotal multi centred European PRECISE1 study which allowed the licensing of this CGM system. As this system is safe and effective, this could potentially be used in the ongoing closed loop (CL) studies. It has recently been reported that this implantable continuous glucose system may be integrated with automated insulin delivery including the Boston group's "bionic pancreas" system. The data I present in this thesis have helped pave the way for exciting and advanced technological approaches to glucose monitoring and insulin delivery already. Hypothetically, there are no scientific reasons why one could not extend the system for the use in people with Type 2 diabetes as well.

Although CGM systems have advanced in the last 2 decades with improved accuracy and connectivity, these systems still need further refinement to facilitate further development of AID system. CGM's signalling error and signal drop-outs needs to be minimised[70, 365]. Further accuracy metrics of CGM needs to be based on direct interstitial glucose measurement as reference glucose rather than currently used venous glucose reference [89, 365, 366]. The latest CGMs systems that do not need calibration is without a doubt an important step towards AID advancement. Longevity of sensor such as Eversense® CGM system has paved the way for sensors with longer life. This system is still limited

by the fact that it needs SMBG for calibration and that the sensor needs warm up phase which again is hindrance to the AID[365] .

In people with T2D benefits of advance diabetes technologies has not been investigated as much as in people with T1D and the lack of data makes it difficult to predict its benefit[367, 368]. One of the challenges seems to be that hyperglycaemia is more pronounced than hypoglycaemia in T2D in contrast to those with T1D[81]. There are reports suggesting that fully automated system is not necessarily the best way to achieve better glycaemic controls as similar results were achieved in some studies through fixed bolus using pen device [367]. Further question remains if the costs and complexity of AID are justified in people with T2D [367].

My data from our study of exhaled breath Volatiles Organic Compounds is encouraging for those who are still exploring non-invasive approaches of glucose monitoring. The rise in exhaled isoprene during hypoglycemia is indeed very interesting. This has gained interest in the public and media as well. A major limitation to this approach for the future studies is the gap in our understanding of the basic biology of isoprene metabolism in the human body.

These limitations notwithstanding, I speculate that medically trained dogs which detect hypoglycaemia may have been detecting (at least in part) exhaled volatile compounds (including perhaps isoprene) in the breath of their owners. One could scientifically explore dog's response to experimentally controlled hypoglycaemia in human T1D subjects in a controlled environment and indeed also study the response of trained dogs to exhaled volatile compounds[369].

Recent studies (published after my study was completed) however did not support the popular anecdotal reports of diabetes trained dogs reliably alerting

humans to hypoglycaemia[369, 370]. Gonder-Frederick et al. examined dog's alerts records by owners and compared these with blinded CGM readings in 14 people with T1D over 29 median days. In this study dogs did not accurately detect either hypoglycaemia or hyperglycaemia. Dogs only detected 35.9% of low blood glucose events during waking hours and 22.2% during sleeping hours[370]. In a separate similar study of the dogs trained to detect hypoglycaemia owned by people with T1D using blinded CGM, dogs provided timely alerts only in 36% of all hypoglycemia events. However this study was limited by small sample size of 8 and that there were variations in breed, age, training duration of the dogs that could influence accuracy of the alerts and hence the result of the study [369].

Future research could also look into development of innovative breath sensing technology for earlier detection of hypoglycemia to prevent severe hypoglycemia, perhaps using an individualised fingerprint of individual volatile compound or indeed multitude of compounds. One could speculate that an "artificial nose" could be designed to help detect hypoglycaemia early. Similarly, breath sensing smart pillows could be developed to detect hypoglycaemia during sleep. Tiny sensor devices that could detect exhaled breath compounds could be implanted in the nasal cavity. Such an approach could particularly be of huge benefit for those who have little or no awareness of hypoglycaemia during sleep. Parents or care givers of children with T1D would welcome future non-invasive or minimally invasive devices that reliably detect hypoglycaemia.

8.3 Better Insulin Delivery

Some people with T1D are prone to recurrent hypoglycaemia despite relatively lower basal insulin use. It is possible that reducing the variation in insulin absorption may address the problem. Results of my study on the stability of diluted insulin aspart over 33 days raises the possibility that diluted insulin aspart could be used in these groups of people.

One recently published overnight study on diluted insulin showed short term (<24h) stability and no effect of dilution on pharmacokinetics [359]. However, a recent study by Freckmann observed that higher volume of insulin was associated with better accuracy of bolus doses supporting my finding[357]. This needs to be replicated in larger randomized studies. This observation was however not consistent with all other examined bolus and basal and bolus doses.

This finding could potentially be utilised widely. Diluted insulin use could be utilised in those with low basal insulin requirement to prevent hypoglycaemia. Furthermore, this could be used in children who by nature require much less insulin compared to adults and so need much finer insulin dose adjustments. Broadly this could also be used in closed loop insulin delivery system to allow for much finer insulin dosing increments.

8 Appendices

Appendix 1

ADA/WHO Definition of Diabetes Mellitus

1. FPG ≥ 7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.

OR

2. Symptoms of hyperglycemia and a casual plasma glucose ≥11.1 mmol/l. Casual is defined as any time of day without regard to time since last meal. The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss.

OR

3. 2-h plasma glucose ≥ 11.1 mmol/l during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day.

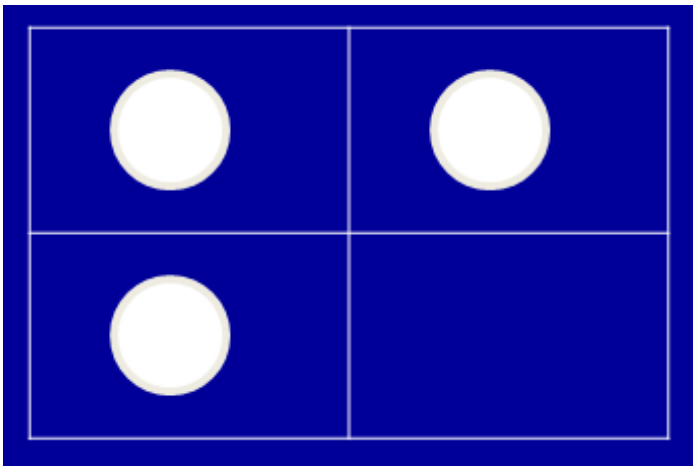
Appendix 2

Cognitive function Tests

A. Four choice reaction time

In this test, participant is presented with a computer screen which is divided into four quadrants. A computer-generated signal appears randomly in one quadrant at a time and the participant is asked to clear each signal that appear by pressing a corresponding button on a box [371].

Sample of Four Choice Reaction Time Test



B. Stroop Black & White

In this test, participant is presented with names of colour written in Black & White and is asked to name the colour[246].

Sample of Stroop Black & White

BLUE	**RED**	**GREEN**	**TAN**
RED	**GREEN**	**BLUE**	**RED**
GREEN	**TAN**	**RED**	**GREEN**
TAN	**BLUE**	**GREEN**	**RED**
RED	**TAN**	**BLUE**	**TAN**
BLUE	**GREEN**	**RED**	**RED**
GREEN	**TAN**	**TAN**	**BLUE**
BLUE	**RED**	**GREEN**	**TAN**
RED	**BLUE**	**RED**	**BLUE**
GREEN	**RED**	**GREEN**	**RED**
TAN	**GREEN**	**TAN**	**GREEN**
BLUE	**RED**	**TAN**	**TAN**
RED	**BLUE**	**GREEN**	**BLUE**
GREEN	**TAN**	**RED**	**GREEN**

C. Stroop Colour

In this test participant is presented with series of ‘X’ in different color inks and is asked to name the color of each ‘X’ [246].

Sample of Stroop Colour Test

X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X
X	X	X	X

D. Stroop Colour Word

In this test participant is presented with series of the words ‘blue’, ‘red’, ‘green’, ‘tan’ printed in ink of a contrasting colour. Participant is asked to name the colour of the ink.

Sample of Stroop Colour Word

BLUE	RED	GREEN	TAN
RED	GREEN	BLUE	RED
GREEN	TAN	RED	GREEN
TAN	BLUE	GREEN	RED
RED	TAN	BLUE	TAN
BLUE	GREEN	RED	RED
GREEN	TAN	TAN	BLUE
BLUE	RED	GREEN	TAN
RED	BLUE	RED	BLUE
GREEN	RED	GREEN	RED
TAN	GREEN	TAN	GREEN
BLUE	RED	TAN	TAN
RED	BLUE	GREEN	BLUE
GREEN	TAN	RED	GREEN

Appendix 3

Table: Baseline characteristics of the participants who took part in main HypoCOMPaSS study

	All	Insulin comparison		Monitoring comparison	
		MDI	CSII	SMBG	RT
Baseline HbA_{1c}					
<8%	41 (43)	22 (44)	19 (41)	21 (44)	20 (42)
≥8%	55 (57)	28 (56)	27 (59)	27 (56)	28 (58)
HbA_{1c} (%)	8.2 ± 1.2	8.2 ± 1.3	8.2 ± 1.2	8.3 ± 1.3	8.2 ± 1.1
HbA_{1c} (mmol/mol)	66 ± 12	66 ± 13	66 ± 12	67 ± 13	66 ± 11
Age (years)	49.0(23-73)	46.7(25-73)	50.0(23-72)	47.0(23-72)	53.5(25-73)
Male	35 (36)	16 (32)	19 (41)	20 (42)	15 (31)
Diabetes duration (years)*	28.9 ± 12.3	29.5 ± 12.5	28.2 ± 12.2	26.7 ± 12.1	31.0 ± 12.2
Body weight (kg)	74.7 ± 14.2	74.9 ± 13.9	74.5 ± 14.6	74.5 ± 14.6	75.0 ± 13.9
BMI (kg/m^2)	26.5 ± 4.4	26.7 ± 4.6	26.3 ± 4.4	26.1 ± 4.3	26.9 ± 4.7
Insulin dose (units/kg/24 h)	0.64 ± 0.23	0.63 ± 0.21	0.66 ± 0.26	0.61 ± 0.19	0.68 ± 0.27
Mean study visits/person (maximum 7)	6.6	6.6	6.6	6.5	6.0

Appendix 4

Edinburgh Hypoglycaemia Survey (including The Gold Score)

1. Please score the extent to which you experience the following symptoms during a typical daytime hypoglycaemic episode (circle a number for each symptom)

	Not present						Present a great deal
Confusion	1	2	3	4	5	6	7
Sweating	1	2	3	4	5	6	7
Drowsiness	1	2	3	4	5	6	7
Weakness	1	2	3	4	5	6	7
Dizziness	1	2	3	4	5	6	7
Warmth	1	2	3	4	5	6	7
Difficulty Speaking	1	2	3	4	5	6	7
Pounding heart	1	2	3	4	5	6	7
Inability to concentrate	1	2	3	4	5	6	7
Blurred vision	1	2	3	4	5	6	7
Hunger	1	2	3	4	5	6	7
Nausea	1	2	3	4	5	6	7
Anxiety	1	2	3	4	5	6	7
Tiredness	1	2	3	4	5	6	7
Tingling lips	1	2	3	4	5	6	7
Trembling	1	2	3	4	5	6	7
Headache	1	2	3	4	5	6	7

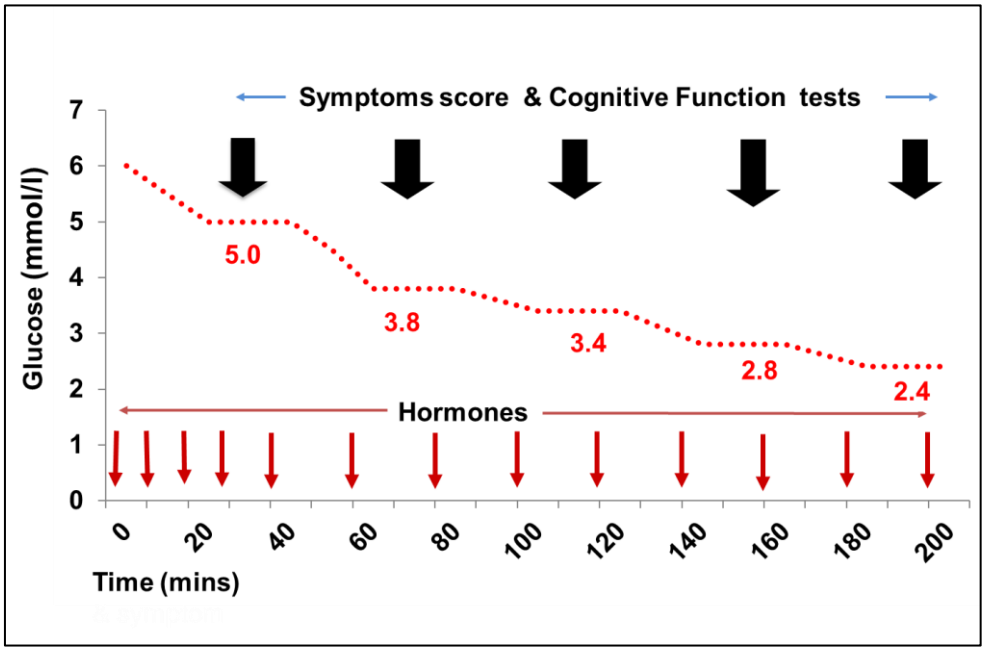
2. Do you know when your hypos are commencing? Please circle a number: (The Gold Score)

	Always aware						Never aware
Awareness	**1**	**2**	**3**	**4**	**5**	**6**	**7**

Comments:

Appendix 5

Clamp activity of HypoCOMPaSS clamp sub study



Appendix 6

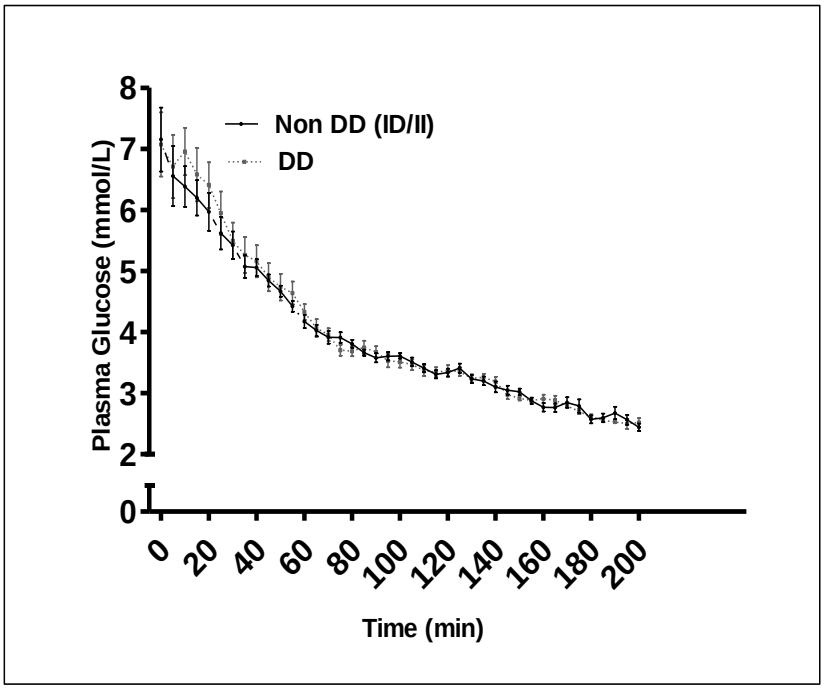
Additional characteristics and analysis of all participants who took part in clamp procedure at the end of 6 months RCT

Baseline characteristics of all participants who took part in clamp procedure at end of the 6 months RCT

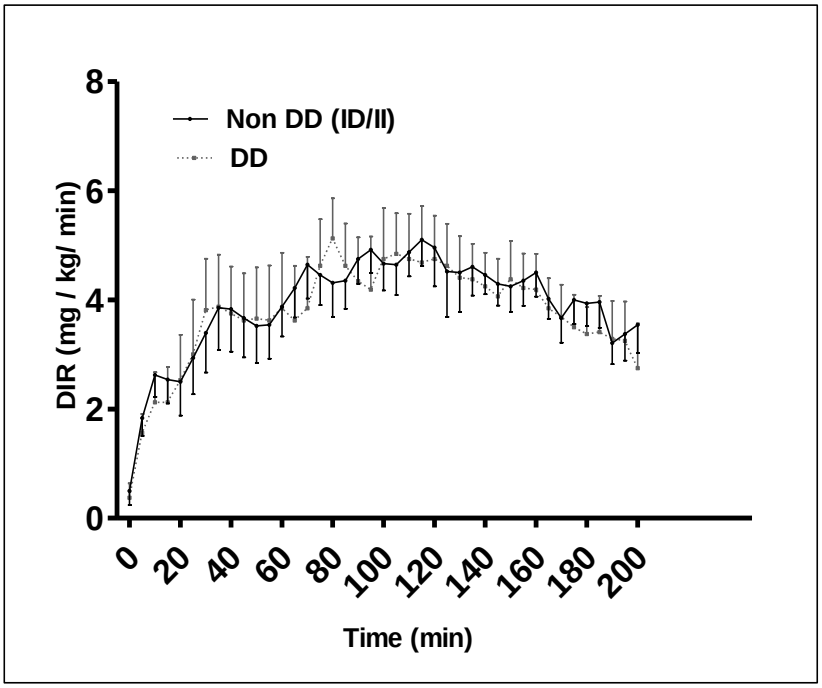
	All n=21	DD n= 8	Non DD (ID/II) n=13	p value*
Female Sex (n=14)	21	5	9	0.55 [a]
Age (years)	48.6 ± 1.8	45.3±3.9	50.9±1.6	0.11
Duration of diabetes (years)	35.7±2.1	30.6.9±4.1	38.7±1.9	0.06
$HbA_{1c\ (\%)}$ baseline	7.9±0.2	8..0±0.3	7.9±0.3	0.97
$HbA_{1c\ (mmol/mol)}$ baseline	63.8±2.1	63.9±3.4	63.8±2.8	0.97
Severe Hypoglycaemia at baseline (episodes/person/year)	11.5±3.5	5.5(-0.46, 23.36)	7(3.01-22.7	0.64
Gold Score at baseline	5.3±0.1	5.4±0.2	5.4±0.1	0.27

Data shown are mean ±SEM or Median (IQR)
* Independent sample T-Test or Mann Whitney U Test
a Fisher Exact test

Plasma glucose for all participants who took part in clamp procedure at end of the 6 months RCT



Dextrose Infusion Rate (DIR) for all participants who took part in clamp procedure at end of the 6 months RCT



Symptoms and hormonal responses at end RCT clamped hypoglycaemia in all participants - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes

	DD N=8	Non DD (II/ID) N= 13	*p value
Glucose levels subjects felt low (mmol/l)	2.8±0.2	3.1±0.2	0.39
Symptoms AUC			
Total	1420.0±523.9	483.9±134.2	0.22
Autonomic	812.5±323.4	358.5±84.8	0.11
Neuroglycopenic	607.5±222.5	513.8±84.5	0.65
Total Cognitive Dysfunction Symptoms§	6(-0.81,22)	2(0.06, 7.02)	0.30
Hormones AUC			
Metanephrines	55745.0±8083.0	49325.4±6662.5	0.55

Data shown are mean ±SEM or Median (IQR)
* Independent sample T-Test or Mann Whitney U Test
§ Confusion, Difficulty in speaking, ClumsinesS

Glucose (mmol/l) threshold at which cognitive function deteriorated in End RCT clamped hypoglycaemia in all participants who took part in ACE genotype sub study

	DD	Non DD (ID/II)	*p value
Stroop Colour word	3.4±0.2 N=7	2.9±0.2 N= 12	0.18
Four choice reaction time	3.1±0.2 N=7	3.0±0.1 N=11	0.75

Data shown are mean ±SEM
* Independent sample T-Test

Cognitive Function Tests in End RCT clamped ypoglycaemia in all participants - at risk 'DD' Vs 'Non DD' (ID/II) ACE genotypes

	DD N=8	Non DD (II/ID) N=13	*p value
Cognitive Function AUC			
Four Choice Reaction Time	6649.2±1250.5	5572.8±886.4	0.48
Stroop Black & White	9567.5±279.3	9833.1±288.9	0.54
Stroop Colour	14352.5±1040.9	14556.9±557.8	0.65
Stroop Colour Word	20265.0±1056.9	19876.9±756.8	0.76

Data shown are mean ±SEM
* Independent sample T-Test

Appendix 7

Publications during PhD

- **Exhaled Breath Isoprene Rises During Hypoglycemia in Type 1 Diabetes**: ***Sankalpa Neupane****, Robert Peverall, Graham Richmond, Tom P.J. Blaikie, David Taylor, Gus Hancock, Mark L. Evans*, Diabetes Care 2016 Jul; 39(7): 97-98.

- **Accuracy and Longevity of an Implantable Continuous Glucose Sensor in the PRECISE Study**: A 180-Day, Prospective, Multicenter, Pivotal Trial, *Jort Kropff, Pratik Choudhary,* ***Sankalpa Neupane****, Katharine Barnard, Steve C. Bain, Christoph Kapitza, Thomas Forst, Manuela Link, Andrew Dehennis, J. Hans DeVries*, Diabetes Care 2017 Jan; 40 (1): 63-68.

- **Acceptability of Implantable Continuous Glucose Monitoring Sensor:** *Barnard KD, Kropff J, Choudhary P,* ***Neupane S****, Bain SC, Kapitza , Forst T, Link M, Mdingi C, DeVries JH,* J Diabetes Sci Technol. 2017 Oct:1-5

Appendix 8

Presentations during PhD

- **Detecting Hypoglycaemia in Breath- Exhaled Isoprene and Acetone as Biomarkers of Blood Glucose**. ***S. Neupane****, G. Richmond, T. Blaikie, R. Peverall, D. Taylor, I. Campbell, G. Hancock, M.L. Evans*, European Association of Study of Diabetes, Stockholm, Sweden , September 2015(Poster)[372] .

- **Risk of hypoglycaemia in Type 1 diabetes is associated with homozygous deletion (DD) allelic variation in the angiotensin-**

converting enzyme (ACE) in the Hypo-COMPASS study. ***S Neupane****, L Leelarathna, S Little, E Walkinshaw, HK Tan, A Lubina-Solomon, S Barendse, J Speight, D Kerr, D Flanagan, S Heller, J Shaw, M L Evan*s, Diabetes UK Annual Professional Conference, Liverpool, March 2014 (Oral)[373].

- **Accuracy and Longevity of an Implantable Continuous Glucose Sensor in the PRECISE Study**: **A 180-Day, Prospective, Multicenter, Pivotal Trial**. *J H Devries, J Kropff, P Choudhary,* ***S Neupane****, S C Bain, C Kapitza, T A Forst, M Link, A D Dehennis*, American Diabetes Association, 76th Scientific Sessions, New Orleans, Louisiana, June 2016 (Poster)[374].

Appendix 9

Study related responsibilities

A. *HypoCOMPaSS sub Analysis: Examination for potential predictors of severe hypoglycaemia from baseline hypoglycaemic clamp study (chapter 2)*

The main HypoCOMPaSS was planned and commenced before I started my PhD.

1. Development of statistical analysis plan
2. Data cleaning and statistical analysis
3. Writing manuscript

B. *HypoCOMPaSS ACE genotype sub study (chapter 4)*

1. Genotyping of the DNA samples
2. Development of statistical analysis plan
3. Data cleaning and statistical analysis
4. Providing report and update to the Ethics Committee
5. Writing manuscript
6. Presentation of results at the diabetes UK Annual Professional Conference 2014 and Institute of Institute of Metabolic Science, Away Day meeting November 2013.

C. Novel Accuracy & Use of a novel Continuous Implanted Glucose Sensor (chapter 4)

1. Producing study-related documents, such as study guidelines booklet, Hypoglycaemia and Hyperglycaemia SOP etc.
2. Recruitment of participants
3. Liaising with participants and WTCRF and coordinating multiple in-clinic visits
4. Leading the study and conducting all aspects of the study including Insertion & removals of sensors, Hyperglycaemia & hypoglycaemia challenge, training subjects on using the system etc.
5. Providing report and update to the Data & Safety Monitoring Board
6. Development of statistical analysis plan
7. Data cleaning and statistical analysis
8. Contribution to manuscript
9. Co-author when overall PRECISE I study findings presented in American Diabetes Association meeting 2015

D. Exhaled Breath Volatiles Organic Compounds during Hypoglycaemia in Type 1 Diabetes (chapter 5)

1. Contribution to study design and protocol
2. Producing study-related documents, such as Participant Information Sheet, Consent form and GP letter
3. Application for ethics application and gaining approval
4. Recruitment of participants
5. Contacting the subjects to make any necessary changes in insulin regimen prior to the participants attending the study in the Wellcome Trust Clinical Research Facility (WTCRF)
6. Performing all aspects of the clamp procedure including sampling of blood, breath and making changes to the continuous dextrose infusion (initially under supervision by Dr Mark Evans, subsequently independently)
7. Providing report and update to the Ethics Committee.
8. Development of statistical analysis plan
9. Data cleaning and statistical analysis
10. Writing manuscript
11. Presentation of results at the 51st EASD Annual Meeting, Stockholm, Sweden, 2015

E. In vitro stability of diluted insulin aspart in insulin pump (CSII) (chapter 7)

1. Contribution to study design
2. Liaising with biochemistry lab
3. Development of statistical analysis plan
4. Data cleaning and statistical analysis
5. Writing manuscript

Appendix 10

HypocCOMPaSS Study Protocol

Little *et al. BMC Endocrine Disorders* 2012, **12**:33
http://www.biomedcentral.com/1472-6823/12/33

BMC Endocrine Disorders

STUDY PROTOCOL Open Access

Comparison of Optimised MDI versus Pumps with or without Sensors in Severe Hypoglycaemia (the Hypo COMPaSS trial)

Stuart Little[1], Thomas Chadwick[2], Pratik Choudhary[3], Cath Brennand[2], Julia Stickland[2], Shalleen Barendse[4], Tolulope Olateju[5], Lalantha Leelarathna[6], Emma Walkinshaw[7], Horng K Tan[8], Sally M Marshall[1], Reena M Thomas[1], Simon Heller[7], Mark Evans[6], David Kerr[5], Daniel Flanagan[8], Jane Speight[4,9,10] and James AM Shaw[1*]

Abstract

Background: Severe hypoglycaemia (SH) is one of the most feared complications of type 1 diabetes (T1DM) with a reported prevalence of nearly 40%. In randomized trials of Multiple Daily Injections (MDI) and Continuous Subcutaneous Insulin Infusion (CSII) therapy there is a possible benefit of CSII in reducing SH. However few trials have used basal insulin analogues as the basal insulin in the MDI group and individuals with established SH have often been excluded from prospective studies. In published studies investigating the effect of Real Time Continuous Glucose Monitoring (RT-CGM) benefit in terms of reduced SH has not yet been demonstrated. The primary objective of this study is to elucidate whether in people with T1DM complicated by impaired awareness of hypoglycaemia (IAH), rigorous prevention of biochemical hypoglycaemia using optimized existing self-management technology and educational support will restore awareness and reduce risk of recurrent SH.

Methods/design: This is a multicentre prospective RCT comparing hypoglycaemia avoidance with optimized MDI and CSII with or without RT-CGM in a 2×2 factorial design in people with type 1 diabetes who have IAH. The primary outcome measure for this study is the difference in IAH (Gold score) at 24 weeks. Secondary outcomes include biomedical measures such as HbA1c, SH incidence, blinded CGM analysis, self monitored blood glucose (SMBG) and response to hypoglycaemia in gold standard clamp studies. Psychosocial measures including well-being and quality of life will also be assessed using several validated and novel measures. Analysis will be on an intention-to-treat basis.

Discussion: Most existing RCTs using this study's interventions have been powered for change in HbA1c rather than IAH or SH. This trial will demonstrate whether IAH can be reversed and SH prevented in people with T1DM in even those at highest risk by using optimized conventional management and existing technology.

Trial Registration: ISRCTN52164803 Eudract No: 2009-015396-27

Background

Type 1 diabetes mellitus (T1DM) accounts for 5–10% [1] of those with diabetes and is characterised by an absolute deficiency of insulin caused by immunologically mediated damage to the beta-cells in the pancreas. Onset can occur at any age but is most commonly in children, adolescents and young adults. Complications include microvascular disease of the kidneys, eyes and nervous tissue in addition to macrovasular disease such as ischaemic heart disease, cerebrovascular disease and peripheral vascular disease.

As there is no cure for T1DM, management entails regulation of blood glucose levels with insulin replacement therapy and dietary modification. There is incontrovertible evidence from the landmark DCCT [2] and the follow-up EDIC [3] study that microvascular and macrovascular complications can be prevented by rigorous avoidance of high glucose levels.

* Correspondence: jim.shaw@ncl.ac.uk
[1]Institute of Cellular Medicine, The Medical School, Newcastle University, 4th Floor William Leech Building, Framlington Place, Newcastle upon Tyne NE2 4HH, UK
Full list of author information is available at the end of the article

Despite 90 years' clinical experience with insulin replacement therapy, however, severe hypoglycaemia (SH) remains the major factor limiting optimal glycaemic control [4]. In a retrospective epidemiological survey of an unselected population with T1DM the prevalence of SH was reported to be 37% over a one year recall period [5]. SH remains one of the most feared complications of insulin therapy as it can result in collapse without warning, fits, or even sudden death [6,7]. Tight glycaemic control in the DCCT attained by MDI (multiple daily injections) or CSII (continuous subcutaneous insulin infusion) was associated with a three-fold increase in SH [2].

Established risk factors for SH include age, duration of diabetes, tight glycaemic control, previous SH and impaired awareness of hypoglycaemia (IAH) [8]. IAH occurs in 20% of those with T1DM and is characterised by diminished autonomic warning symptoms of impending hypoglycaemia and associated with a six-fold increased risk of SH [9-11]. Antecedent biochemical hypoglycaemia (BH) including silent nocturnal hypoglycaemia can induce IAH in addition to diminished counter-regulatory hormone response in people with established diabetes [12].

In insulinoma patients, surgical resection restores normal symptomatic and neuroendocrine response to hypoglycaemia providing further evidence of the direct causative role of BH in IAH and SH [13]. Rigorous avoidance of hypoglycaemia by relaxing glycaemic targets while maintaining conventional MDI therapy has been shown to restore hypoglycaemia awareness with normalisation of glycaemic thresholds for symptoms and neuroendocrine responses during a stepped hyperinsulinemic–hypoglycaemic clamp study [14,15]. This was, however, associated with a 0.4–1.1% (4–12 mmol/mol) increase in HbA_{1c}. Moreover, success has previously been confined to those with relatively short duration of diabetes [7] or transiently following a brief period of absolute hypoglycaemia avoidance in those with longer duration diabetes [8].

The potential for reducing nocturnal and late postprandial hypoglycaemia by employing rapid-acting insulin analogues pre-prandially has been demonstrated [16-18]. In addition, reduced nocturnal hypoglycaemia has been reported in insulin glargine trials [19-23] and insulin detemir trials [24-27]. However, individuals with a previous history of IAH and SH and longer duration of diabetes have typically been excluded from randomized clinical trials investigating insulin analogues. The National Institute for Health and Clinical Excellence (NICE) in the UK has recommended further studies to assess the impact of insulin analogues on duration and severity of hypoglycaemia and on quality of life (QoL) [28].

In randomized trials of CSII versus MDI, a relatively modest improvement in HbA_{1c} has been demonstrated in addition to the potential for reduction in the incidence of SH [29,30]. There have been relatively few trials to date with glargine as the basal insulin in the MDI comparator group [30,31]. A Cochrane review has indicated a possible benefit of CSII as compared to MDI in reducing SH but data also indicated no benefit of CSII in reducing non-severe hypoglycaemic events [32]. Individuals with established SH have again often been excluded from prospective studies, despite a reported sustained reduction in the incidence of SH in a non-randomized, retrospective study [33]. NICE recommends CSII therapy where achievement of optimal glycaemic control has been precluded by disabling hypoglycaemia but has emphasized the absence of studies in high-risk individuals together with the need for randomized control trials to assess biomedical and psychosocial outcomes of both analogue MDI and CSII in those with established SH [34].

The potential role of real time continuous glucose monitoring (RT-CGM) has generated considerable interest among clinicians and those with T1DM since its introduction. Improved overall glycaemic control has been reported, though benefit in terms of reduced SH has not yet been demonstrated [35,36]. Studies that have compared RT-CGM integrated with CSII with analogue regimens have also failed to demonstrate a difference in rates of SH although they have suggested reduced HbA1c with the technology [37,38]. There is evidence that RT-CGM can significantly reduce the time spent with a blood glucose <3.5 mmol/l [39], however there is no evidence from the major RCTs published that it can prevent SH. This may be due to the study design and participant selection criteria of these trials. Sustained avoidance of BH achieved through feedback from RT-CGM use with the aim of restoring hypoglycaemia awareness and preventing risk of further SH in high-risk individuals with T1DM has not been assessed.

Despite implicit acknowledgement amongst healthcare professionals that SH impairs an individual's QoL, there is little formal evidence for this in the literature. Davis *et al.*, have demonstrated the major impact of SH on perceived health and well being [40] but the full impact of SH on QoL has not been assessed adequately. Relatively few studies have directly assessed impact of successful prevention of further SH in addition to differential effects according to therapeutic intervention.

We have previously conducted a 6-month randomised prospective pilot study in individuals with T1DM complicated by SH, comparing rigorous BH avoidance with optimised analogue MDI; CSII; or education alone (EDUC) [41]. This demonstrated absolute prevention of recurrent SH in 71% in all groups. Quantitative improvement in IAH was confirmed using the validated Clarke questionnaire [42] in addition to restored symptomatic response to clamp-induced hypoglycaemia. Concomitant improvement in glycaemic control (HbA1c) was achieved with MDI

(baseline: 8.6 ± 1.1%, endpoint: 7.6 ± 0.7%, p = 0.04) and CSII (baseline: 8.5 ± 1.9%, endpoint: 7.4 ± 1.0%, p = 0.06) but not EDUC (baseline: 8.5 ± 1.1%, endpoint: 8.3 ± 1.0%, p = 0.54). Significant improvements in diabetes-specific QoL and fear of hypoglycaemia were also demonstrated in MDI and CSII groups. Although in this study RT-CGM was not used, these pilot data provide the rationale, robust power calculation and proven study design for a definitive RCT, without the requirement for an education alone arm.

Study objectives

Primary objective

- To demonstrate that by optimising conventional management, including the use of real time continuous glucose monitoring (RT-CGM), in individuals with T1DM complicated by IAH, rigorous prevention of BH will restore awareness and reduce risk of recurrent SH.

Secondary objectives

- To quantify and compare BH identified by self-monitored blood glucose (SMBG) and blinded CGM profiles during each intervention.
- To quantify and compare overall glycaemic control and glucose lability in each group by analysis of HbA1c, SMBG and blinded CGM.
- To quantify and compare total daily doses of insulin before and after the intervention period.
- To compare health utility, well-being and QoL during each intervention using validated and novel measures.
- To perform secondary analyses of those who continue to experience IAH regardless of study intervention, to determine factors associated with absence of response. It is hypothesised that these will include two sub-groups: one in whom an absolute focus on avoidance of high glucose (evidenced from patient-reported outcome (PRO) measures) leads to continued biochemical hypoglycaemia despite the study goals; and a second with severe autonomic neuropathy (evidenced from clinical history) who are unable to recover autonomic warning symptoms of hypoglycaemia despite effective reduction in biochemical hypoglycaemia.
- To determine symptomatic, counter-regulatory hormone and cognitive response to hypoglycaemia in gold standard clamp studies. Comparisons will be made between those randomised to CSII and those randomised to MDI; in addition to RT-CGM versus no RT-CGM; and responders with restored hypoglycaemia awareness versus non-responders with persistent IAH despite study intervention.

Methods/design

Ethical and governance approval

Ethical approval for this study has been granted by Sunderland Research Ethics Committee (09/H0904/63) and Clinical Trial Authorisation has been given by the Medicines and Healthcare products Regulatory Agency (17136/0246/001-0001). Site Specific Approval has been granted by all participating Acute Hospital Trust Research and Development Departments.

Study design

The study is an interventional multicentre prospective RCT comparing hypoglycaemia avoidance with optimised subcutaneous insulin analogue regimen (MDI) and insulin pump therapy (CSII) with or without adjunctive RT-CGM in a 2×2 factorial design (Figure 1). The trial design is consistent with the CONSORT Statement [43].

Participants

Participants will be recruited from the diabetes clinics of the five participating UK tertiary referral and academic hypoglycaemia/CSII centres (Royal Bournemouth Hospital, Bournemouth; Addenbrooke's Hospital, Cambridge; Newcastle Diabetes Centre, Newcastle upon Tyne, UK; Derriford Hospital, Plymouth; Northern General Hospital, Sheffield). Patients with T1DM, a history of IAH and increased risk of SH will be assessed to determine if they are eligible for the study. It is envisaged that the majority of these will have experienced SH within the preceding year, although this is not mandatory for study participation. Those who are considered potentially eligible will be approached to give their written informed consent before attending for a screening visit at which inclusion criteria will be checked.

Inclusion criteria

Individuals who are aged 18–74 years and have a diagnosis of diabetes mellitus according to ADA [44] / WHO [45] criteria and consistent with a clinical diagnosis of T1DM. Participants will have:

- serum C-peptide below the quality assured limit of detection for the assay and laboratory (<50 pmol/L) with simultaneous exclusion of BH (glucose <4.0 mmol/l) by laboratory plasma glucose assay.
- impaired awareness of hypoglycaemia, as confirmed by a Gold score ≥4 [11].

Exclusion criteria

- Any condition that in the investigator's judgement is likely to cause the participant to be unable to understand the information in the Informed Consent Document or to provide informed consent.

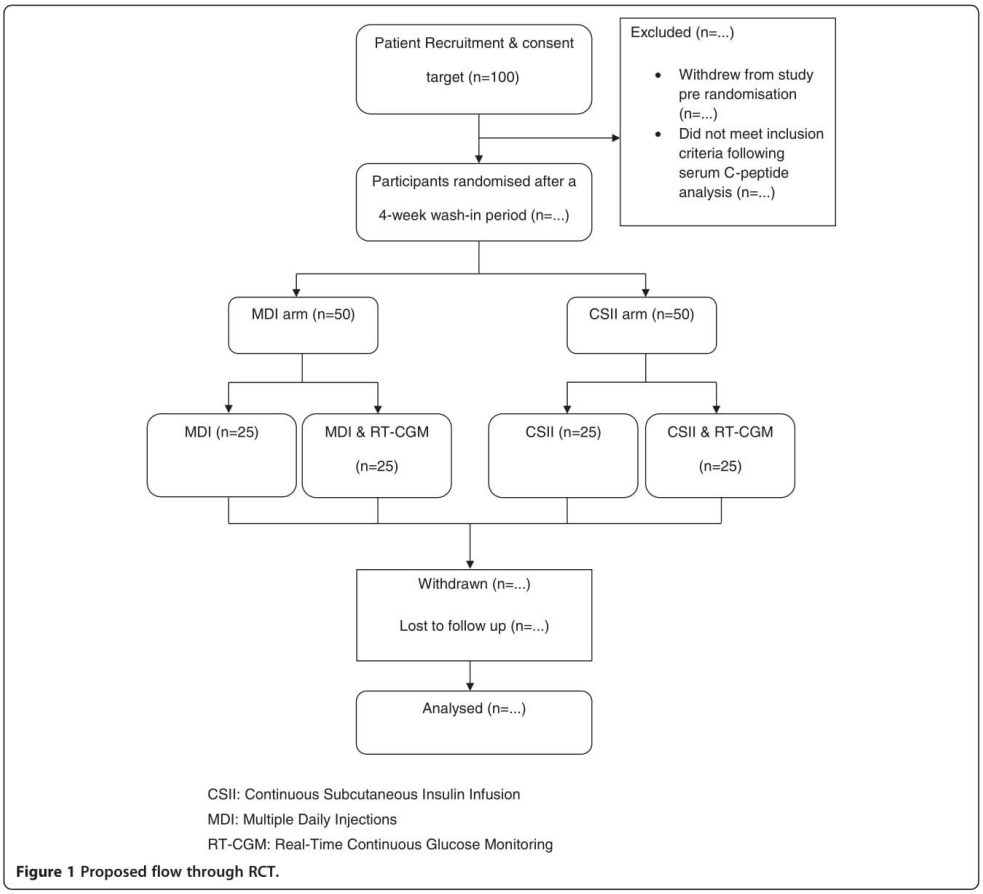


Figure 1 Proposed flow through RCT.

- Insufficient proficiency in English, below that to enable the participant to understand both verbal and written information during the study. This is due to the complexity of the education programme, the need for independent completion of the questionnaire measures, and the degree of communication required between participants and clinicians during the study.
- Unwilling to undertake intensive insulin therapy, including randomisation to use of CSII, optimised MDI regimen or RT-CGM.
- Unwilling to undertake glucose profiles using the subcutaneous continuous glucose monitoring (CGM) equipment.
- Unwilling to use SMBG at least 4 times daily.
- Unwilling to monitor and record signs and symptoms of hypoglycaemia.
- A history of intolerance to insulin glargine.

Trial intervention and study procedures

Schedule for study visits is given in Table 1.

Four week baseline period

After consent, participants will undertake a 4-week wash-in period before the 24-week RCT period. Participants will be educated in the use of the study-specific prospective SMBG hypoglycaemia diary. All participants will be provided with a study hand-held glucometer (Contour link®, Bayer Healthcare) to measure daily 4-point and weekly 8-point profiles. They will be asked to record clinical

Table 1 Visit schedule

Study period	4 week wash in period			24 week primary RCT																	18 month RT-CGM RCT continuation					
Visit number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
informed consent	x																										
eligibility criteria	x																										
given information sheet on clamp study	x																		x								
hypoglycaemia screening questionnaire	x		x																								
HbA1c	x		x							x		x		x		x		x		x			x		x		x
C-peptide and plasma glucose	x																										
retinal photographs		x																									
urine albumin:creatinine ratio		x																									
demographic info			x																								
concomitant medication			x							x		x		x		x		x		x			x		x		x
full physical examination			x																	x							
history of glycaemic control										x		x		x		x		x									
full medical history including glycaemic control			x																	x			x		x		x
vital signs			x							x		x		x		x		x		x			x		x		x
height			x																								
weight			x							x		x		x		x		x		x			x		x		x
TFTs					x																						
Coeliac antibody					x																						
short synacthen test					x																						
detailed SH history			x							x		x		x		x		x		x			x		x		x
Modified Clarke/Edinburgh			x																	x			x		x		x
QoL questionnaires			x																	x			x		x		x
week 4 short questionnaire pack										x																	
4 week Blood Glucose / hypo diary	x		x							x		x		x		x		x		x							
7 day CGMS placement		x							x		x		x		x		x		x			x		x		x	
Autonomic function tests				x																	x						
clamp study			x																x								
education programme						x																					
Insulin administration education session							x																				
Home glucose monitoring/RT education session								x																			

details of all glucose levels <4 mmol/l and symptomatic hypoglycaemic events. All participants will wear a blinded CGM device (iPro™ 1, Medtronic) for 7 days during the baseline period.

At the end of the wash-in period, participants will attend for a baseline study visit where the investigator will complete the GCP-compliant baseline Case Report Form (CRF). This will record demographic information, full diabetes-specific clinical history including frequency and consequences of SH events over the preceding 12 months and full clinical examination assessing complication status. The study SMBG / hypoglycaemia diary will be collected and CGM device downloaded. In addition, hypoglycaemia awareness will be re-evaluated using the validated Gold Score [11] and Clarke questionnaire [42].

A first morning urine sample for albumin: creatinine ratio will be collected together with dilated retinal photography if not performed within the preceding 6 months. A

blood sample will be taken for HbA1c (DCCT-aligned), urea and electrolytes, liver function tests and lipid profile analysed at local site quality-assured clinical laboratory. Study-specific PRO questionnaire booklets will be completed comprising several validated and novel measures. During design of and preparation for the study, new measures were developed by the team to capture aspects of participant experience for which validated measures did not exist (e.g. hypoglycaemia cues) or where there was specific concern that existing measures were insensitive (e.g. the Gold and the Clarke have been demonstrated to lack sensitivity to improvements in awareness). All PRO measures included in the evaluation are listed in Table 2.

Baseline hypoglycaemic clamp study

A stepped hyperinsulinaemic hypoglycaemic clamp study will be conducted at baseline and at 24 weeks with participants who are willing to undergo this procedure for which specific consent will be sought separately. It is envisaged that approximately 25% of participants will be studied. The method is described in detail later.

Autonomic function testing

Before the start of the RCT, participants will attend for non-invasive detailed cardiac autonomic function testing using the recommendations in the review by Tesfaye et al. [46]. These will include heart rate response to deep breathing, a Valsalva maneuver and heart rate and blood pressure response to standing interpreted using age normative values. Spectral analysis of heart rate variability and assessment of cardiac vagal baroreflex sensitivity (BRS) will be carried out over a five minute period of controlled breathing. Structured assessment of global autonomic function will be made using the Autonomic Symptom Profile Questionnaire [47].

Concomitant autoimmune disease screening

Participants will attend for a short synacthen test to screen for adrenocortical insufficiency. At this visit a sample will also be taken for serum thyroid stimulating hormone assay to exclude thyroid disease and for anti-endomysial antibody analysis to exclude coeliac disease. New diagnoses of other autoimmune diseases will not preclude participation in the study. If indicated participants with newly diagnosed autoimmune disease will be referred to an appropriate specialist for further investigation and management.

Education visit

Following experience in the pilot study [41] and informed by insights from the qualitative study undertaken in preparation for the RCT (paper submitted), a brief education programme (with formal curriculum and workbook, referred to as the 'My Hypo COMPaSS' tool), was developed. Participants will attend a brief education session (approximately 3 h) individually or in small groups of up to four. During the session, a trained research fellow, specialist nurse or dietician will facilitate discussions and exercises targeted specifically at rigorous avoidance of BH while maintaining overall glycaemic control [48], including four key elements forming the four points of the 'Hypo COMPaSS' establishing the imperatives: to never delay the treatment of hypoglycaemia and the optimal treatments for hypoglycaemia; to recognise the individual's unique times of increased risk; to recognise hypoglycaemia by the presence of subtle symptoms; to be particularly careful about detecting and preventing nocturnal hypoglycaemia. Also included will be advice on self-adjustment of insulin doses according to carbohydrate intake, SMBG and planned activity and recommendation for oral carbohydrate administration for all glucose levels less than 4 mmol/l.

Table 2 Validated and novel patient reported outcome measures assessed during the Hypo COMPaSS trial

Validated PRO measures	Novel PRO measures undergoing validation
• The Gold Score [44]	• The Hypoglycaemia Awareness Questionnaire (HypoA-Q)*
• The Edinburgh Hypoglycaemia Survey [54]	• The Hypoglycaemia Burden Questionnaire (HypoB-Q)* – part A only
• Clarke Hypoglycaemia Awareness Questionnaire (minimally modified version)	
• The Hypoglycaemia Fear Survey II (HFS II) [55]	• The Hypoglycaemia Cues Questionnaires (HypoC-Q)*
• The Hyperglycaemia Avoidance Scale [56]	• The Blood Glucose Monitoring Questionnaire (BGM-Q)*
• The Diabetes Treatment Satisfaction Questionnaire – status version (DTSQ(s)) [57]	• The Quality of Life Questionnaire Diabetes (QoL-Q Diabetes)^
• Insulin Treatment Satisfaction Questionnaire [58] – abridged version including two subscales only	• The Attitudes to Awareness of Hypos Questionnaire#
• EuroQoL EQ-5D [59]	
• Perceived Control of Diabetes scales (type 1) [60]	

* designed by Prof Jane Speight and Dr Shalleen Barendse (© AHP Research, 2010).
^ designed by Prof Jane Speight, Dr Alison Woodcock and Matthew Reaney (© AHP Research, 2007).
# designed by Dr Nicole DeSoyza, Helen Rogers and Prof Stephanie Amiel (King's College London).

Intervention period

Randomisation

Participants will be allocated by third party concealed randomisation by centre and baseline HbA1c (with stratification cut-off of 64 mmol/mol (8%) to one of four groups for a period of 24 weeks):

- MDI with SMBG (group 1; n = 25).
- MDI with SMBG and RT-CGM (group 2; n = 25).
- CSII with SMBG (group 3; n = 25).
- CSII with SMBG and RT-CGM (group 4; n = 25).

As this is an open study it is not possible for participants to be blinded to study treatment arm. Randomisation will be administered centrally by Newcastle Clinical Trial Unit (NCTU) using a secure web based system.

Study Interventions

The primary goal of titration throughout the 24-week RCT period will be the absolute avoidance of all glucose levels <4 mmol/l as determined by CGM and SMBG. This will be achieved by setting '4 as the floor' with all glucose levels <4 mmol/l treated by 15 g glucose with repeat SMBG every 15 min until glucose >4 mmol/l, in addition to consideration of insulin dose reduction.

The trial is designed to prevent any potential bias from additional educational support provided to those randomised to CSII or RT-CGM. All participants will be provided with a Medtronic Veo insulin pump to enable use of the bolus prandial insulin dose wizard calculator whether or not they are administering insulin by CSII. All will be provided with a Contour link SMBG meter enabling direct transmission to the pump dose calculator. Access to Carelink glucose self-management software will also be provided to all participants to be used optionally to support attainment of study targets without any specific additional goals / training.

All participants (regardless of treatment arm) will attend an education session solely on the technical aspects of the insulin administration or glucose monitoring equipment they will be using during the intervention period, i.e. participants randomised to CSII will receive education restricted to technical aspects of insulin pump management including on the need to change the infusion set at least every 72 h; participants randomised to MDI will receive education restricted to insulin device (pen) use and injection site care.

Participants randomised to RT-CGM will receive education restricted to the technical aspects of using the RT monitors including trend analysis and the use of the hypoglycaemia and hyperglycaemia alarms. They will be encouraged to wear the sensor continuously (re-siting every 7 days) but flexibly with a minimum of 7 days continuous monitoring in the last week of each month. Participants not randomised to RT-CGM will receive education restricted to the technical aspects of using the Contour link meter with the bolus calculator on the pump.

Participants will be seen one week after starting the study intervention to review progress over the first week, using glucose data to achieve the primary goal of avoiding biochemical hypoglycaemia.

Study drugs and devices

In accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004 and Directive 2001/20/EC, the drugs under investigation in this trial fall under the definition of 'investigational medicinal product' (IMP). Although the insulins used during this trial fall under the definition of IMPs they will be used under existing licence.

Insulin for MDI participants

For the participants randomised to MDI, insulin aspart will be given as 3 ml cartridge 100 Units/mL in a pre-filled pen (*Flexpen*®). Insulin glargine will be given as 3 ml cartridge 100 Units/mL in a pre-filled pen (*SoloStar*®). However, for those participants who have had a previous negative experience or adverse effect with insulin aspart, insulin lispro will be offered as 3 ml cartridge 100 Units/mL in a pre-filled pen (*Kwikpen*®).

Insulin for CSII participants

For participants randomised to CSII, insulin aspart will be the insulin used as 10 ml vial 100 Units/mL. Insulin lispro can be used instead of insulin aspart for those who have had previous negative experience / adverse effects.

Insulin pumps

All participants will be given an insulin pump that can receive and display CGM data (Mini-Med Paradigm Veo insulin pump) and will be taught how to use the on-board bolus calculator. Only those randomised to CSII will use the pump for insulin administration, the MDI groups will only use the bolus calculator feature and, if randomised to RT-CGM, the CGM feature. All participants randomised to CSII (insulin pump therapy) will be given a single additional session restricted to technical aspects of pump management.

Self Monitoring of Blood Glucose (SMBG)

All participants will be required to undertake daily 4-point and weekly 8-point self-monitored capillary glucose profiles. All participants will use the Contour link®, Bayer Healthcare meter and will undertake this whether or not concurrent RT-CGM is being used.

Real time—continuous glucose monitoring

Fifty per cent of all participants will be randomised to real time monitoring, using the CE-marked REAL time continuous glucose monitor (Medtronic).

Advice on interpretation and action from SMBG / RTCGM

All participants will attend an education session on the recording of SMBG but, for those participants randomised to RT-CGM, this session will also include the technical aspects of using the monitor. This includes trend analysis, hypoglycaemia and hyperglycaemia alarms. Participants will be given written instructions on how to use the data provided by continuous glucose monitors to make real-time adjustments of insulin doses and on the use of computer software (for those with a home computer) to retrospectively review the glucose data to alter future insulin doses. Participants randomised to RT-CGM will be encouraged to wear the sensor continuously but flexibly with a minimum of 7 days continuous monitoring in the final week of each month. Those participants randomised to RT-CGM will be advised to re-site the sensor every 7 days.

Blinded continuous glucose monitors

Blinded CGM will be undertaken using the CE-marked Medtronic wireless iPro system. These will be used in the 4-week wash in period and during the last seven days of every month. The patients and investigators are blinded to all of this data until the end of the primary 24 week RCT.

Insulin titration protocol

The blood glucose targets (for all patients in CSII and MDI groups) will be as follows:

Fasting blood glucose (FBG): 5.0 - 7.0 mmol/l.
Pre-prandial blood glucose: 4.5 - 7.0 mmol/l.
Post-prandial glucose*: 6.0 – 8.0 mmol/l.
Bedtime blood glucose**: 6.0 – 8.0 mmol/l.
4 am blood glucose: 5.0 – 7.0 mmol/l.

*postprandial blood glucose: measurement made 2 h after the start of a meal.

**bedtime blood glucose: measurement made within 30 min of retiring to bed for the night.

Glargine titration in MDI group

Insulin glargine will be self-administered and the following titration protocol will be followed:

- Take within 30 min of retiring to bed for night / no need for snack.
- Aim for stable (not falling) glucose through the night.
- Reduce dose if any hypoglycaemic episodes or glucose <5.0 mmol/l between 4 am and before breakfast.
- Target glucose of 5–7 mmol/l before breakfast—adjust dose by 1–2 units to maintain target if necessary with primary aim being absolute avoidance of BH (Biochemical Hypoglycaemia).
- During periods of illness, basal insulin doses may need to be altered and this will be guided by SMBG levels.

Introduction of twice daily glargine

Participants randomised to MDI already on twice daily glargine will continue on this from the outset of the RCT. In other MDI participants, if glucose is consistently >7 mmol/l before evening meal or highly variable between breakfast and evening meal, add second dose of insulin glargine before breakfast. Initial dose will be 4 units but can be adjusted in light of participant's current insulin doses. If glucose has been falling through the night, a 2–4 unit reduction in evening glargine dose will be actioned before bed on the day of commencing the morning dose. The addition of a second daily glargine dose will be considered for all participants in the MDI group. This can be initiated between study visits if necessary, e.g. after telephone advice.

Morning insulin glargine will be self-administered and adjusted as follows:

- Take within 30 min of rising from bed for the morning.
- Aim for stable (not falling) glucose through the afternoon.
- Reduce dose if any hypoglycaemic episodes or glucose <5 mmol/l between 2 h after lunch and evening meal.
- Target glucose of 5-7 mmol/l before evening meal – adjust dose by 1–2 units to maintain target if necessary with primary aim being absolute avoidance of BH.

Basal insulin titration in CSII group

The basal insulin delivery rate will be titrated according to fasting, bedtime, pre-prandial and 4 am glucose levels ensuring absence of recurrent low glucose levels at these times (checkpoints). Increased or decreased delivery will be commenced from the previous basal insulin checkpoint level, i.e. if low at 4 am—decrease from bedtime; if high fasting increase from 4 am.

Mean fasting; bedtime; 4 am and pre-prandial blood glucose:

- Within target: No change to basal delivery rate.
- Above target: Increase basal insulin by 0.1 U/hr from previous check point.

- Below target or unexplained late post-prandial hypoglycaemia: Decrease basal insulin by 0.1 U/hr from previous check point.

During periods of illness, basal insulin rates may need to be altered and this will be guided by SMBG levels.

Meal-time insulin bolus in all groups (CSII and MDI)

Carbohydrate counting skills and bolus dose adjustment in light of current blood glucose level / individualised insulin carbohydrate ratios will be reviewed in all participants. Aspart or lispro will be delivered either by subcutaneous injection or as a subcutaneous pump bolus before all meals and snacks with substantial carbohydrate content.

Insulin: carbohydrate ratios will be calculated for all individuals using the '500 rule' and using total daily insulin doses pre-randomisation. The '500 rule' is:

500 divided by the TDD (Total Daily Dose of insulin) = grams of carbohydrate covered by one unit of aspart or lispro.

In the event of high pre-prandial glucose levels corrective doses will also be recommended with meals as part of the meal time bolus. This will be calculated using the '100 rule' for estimation of Insulin Sensitivity Factor. The '100 rule' is:

100 divided by the TDD (Total daily Dose of insulin) = glucose drop in mmol/l per 1 unit of aspart or lispro.

This will be presented to all participants as '*1 unit of aspart / lispro will reduce your blood glucose by x mmol/l*'.

Corrective doses with all pre-main meal boluses / prandial insulin injections will be encouraged according to the 100 rule when glucose level is above target.

The insulin: carbohydrate ratio and Insulin Sensitivity Factor for that period of the day will be adjusted accordingly in the event that:

- The glucose level is consistently below or above target 2 h after a bolus / prandial insulin injection.
- If any unexplained hypoglycaemic event occurs 2 h after a bolus / prandial insulin injection.

Telephone contact

Participants will be contacted by telephone daily for the first week after starting the study intervention and thereafter weekly throughout the RCT to reinforce the primary goal of BH avoidance, provide clinical review / support, and ensure diary completion.

Study follow up

Participants will attend for a study visit every four weeks during the RCT for collection of SMBG/hypoglycaemia diary and HbA1c. One week prior to each visit participants will have a blinded CGM device fitted and these data will be downloaded at the visit. Participants will have their weight measured at each follow up visit and this, along with details of insulin dosage, will be recorded on visit specific CRFs. Both investigator and participant will remain blinded to the results of the blinded CGM data during the RCT period. Clinical review at each follow up visit will reinforce the primary goal of BH avoidance. At each follow up visit information will be collected on any episodes of hypoglycaemia experienced, duration of RT-CGM monitoring usage and RT-CGM alarm settings.

At week 24, participants will attend for the primary RCT completion visit. This will include blinded CGMS data download, collection of SMBG/hypoglycaemia diary, and HbA1c. Participants will also be asked to complete the 'end of RCT' study-specific questionnaire booklets assessing hypoglycaemia experience and other PROs (Table 2).

On the same day, participants will be invited to attend for 'end of RCT' stepped hyperinsulinaemic hypoglycaemic clamp as described below.

At the end of this intervention period, participants will be asked to attend for repeat detailed cardiac autonomic function testing and subjective assessment of global autonomic symptoms.

Post primary RCT follow-up

At the end of the 24-week RCT, participants will return to routine clinical care. Those randomised to CSII may stop this if they and their clinical team wish. Commencement of CSII according to NICE guidance [34] will be considered in those previously randomised to MDI. Those participants who were randomised to RT-CGM will continue with this intervention for a further 18 months constituting an overall 24 month RCT of RT-CGM augmented glucose monitoring vs SMBG alone. RT-CGM will not be offered to those not randomised to this intervention, as it is not currently recommended by NICE. All participants will be invited to attend three follow-up visits 6, 12 and 18 months post primary RCT (Table 1). Participants will be fitted with a blinded CGM device for 7 days before each follow-up visit. This visit will include collection of SMBG/hypoglycaemia diary; SMBG / CGM data download, HbA1c measurement. Participants will be required to complete questionnaire booklets (Table 2) at each follow-up visit.

The end of the study will be the last follow-up visit of the last participant scheduled for 24 months after the commencement of study interventions.

Hypoglycaemic clamp study

A stepped hyperinsulinaemic hypoglycaemic clamp study [49] will be conducted in participants who are willing to undergo this procedure for which separate informed consent will be obtained. It is envisaged that approximately 25% of participants will be studied. Additional exclusion criteria will be in place to ensure participant safety:

- Age >60 years.
- History of epilepsy (seizures not primarily induced by hypoglycaemia).
- Known ischaemic heart disease.
- Other significant disease which in the judgement of the investigator precludes participation.

Participants will be fitted with a retrospective CGM sensor to be worn typically for five to seven days (at least 24 h) preceding the study day. This will be downloaded on the morning of the study to determine whether any antecedent biochemical hypoglycaemia (BH) occurred over the 24 h period prior to the clamp. Studies will be postponed to another day if any CGM and/or self-monitored capillary glucose below 3.0 mmol/l are detected during the preceding 24 h. For participants who required rescheduling, a further 72 h of CGM will be organised. All participants will be advised to fast from 22:00 h and to avoid caffeine for 24 h before the study.

The participant will be admitted to the clinical research facility at 7 am on the day of the study. On arrival, an intravenous cannula will be inserted in the ante-cubital vein of the non-dominant arm and blood glucose will be stabilized using sliding scale insulin infusion aiming initially for blood glucose 6.0–7.0 mmol/l and then 5.0–6.0 mmol/l between 10.30 am and 11 am for clamp initiation.

A second retrograde cannula will be inserted into a vein on the dorsum of the non-dominant hand for the sampling of arterialised venous blood. The hand will be kept in a purpose-built heated box (Temp 50–60°Celsius) before inserting the retrograde cannula and throughout the clamp study. A slow intravenous infusion of saline will be used as needed to keep the sampling line patent. During this period of stabilization, participants will be shown how to perform specific cognitive function tests (Four choice reaction time [50-52] and Stroop tests [53,54]) and asked to practise the tests until they achieve consistent results (typically 5 practice sessions). Four-choice reaction time is a test of attention, discrimination and motor speed reaction while Stroop tests are a group of related sub-tests all requiring selective attention and mental tracking.

At the start of the clamp a primed infusion of 60 mU/m^2/min soluble human Actrapid insulin will be started via the non-dominant antecubital vein catheter. Dextrose infusion rates will be adjusted as needed, aiming to stabilise plasma glucose at 5.0 mmol/l at 40mins followed by step-wise lowering to 3.8 mmol/l, 3.4 mmol/l 2.8 mmol/l and 2.4 mmol/l. Each step will last 40 min allowing 20 min to achieve new target and 20 min for stabilization at that level. Samples for plasma glucose will be obtained every 5 min and analysed in real-time but participants will be blinded to these glucose levels throughout the study.

At the end of each clamp stage participants will be asked to complete a validated symptom questionnaire [55,56] followed by the four choice reaction time test and Stroop test. Each symptom will be graded on a visual analogue scale from 1 (not at all) to 7 (very severe).

Arterialised venous blood samples for insulin, catecholamines, growth hormones, glucagon and cortisol will be obtained every 10 min during the first 40 min of the study (euglycaemia) followed by every 20 min during progressive hypoglycaemia. Heart rate and blood pressure will be recorded every 20 min. In addition, spectral analysis of heart rate variability and assessment of cardiac vagal baroreflex sensitivity (BRS) will be carried out during each clamp stage. At the end of the study, insulin infusion will be reduced to basal insulin requirements and dextrose infusion increased to raise blood glucose to euglycaemia. Participants will be provided with lunch with post-meal insulin bolus and re-established on their usual insulin regimen thereafter.

Sample size

The recruitment target for the overall RCT is n = 100 participants (n = 20 from each of the five participating centres). From the pilot study data [41], the sample size of n = 100 (n = 25 in each of the four study arms) would give 80% power at a significance level of 0.05 to detect a difference of 1.1 between the IAH scores (assessed using the Gold Score) of the 50 participants randomised to either of the CSII arms and the 50 randomised to either of the MDI arms. A difference of at least one point (on the 7-point scale) is considered to be a clinically relevant change on the Gold Score. The calculation is based on the use of the 2-sample t-test and the assumption, taken from the pilot data, that the standard deviation of the IAH score is 2 (mean ± SD Gold Score in the pilot study was 2.57 ± 1.90 in the CSII arm and 4.0 ± 1.79 in the MDI arm).

Outcomes / statistical analysis

The principal analysis will examine the factorial structure of the treatment and monitoring regimen effects on the difference in IAH (Gold score) at 24 weeks using analysis of covariance (ANCOVA). Baseline IAH (Gold score) and stratification (centre and baseline HbA1c) variables will be included among the covariates to be considered in addition to suitable summaries of

questionnaire scores and glucose monitoring data collected at baseline prior to randomisation. The glucose monitoring data to be collected include time spent for the following separate ranges: <2.5 mmol/l, <3 mmol/l, <4 mmol/l, >7 mmol/l, >10 mmol/l, between 4 and 7 mmol/l and between 3 and 10 mmol/l. The inclusion of baseline HbA1c as a covariate will enable the examination of possible interactions between effects observed and these values.

Further analyses will be undertaken concerning IAH to corroborate the Gold Score; the Gold Score will be compared with scale and subscale scores derived from the Clarke Questionnaire and the Hypoglycaemia Awareness Questionnaire (HypoA-Q) at 24 weeks.

These measures will also be subject to analysis as for the primary outcome. Additionally, a binary indicator of IAH response (defined as a Gold Score of <4 or ≥4) at 24 weeks will be analysed using logistic regression making use of the covariates used for the primary outcome analysis.

There will also be an additional analysis of the (paired) change in IAH (Gold Score) over the 24-week duration of the trial using the t-test without consideration of the intervention or monitoring groups in order to evaluate the effect of undergoing any intervention or monitoring over the 24-week period.

Other outcomes will be assessed at baseline and 24 weeks. Analysis methods will generally be similar to that described for the primary analysis but alternative techniques such as McNemar's test and logistic regression will be used as appropriate.

Further analyses will be undertaken using HbA1c and the separate continuous glucose monitoring measures (time spent in the following separate ranges: <2.5 mmol/l, <3 mmol/l, <4 mmol/l, >7 mmol/l, >10 mmol/l, between 4 and 7 mmol/l and between 3 and 10 mmol/l) as outcome variables.

Similar analyses will be undertaken on scores from all PRO measure scores used in the study (Table 2).

A number of measures relating to SH (ADA criteria) will be analysed: number of episodes of SH at 24 weeks, change in SH between baseline and 24 weeks (reported as difference in annualised rate pre and post-intervention), change in SH between baseline and 24 weeks (reported as the proportion of participants with reduction in number of SH events compared between the timepoints) and change in proportion without SH between baseline and 24 weeks.

Changes in weight, total daily dose of insulin, and in glucose lability will be subject to analysis in a similar manner to the primary outcome.

Wherever possible participants who elect to withdraw from the study will be followed up so that final outcome data are obtained, enabling their inclusion in an Intention to Treat (ITT) analyses. This will form the analysis groups for the analyses described above.

Analyses restricted to those participants who were allocated to use RT-CGM will be considered, in order to allow use of the further covariate of low or high CGM use (defined by consideration of a pre-defined cut-off value) throughout the 24-week period. Variables analysed in this manner will include IAH (Gold Score), episodes of SH, HbA1c and several of the glucose monitoring measures.

Variables with missing data will be examined and the amount of missing data described. Data analysis will take the form of a complete case analysis, although imputation of values may be considered for the primary outcome variable alone should this be missing to a sufficient extent.

Significance levels will be set at $\alpha=0.05$ throughout.

Safety data are to be documented but will not be subject to statistical analysis.

A detailed analysis plan will be finalised prior to the commencement of data analysis.

Data from the clamp study will be analysed separately; however a comparison will be made between the IAH (Gold) based definition of response and a response measure derived from the clamp results.

Trial Governance

Trial management will be overseen by a Trial Management Group who will meet regularly to discuss the operational aspects of the trial. An independent Data Monitoring and Ethics Committee (DMEC) will be convened to undertake independent review, monitoring safety and efficacy endpoints. The DMEC will comprise two physicians not connected to the trial (at least one of whom will have expertise in hypoglycaemia), one statistician and one patient representative. The DMEC will have full access to unblinded study data.

A Trial Steering Committee (TSC) will supervise the trial, ensuring it is conducted to high standards in accordance with the protocol, the principles of GCP, and with regard to participant safety. This committee will have an independent chair with expertise in hypoglycaemia. In addition to the Chief Investigator (Professor James Shaw) and Principal Investigators, the TSC will consist of a sponsor/funder representative, representatives of the Newcastle Clinical Trials Unit and two consumer representatives. The TSC will also consider safety issues for the trial and relevant information from other sources, ensuring at all times that ethical considerations are met when recommending the continuation of the trial.

Discussion

Over recent years, it has become increasingly clear that SH is the major factor limiting the overall level of

glucose control achievable in those with established T1DM. We believe that this study will provide definitive evidence that recurrent SH is preventable even in the majority of those at highest risk. This will enable a truly evidence-based approach to improving day-to-day diabetes management for all those currently living with the risk and fear of SH. In addition, characteristics of the minority in whom optimised conventional therapy is likely to prove inadequate will be defined, enabling early identification and intervention.

While the interventions in this trial are widely used in an attempt to reduce the incidence of SH, there is little evidence from adequately powered RCTs supporting their use, with most existing RCTs having been powered for change in glycated haemoglobin rather than SH or IAH. The primary objective of this trial is to determine whether these treatments improve awareness of hypoglycaemia and, secondarily, to determine whether they have an impact on SH events and overall glycaemic control. Crucially, this unique trial will also determine the impact of these intensive interventions on a range of patient-reported outcomes.

The trial does pose challenges, perhaps foremost the need to ensure equal input to all study participants and not provide additional care to those randomised to pump therapy. The protocol was designed to take this into consideration. Participants randomised to MDI will also be provided with insulin pumps so that this group is not disadvantaged by not having access to a bolus calculator. Secondly, study investigators will need to remain absolutely focused on biochemical hypoglycaemia avoidance, undiluted by attempts to tighten or maintain overall glycaemic control. This may be made more challenging by existing investigator and participant behaviours. For this reason, detailed insulin titration protocols (detailed above) will be used across all sites.

This ambitious and intensive trial will demonstrate definitively whether SH, a cause of major morbidity in up to 5000 adults with T1DM in the UK, can be prevented successfully in even those at highest risk by optimised conventional management using existing technology. Overall biomedical, psychosocial and health utility impact of MDI, CSII and RT in this group will be determined employing optimised measures as detailed above.

Competing interests

No pharmaceutical company or medical device manufacturer has had any role in the design or funding of this trial.
DK has received honoraria for participation in educational events and consultancy fees from Abbott Diabetes Care, manufacturers of glucose sensors.
JAMS has in the past taken part in Medical Advisory Boards for Novo Nordisk, Sanofi Aventis, Johnson and Johnson and Medtronic.
JSp is a member of the Accu-Chek Advisory Board. In relation to this activity, JSp's research group, The Australian Centre for Behavioural Research in Diabetes, has received consultancy fees from Roche Diagnostics Australia. It is also the recipient of unrestricted educational grants from Sanofi Aventis and Medtronic, and speaker fees from Abbott Diabetes Care.
PC has received speaker fees and been on Advisory Boards for Medtronic, Johnson and Johnson and Roche.
ME has acted on Advisory Boards for Medtronic, Roche and Cellnovo and received travel support/speakers fees from Medtronic and Animas.
SH has carried out consultancy work for pump/meter, insulin companies, Lifescan, Sanofi-Aventis, NovoNordisk and Lilly. Medtronic has supplied pumps for use in one of his studies.

Authors' contribution

JAMS, JSp, DK, PC, SH, ME, DF, TO, RT, SMM and SB conceived the study and participated in its design. TC advised on the statistical analysis plan. SL, LL, HKT and EW have responsibility regarding the clinical management of the participants and helped to draft the manuscript. CB and JSt participated in the study design and coordination. All authors read and approved the final manuscript.

Acknowledgements

We thank Prof B Frier, University of Edinburgh for his advice and experience in planning this study. We also thank Prof J Matthews, Newcastle University for his advice on the statistical analysis plan. The study is funded by a peer-reviewed grant from Diabetes UK.

Author details

[1]Institute of Cellular Medicine, The Medical School, Newcastle University, 4th Floor William Leech Building, Framlington Place, Newcastle upon Tyne NE2 4HH, UK. [2]Institute of Health and Society, Newcastle University, Newcastle, UK. [3]Diabetes and Nutritional Sciences Division, Kings College London, London, UK. [4]AHP Research, Hornchurch, UK. [5]Bournemouth Diabetes Centre, Bournemouth, UK. [6]Institute of Metabolic Science, University of Cambridge, Cambridge, UK. [7]School of Medicine and Biomedical Sciences, Sheffield University, Sheffield, UK. [8]Peninsula College of Medicine and Dentistry, Plymouth, UK. [9]The Australian Centre for Behavioural Research in Diabetes, Diabetes Australia – Vic, Melbourne, Australia. [10]Centre for Mental Health and Wellbeing Research, School of Psychology, Deakin University, Burwood, Australia.

Received: 27 October 2012 Accepted: 19 November 2012
Published: 13 December 2012

References

1. Forouhi NG, Merrick D, Goyder E, Ferguson BA, Abbas J, Lachowycz K: **Diabetes prevalence in England, 2001-estimates from an epidemiological model.** *Diabet Med* 2006, **23**(2):189–197. PubMed PMID: 16433718. Epub 2006/01/26. eng.
2. The Diabetes Control and Complications Trial Research Group: **The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group.** *N Engl J Med* 1993, **329**(14):977–986. PubMed PMID: 8366922. Epub 1993/09/30. eng.
3. The Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group: **Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study.** *JAMA* 2003, **290**(16):2159–2167. PubMed PMID: 14570951. Pubmed Central PMCID: 2622725. Epub 2003/10/23. eng.
4. Cryer PE: **Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes.** *Diabetologia* 2002, **45**(7):937–948. PubMed PMID: 12136392. Epub 2002/07/24. eng.
5. Pedersen-Bjergaard U, Pramming S, Heller SR, Wallace TM, Rasmussen AK, Jorgensen HV, *et al*: **Severe hypoglycaemia in 1076 adult patients with type 1 diabetes: influence of risk markers and selection.** *Diabetes Metab Res Rev* 2004, **20**(6):479–486. PubMed PMID: 15386817. Epub 2004/09/24. eng.
6. Tattersall RB, Gill GV: **Unexplained Deaths of Type-1 Diabetic-Patients.** *Diabet Med* 1991, **8**(1):49–58. PubMed PMID: ISI:A1991ET26600010. English.
7. Cryer PE, Davis SN, Shamoon H: **Hypoglycemia in diabetes.** *Diabetes Care* 2003, **26**(6):1902–1912. PubMed PMID: 12766131. Epub 2003/05/27. eng.
8. DCCT: **Epidemiology of severe hypoglycemia in the diabetes control and complications trial. The DCCT Research Group.** *Am J Med* 1991, **90**(4):450–459. PubMed PMID: 2012085. Epub 1991/04/01. eng.

9. Geddes J, Wright RJ, Zammitt NN, Deary IJ, Frier BM: *Diabetes Care* 2007, **30**(7):1868–1870. PubMed PMID: 17416785. Epub 2007/04/10. eng.
10. Geddes J, Schopman JE, Zammitt NN, Frier BM: **Prevalence of impaired awareness of hypoglycaemia in adults with Type 1 diabetes.** *Diabet Med* 2008, **25**(4):501–504.
11. Gold AE, MacLeod KM, Frier BM: **Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia.** *Diabetes Care* 1994, **17**(7):697–703. PubMed PMID: 7924780. Epub 1994/07/01. eng.
12. Veneman T, Mitrakou A, Mokan M, Cryer P, Gerich J: **Induction of hypoglycemia unawareness by asymptomatic nocturnal hypoglycemia.** *Diabetes* 1993, **42**(9):1233–1237. PubMed PMID: 8349033. Epub 1993/09/01. eng.
13. Mitrakou A, Fanelli C, Veneman T, Perriello G, Calderone S, Platanisiotis D, *et al*: **Reversibility of unawareness of hypoglycemia in patients with insulinomas.** *N Engl J Med* 1993, **329**(12):834–839. PubMed PMID: 8355741. Epub 1993/09/16. eng.
14. Fanelli CG, Epifano L, Rambotti AM, Pampanelli S, Di Vincenzo A, Modarelli F, *et al*: **Meticulous prevention of hypoglycemia normalizes the glycemic thresholds and magnitude of most of neuroendocrine responses to, symptoms of, and cognitive function during hypoglycemia in intensively treated patients with short-term IDDM.** *Diabetes* 1993, **42**(11):1683–1689. PubMed PMID: 8405713. Epub 1993/11/01. eng.
15. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA: **Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes.** *Lancet* 1994, **344**(8918):283–287. PubMed PMID: 7914259. Epub 1994/07/30. eng.
16. Gale EA: **A randomized, controlled trial comparing insulin lispro with human soluble insulin in patients with Type 1 diabetes on intensified insulin therapy. The UK Trial Group.** *Diabet Med* 2000, **17**(3):209–214. PubMed PMID: 10784225. Epub 2000/04/28. eng.
17. Heller SR, Amiel SA, Mansell P: **Effect of the fast-acting insulin analog lispro on the risk of nocturnal hypoglycemia during intensified insulin therapy. U.K. Lispro Study Group.** *Diabetes Care* 1999, **22**(10):1607–1611. PubMed PMID: 10526722. Epub 1999/10/20. eng.
18. Holleman F, Schmitt H, Rottiers R, Rees A, Symanowski S, Anderson JH: **Reduced frequency of severe hypoglycemia and coma in well-controlled IDDM patients treated with insulin lispro. The Benelux-UK Insulin Lispro Study Group.** *Diabetes Care* 1997, **20**(12):1827–1832. PubMed PMID: 9405901. Epub 1997/12/24. eng.
19. Ratner RE, Hirsch IB, Neifing JL, Garg SK, Mecca TE, Wilson CA: **Less hypoglycemia with insulin glargine in intensive insulin therapy for type 1 diabetes. U.S. Study Group of Insulin Glargine in Type 1 Diabetes.** *Diabetes Care* 2000, **23**(5):639–643. PubMed PMID: 10834423. Epub 2000/06/02. eng.
20. Pieber TR, Eugene-Jolchine I, Derobert E: **Efficacy and safety of HOE 901 versus NPH insulin in patients with type 1 diabetes. The European Study Group of HOE 901 in type 1 diabetes.** *Diabetes Care* 2000, **23**(2):157–162. PubMed PMID: 10868824. Epub 2000/06/27. eng.
21. Fulcher GR, Gilbert RE, Yue DK: **Glargine is superior to neutral protamine Hagedorn for improving glycated haemoglobin and fasting blood glucose levels during intensive insulin therapy.** *Intern Med J* 2005, **35**(9):536–542. PubMed PMID: 16105155. Epub 2005/08/18. eng.
22. Porcellati F, Rossetti P, Pampanelli S, Fanelli CG, Torlone E, Scionti L, *et al*: **Better long-term glycaemic control with the basal insulin glargine as compared with NPH in patients with Type 1 diabetes mellitus given meal-time lispro insulin.** *Diabet Med* 2004, **21**(11):1213–1220. PubMed PMID: 15498088. Epub 2004/10/23. eng.
23. Rossetti P, Pampanelli S, Fanelli C, Porcellati F, Costa E, Torlone E, *et al*: **Intensive replacement of basal insulin in patients with type 1 diabetes given rapid-acting insulin analog at mealtime: a 3-month comparison between administration of NPH insulin four times daily and glargine insulin at dinner or bedtime.** *Diabetes Care* 2003, **26**(5):1490–1496. PubMed PMID: 12716810. Epub 2003/04/30. eng.
24. De Leeuw I, Vague P, Selam JL, Skeie S, Lang H, Draeger E, *et al*: **Insulin detemir used in basal-bolus therapy in people with type 1 diabetes is associated with a lower risk of nocturnal hypoglycaemia and less weight gain over 12 months in comparison to NPH insulin.** *Diabetes Obes Metab* 2005, **7**(1):73–82. PubMed PMID: 15642078. Epub 2005/01/12. eng.
25. Bartley PC, Bogoev M, Larsen J, Philotheou A: **Long-term efficacy and safety of insulin detemir compared to Neutral Protamine Hagedorn insulin in patients with Type 1 diabetes using a treat-to-target basal-bolus regimen with insulin aspart at meals: a 2-year, randomized, controlled trial.** *Diabet Med* 2008, **25**(4):442–449. PubMed PMID: 18387078. Pubmed Central PMCID: 2327220. Epub 2008/04/05. eng.
26. Kolendorf K, Ross GP, Pavlic-Renar I, Perriello G, Philotheou A, Jendle J, *et al*: **Insulin detemir lowers the risk of hypoglycaemia and provides more consistent plasma glucose levels compared with NPH insulin in Type 1 diabetes.** *Diabet Med* 2006, **23**(7):729–735. PubMed PMID: 16842476. Epub 2006/07/18. eng.
27. Vague P, Selam JL, Skeie S, De Leeuw I, Elte JW, Haahr H, *et al*: **Insulin detemir is associated with more predictable glycemic control and reduced risk of hypoglycemia than NPH insulin in patients with type 1 diabetes on a basal-bolus regimen with premeal insulin aspart.** *Diabetes Care* 2003, **26**(3):590–596. PubMed PMID: 12610006. Epub 2003/03/01.
28. National Institute for Clinical Excellence: *Technology Appraisal No. 53. Guidance on the use of long-acting insulin analogues for the treatment of diabetes – insulin glargine.* NICE; 2002. http://www.nice.org.uk/nicemedia/live/11482/32518/32518.pdf.
29. Boland EA, Grey M, Oesterle A, Fredrickson L, Tamborlane WV: **Continuous subcutaneous insulin infusion. A new way to lower risk of severe hypoglycemia, improve metabolic control, and enhance coping in adolescents with type 1 diabetes.** *Diabetes Care* 1999, **22**(11):1779–1784. PubMed PMID: 10546007. Epub 1999/11/05. eng.
30. Doyle EA, Weinzimer SA, Steffen AT, Ahern JA, Vincent M, Tamborlane WV: **A randomized, prospective trial comparing the efficacy of continuous subcutaneous insulin infusion with multiple daily injections using insulin glargine.** *Diabetes Care* 2004, **27**(7):1554–1558. PubMed PMID: 15220227. Epub 2004/06/29. eng.
31. Hirsch IB, Bode BW, Garg S, Lane WS, Sussman A, Hu P, *et al*: **Continuous subcutaneous insulin infusion (CSII) of insulin aspart versus multiple daily injection of insulin aspart/insulin glargine in type 1 diabetic patients previously treated with CSII.** *Diabetes Care* 2005, **28**(3):533–538. PubMed PMID: 15735183. Epub 2005/03/01. eng.
32. Misso ML, Egberts KJ, Page M, O'Connor D, Shaw J: **Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus.** *Cochrane Database of Systematic Reviews* 2010, (Issue 1):CD005103. doi:10.1002/14651858.CD005103.pub2.
33. Bode BW, Steed RD, Davidson PC: **Reduction in severe hypoglycemia with long-term continuous subcutaneous insulin infusion in type I diabetes.** *Diabetes Care* 1996, **19**(4):324–327. PubMed PMID: 8729154. Epub 1996/04/01. eng.
34. National Institute for Health and Clinical Excellence NICE technology appraisal guidance 151: *Continuous subcutaneous insulin infusion for the treatment of diabetes mellitus (review of technology appraisal guidance 57).* NICE; 2008. http://guidance.nice.org.uk/TA151/Guidance/pdf/English.
35. Deiss D, Bolinder J, Riveline JP, Battelino T, Bosi E, Tubiana-Rufi N, *et al*: **Improved glycemic control in poorly controlled patients with type 1 diabetes using real-time continuous glucose monitoring.** *Diabetes Care* 2006, **29**(12):2730–2732. PubMed PMID: 17130215. Epub 2006/11/30. eng.
36. Tamborlane WV, Beck RW, Bode BW, Buckingham B, Chase HP, Clemons R, *et al*: **Continuous glucose monitoring and intensive treatment of type 1 diabetes.** *N Engl J Med* 2008, **359**(14):1464–1476. PubMed PMID: 18779236. Epub 2008/09/10. eng.
37. Bergenstal RM, Tamborlane WV, Ahmann A, Buse JB, Dailey G, Davis SN, *et al*: **Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes.** *N Engl J Med* 2010, **363**(4):311–320. PubMed PMID: 20587585. Epub 2010/07/01. eng.
38. Hermanides J, Nørgaard K, Bruttomesso D, Mathieu C, Frid A, Dayan C, *et al*: **Sensor augmented pump therapy substantially lowers HbA1c; a randomized controlled trial.** *Diabetologia* 2009, **52**(Suppl 1):S43. Abstract.
39. Battelino T, Phillip M, Bratina N, Nimri R, Oskarsson P, Bolinder J: **Effect of continuous glucose monitoring on hypoglycemia in type 1 diabetes.** *Diabetes Care* 2011, **34**(4):795–800. PubMed PMID: 21335621. Pubmed Central PMCID: 3064030. Epub 2011/02/22. eng.
40. Davis RE, Morrissey M, Peters JR, Wittrup-Jensen K, Kennedy-Martin T, Currie CJ: **Impact of hypoglycaemia on quality of life and productivity in type 1 and type 2 diabetes.** *Curr Med Res Opin* 2005, **21**(9):1477–1483. PubMed PMID: 16197667. Epub 2005/10/04. eng.
41. Thomas RM, Aldibbiat A, Griffin W, Cox MA, Leech NJ, Shaw JA: **A randomized pilot study in Type 1 diabetes complicated by severe hypoglycaemia, comparing rigorous hypoglycaemia avoidance with insulin analogue therapy, CSII or education alone.** *Diabet Med* 2007, **24**(7):778–783. PubMed PMID: 17535290. Epub 2007/05/31. eng.

42. Clarke WL, Cox DJ, Gonder-Frederick LA, Julian D, Schlundt D, Polonsky W: **Reduced awareness of hypoglycemia in adults with IDDM. A prospective study of hypoglycemic frequency and associated symptoms.** *Diabetes Care* 1995, **18**(4):517–522. PubMed PMID: 7497862. Epub 1995/04/01. eng.
43. Moher D, Schulz KF, Altman DG: **The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials.** *Lancet* 2001, **357**(9263):1191–1194. PubMed PMID: 11323066. Epub 2001/04/27. eng.
44. **Diagnosis and classification of diabetes mellitus.** *Diabetes Care* 2011, **34**(Suppl 1):S62–S69. PubMed PMID: 21193628. Pubmed Central PMCID: 3006051. Epub 2011/01/14. eng.
45. World Health Organisation: *Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation*. WHO; 2006. http://whqlibdoc.who.int/publications/2006/9241594934_eng.pdf.
46. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, *et al*: **Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments.** *Diabetes Care* 2010, **33**(10): 2285–2293. PubMed PMID: 20876709. Pubmed Central PMCID: 2945176.
47. Suarez GA, Opfer-Gehrking TL, Offord KP, Atkinson EJ, O'Brien PC, Low PA: **The Autonomic Symptom Profile: a new instrument to assess autonomic symptoms.** *Neurology* 1999, **52**(3):523–528. PubMed PMID: 10025781. Epub 1999/02/20. eng.
48. Cox D, Ritterband L, Magee J, Clarke W, Gonder-Frederick L: **Blood glucose awareness training delivered over the internet.** *Diabetes Care* 2008, **31**(8):1527–1528. PubMed PMID: 18477813. Pubmed Central PMCID: 2494647. Epub 2008/05/15. eng.
49. Roden M: *Clinical Diabetes Research: Methods and Techniques; Chapter 4 Glucose Clamp Techniques and Chapter 5 Methods of Assessment of Counterregulation to Hypoglycaemia*. 1st edition. Wiley-Blackwell; 2007 20 July 2007.
50. Cranston I, Lomas J, Maran A, Macdonald I, Amiel SA: **Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes.** *Lancet* 1994, **344**(8918):283–287. PubMed PMID: 7914259. eng.
51. Maran A, Lomas J, Macdonald IA, Amiel SA: **Lack of preservation of higher brain function during hypoglycaemia in patients with intensively-treated IDDM.** *Diabetologia* 1995, **38**(12):1412–1418. PubMed PMID: 8786014. eng.
52. Heller SR, Macdonald IA: **The measurement of cognitive function during acute hypoglycaemia experimental limitations and their effect on the study of hypoglycaemia unawareness.** *Diabet Med* 1996, **13**(7):607–615. PubMed PMID: ISI:A1996UZ37800003. English.
53. Lezak MD: **Orientation and Attention**. In *Neuropsychological Assessment*. 3rd edition. Oxford: Oxford University Press; 1995. Chapter 9.
54. Bench CJ, Frith CD, Grasby PM, Friston KJ, Paulesu E, Frackowiak RSJ, *et al*: **Investigations of the Functional-Anatomy of Attention Using the Stroop Test.** *Neuropsychologia* 1993, **31**(9):907–922. PubMed PMID: ISI: A1993LZ62000003. English.
55. McAulay V, Deary IJ, Frier BM: **Symptoms of hypoglycaemia in people with diabetes.** *Diabet Med* 2001, **18**(9):690–705. PubMed PMID: 11606166. Epub 2001/10/19. eng.
56. Deary IJ, Hepburn DA, MacLeod KM, Frier BM: **Partitioning the symptoms of hypoglycaemia using multi-sample confirmatory factor analysis.** *Diabetologia* 1993, **36**(8):771–777. PubMed PMID: 8405746. Epub 1993/08/01. eng.
57. Bradley C, Lewis KS: **Measures of psychological well-being and treatment satisfaction developed from the responses of people with tablet-treated diabetes.** *Diabet Med* 1990, **7**(5):445–51. Epub 1990/06/01.
58. Anderson RT, Skovlund SE, Marrero D, Levine DW, Meadows K, Brod M, *et al*: **Development and validation of the insulin treatment satisfaction questionnaire.** *Clin Ther* 2004, **26**(4):565–Epub 2004/06/11.
59. The EuroQol Group: **EuroQol-a new facility for the measurement of health-related quality of life.** *Health Policy* 1990, **16**(3):199–208. Epub 1990/11/05.
60. Bradley C, Brewin CR, Gamsu DS, Moses JL: **Development of scales to measure perceived control of diabetes mellitus and diabetes-related health beliefs.** *Diabet Med* 1984, **1**(3):213–8. Epub 1984/09/01.

doi:10.1186/1472-6823-12-33
Cite this article as: Little *et al.*: Comparison of Optimised MDI versus Pumps with or without Sensors in Severe Hypoglycaemia (the Hypo COMPaSS trial). *BMC Endocrine Disorders* 2012 **12**:33.

9 References

1. *IDF Diabetes Atlas Sixth Edition Poster Update 2014*. 2014: Online.
2. Association, A.D., *Diagnosis and classification of diabetes mellitus.* Diabetes Care, 2014. **37 Suppl 1**: p. S81-90.
3. *UK has world's 5th highest rate of Type 1 diabetes in children*. 2013: http://www.diabetes.org.uk.
4. Tuomilehto, J., *The emerging global epidemic of type 1 diabetes.* Curr Diab Rep, 2013. **13**(6): p. 795-804.
5. Bluestone, J.A., K. Herold, and G. Eisenbarth, *Genetics, pathogenesis and clinical interventions in type 1 diabetes.* Nature, 2010. **464**(7293): p. 1293-300.
6. Jahromi, M.M. and G.S. Eisenbarth, *Cellular and molecular pathogenesis of type 1A diabetes.* Cell Mol Life Sci, 2007. **64**(7-8): p. 865-72.
7. Gianani, R., et al., *Dimorphic histopathology of long-standing childhood-onset diabetes.* Diabetologia, 2010. **53**(4): p. 690-8.
8. Lutale, J.J., et al., *Islet cell autoantibodies in African patients with Type 1 and Type 2 diabetes in Dar es Salaam Tanzania: a cross sectional study.* J Autoimmune Dis, 2007. **4**: p. 4.
9. McCarthy, M.I. and A.T. Hattersley, *Learning from molecular genetics: novel insights arising from the definition of genes for monogenic and type 2 diabetes.* Diabetes, 2008. **57**(11): p. 2889-98.
10. von Herrath, M., S. Sanda, and K. Herold, *Type 1 diabetes as a relapsing-remitting disease?* Nat Rev Immunol, 2007. **7**(12): p. 988-94.
11. Pociot, F., et al., *Genetics of type 1 diabetes: what's next?* Diabetes, 2010. **59**(7): p. 1561-71.
12. Lie, B.A., et al., *Application and interpretation of transmission/disequilibrium tests: transmission of HLA-DQ haplotypes to unaffected siblings in 526 families with type 1 diabetes.* Am J Hum Genet, 2000. **66**(2): p. 740-3.
13. Redondo, M.J., et al., *DR- and DQ-associated protection from type 1A diabetes: comparison of DRB1*1401 and DQA1*0102-DQB1*0602*.* J Clin Endocrinol Metab, 2000. **85**(10): p. 3793-7.
14. Kawasaki, E., et al., *Transmission of DQ haplotypes to patients with type 1 diabetes.* Diabetes, 1998. **47**(12): p. 1971-3.
15. Haller, M.J., M.A. Atkinson, and D. Schatz, *Type 1 diabetes mellitus: etiology, presentation, and management.* Pediatr Clin North Am, 2005. **52**(6): p. 1553-78.
16. Ginsberg-Fellner, F., et al., *Congenital rubella syndrome as a model for type 1 (insulin-dependent) diabetes mellitus: increased prevalence of islet cell surface antibodies.* Diabetologia, 1984. **27 Suppl**: p. 87-9.
17. Shaver, K.A., J.A. Boughman, and W.E. Nance, *Congenital rubella syndrome and diabetes: a review of epidemiologic, genetic, and immunologic factors.* Am Ann Deaf, 1985. **130**(6): p. 526-32.
18. Martin, J.M., et al., *Milk proteins in the etiology of insulin-dependent diabetes mellitus (IDDM).* Ann Med, 1991. **23**(4): p. 447-52.
19. Ziegler, A.G., et al., *Early infant feeding and risk of developing type 1 diabetes-associated autoantibodies.* JAMA, 2003. **290**(13): p. 1721-8.

20. Akirav, E., J.A. Kushner, and K.C. Herold, *Beta-cell mass and type 1 diabetes: going, going, gone?* Diabetes, 2008. **57**(11): p. 2883-8.
21. Nathan, D.M., et al., *Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes.* N Engl J Med, 2005. **353**(25): p. 2643-53.
22. *Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group.* Lancet, 1998. **352**(9131): p. 837-53.
23. Michael J. Fowler, M., *Microvascular and Macrovascular Complications of Diabetes*. 2008, Clinical Diabetes: *http://clinical.diabetesjournals.org*. p. 77-82.
24. Nathan, D.M., et al., *The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus.* N Engl J Med, 1993. **329**(14): p. 977-86.
25. *Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. UK Prospective Diabetes Study Group.* BMJ, 1998. **317**(7160): p. 703-13.
26. Stratton, I.M., et al., *Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study.* BMJ, 2000. **321**(7258): p. 405-12.
27. Fiorentino, T.V., et al., *Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases.* Curr Pharm Des, 2013. **19**(32): p. 5695-703.
28. Marathe, P.H., H.X. Gao, and K.L. Close, *American Diabetes Association Standards of Medical Care in Diabetes 2017.* J Diabetes, 2017. **9**(4): p. 320-324.
29. Chamberlain, J.J., et al., *Treatment of Type 1 Diabetes: Synopsis of the 2017 American Diabetes Association Standards of Medical Care in Diabetes.* Ann Intern Med, 2017. **167**(7): p. 493-498.
30. McKnight, J.A., et al., *Glycaemic control of Type 1 diabetes in clinical practice early in the 21st century: an international comparison.* Diabet Med, 2015. **32**(8): p. 1036-50.
31. Berger, W. and G.A. Spinas, *[Practical aspects of intensified insulin therapy using the basal bolus principle].* Ther Umsch, 1990. **47**(1): p. 30-40.
32. Sankalpa Neupane , M.L.E., *Modern strategies for management of glycaemia in type 1 diabetes*. Managing glycaemia in diabetes. Vol. Volume 42. 2014, Medicine: Elsevier. 4.
33. Schilling, L.S., M. Grey, and K.A. Knafl, *The concept of self-management of type 1 diabetes in children and adolescents: an evolutionary concept analysis.* J Adv Nurs, 2002. **37**(1): p. 87-99.
34. Reddy, M., et al., *Type 1 diabetes in adults: supporting self management.* BMJ, 2016. **352**: p. i998.
35. programme, T.D.A.f.N.E.D.e. and P. Mansell, *The Dose Adjustment for Normal Eating (DAFNE) education programme* 2012: Journal of Diabetes Nursing.
36. Mühlhauser, I. and M. Berger, *Patient education - evaluation of a complex intervention.* Diabetologia, 2002. **45**(12): p. 1723-33.

37. Group, D.S., *Training in flexible, intensive insulin management to enable dietary freedom in people with type 1 diabetes: dose adjustment for normal eating (DAFNE) randomised controlled trial.* BMJ, 2002. **325**(7367): p. 746.
38. Howorka, K., et al., *Empowering diabetes out-patients with structured education: short-term and long-term effects of functional insulin treatment on perceived control over diabetes.* J Psychosom Res, 2000. **48**(1): p. 37-44.
39. Grant, L., et al., *Type 1 diabetes structured education: What are the core self-management behaviours?* Diabet Med, 2013. **30**(6): p. 724-30.
40. Speight, J., et al., *Long-term biomedical and psychosocial outcomes following DAFNE (Dose Adjustment For Normal Eating) structured education to promote intensive insulin therapy in adults with sub-optimally controlled Type 1 diabetes.* Diabetes Res Clin Pract, 2010. **89**(1): p. 22-9.
41. Hopkins, D., et al., *Improved biomedical and psychological outcomes 1 year after structured education in flexible insulin therapy for people with type 1 diabetes: the U.K. DAFNE experience.* Diabetes Care, 2012. **35**(8): p. 1638-42.
42. Cooke, D., et al., *Structured type 1 diabetes education delivered within routine care: impact on glycemic control and diabetes-specific quality of life.* Diabetes Care, 2013. **36**(2): p. 270-2.
43. Lawton, J. and D. Rankin, *How do structured education programmes work? An ethnographic investigation of the dose adjustment for normal eating (DAFNE) programme for type 1 diabetes patients in the UK.* Soc Sci Med, 2010. **71**(3): p. 486-93.
44. http://www.dafne.uk.com. 2018 [cited 2018 May 2018].
45. Elliott, J., et al., *Substantial reductions in the number of diabetic ketoacidosis and severe hypoglycaemia episodes requiring emergency treatment lead to reduced costs after structured education in adults with Type 1 diabetes.* Diabet Med, 2014. **31**(7): p. 847-53.
46. Gunn, D. and P. Mansell, *Glycaemic control and weight 7 years after Dose Adjustment For Normal Eating (DAFNE) structured education in Type 1 diabetes.* Diabet Med, 2012. **29**(6): p. 807-12.
47. Marcus, A.O. and M.P. Fernandez, *Insulin pump therapy: acceptable alternative to injection therapy.* Postgrad Med, 1996. **99**(3): p. 125-32, 142-4.
48. Pickup, J.C., et al., *Long-term continuous subcutaneous insulin infusion in diabetics at home.* Lancet, 1979. **2**(8148): p. 870-3.
49. Reece, S.W. and C.L. Hamby Williams, *Insulin Pump Class: Back to the Basics of Pump Therapy.* Diabetes Spectr, 2014. **27**(2): p. 135-40.
50. Jeitler, K., et al., *Continuous subcutaneous insulin infusion versus multiple daily insulin injections in patients with diabetes mellitus: systematic review and meta-analysis.* Diabetologia, 2008. **51**(6): p. 941-51.
51. Thabit, H. and R. Hovorka, *Continuous subcutaneous insulin infusion therapy and multiple daily insulin injections in type 1 diabetes mellitus: a comparative overview and future horizons.* Expert Opin Drug Deliv, 2016. **13**(3): p. 389-400.

52. Cummins, E., et al., *Clinical effectiveness and cost-effectiveness of continuous subcutaneous insulin infusion for diabetes: systematic review and economic evaluation.* Health Technol Assess, 2010. **14**(11): p. iii-iv, xi-xvi, 1-181.
53. Misso, M.L., et al., *Continuous subcutaneous insulin infusion (CSII) versus multiple insulin injections for type 1 diabetes mellitus.* Cochrane Database Syst Rev, 2010(1): p. CD005103.
54. Pozzilli, P., et al., *Continuous subcutaneous insulin infusion in diabetes: patient populations, safety, efficacy, and pharmacoeconomics.* Diabetes Metab Res Rev, 2016. **32**(1): p. 21-39.
55. Yeh, H.C., et al., *Comparative effectiveness and safety of methods of insulin delivery and glucose monitoring for diabetes mellitus: a systematic review and meta-analysis.* Ann Intern Med, 2012. **157**(5): p. 336-47.
56. Ontario, H.Q., *Continuous Subcutaneous Insulin Infusion (CSII) Pumps for Type 1 and Type 2 Adult Diabetic Populations: An Evidence-Based Analysis.* Ont Health Technol Assess Ser, 2009. **9**(20): p. 1-58.
57. Marmolin, E.S., et al., *Better treatment of outpatients with type 1 diabetes after introduction of continuous subcutaneous insulin infusion.* Dan Med J, 2012. **59**(6): p. A4445.
58. Pickup, J., *Insulin pumps.* Int J Clin Pract Suppl, 2010(166): p. 16-9.
59. Pickup, J.C. and A.J. Sutton, *Severe hypoglycaemia and glycaemic control in Type 1 diabetes: meta-analysis of multiple daily insulin injections compared with continuous subcutaneous insulin infusion.* Diabet Med, 2008. **25**(7): p. 765-74.
60. Vigersky, R.A., *The benefits, limitations, and cost-effectiveness of advanced technologies in the management of patients with diabetes mellitus.* J Diabetes Sci Technol, 2015. **9**(2): p. 320-30.
61. Ratheau, L., et al., *How technology has changed diabetes management and what it has failed to achieve.* Diabetes Metab, 2011. **37 Suppl 4**: p. S57-64.
62. https://www.nice.org.uk/guidance/NG17. *Type 1 diabetes in adults: diagnosis and management*. Published date: August 2015 Last updated: July 2016 [cited 2017 01/08/2017]; NICE guideline [NG17]:[
63. Gross, T.M., et al., *Performance evaluation of the MiniMed continuous glucose monitoring system during patient home use.* Diabetes Technol Ther, 2000. **2**(1): p. 49-56.
64. Chen, C., et al., *Current and Emerging Technology for Continuous Glucose Monitoring.* Sensors (Basel), 2017. **17**(1).
65. Gómez, A.M., D.C. Henao Carrillo, and O.M. Muñoz Velandia, *Devices for continuous monitoring of glucose: update in technology.* Med Devices (Auckl), 2017. **10**: p. 215-224.
66. Klonoff, D.C., D. Ahn, and A. Drincic, *Continuous glucose monitoring: A review of the technology and clinical use.* Diabetes Res Clin Pract, 2017. **133**: p. 178-192.
67. Joubert, M. and Y. Reznik, *Personal continuous glucose monitoring (CGM) in diabetes management: review of the literature and implementation for practical use.* Diabetes Res Clin Pract, 2012. **96**(3): p. 294-305.

68. Shapiro, A.R., *The Safety of Nonadjunctive Use of Continuous Glucose Monitors for Insulin Dosing: Still Not Resolved.* J Diabetes Sci Technol, 2017. **11**(4): p. 856-857.
69. Shapiro, A.R., *Nonadjunctive Use of Continuous Glucose Monitors for Insulin Dosing: Is It Safe?* J Diabetes Sci Technol, 2017. **11**(4): p. 833-838.
70. Rodbard, D., *Continuous Glucose Monitoring: A Review of Successes, Challenges, and Opportunities.* Diabetes Technol Ther, 2016. **18 Suppl 2**: p. S3-S13.
71. Rodbard, D., *Continuous Glucose Monitoring: A Review of Recent Studies Demonstrating Improved Glycemic Outcomes.* Diabetes Technol Ther, 2017. **19**(S3): p. S25-S37.
72. Kovatchev, B., et al., *Comparison of the numerical and clinical accuracy of four continuous glucose monitors.* Diabetes Care, 2008. **31**(6): p. 1160-4.
73. Maiorino, M.I., et al., *Continuous glucose monitoring for patients with type 1 diabetes on multiple daily injections of insulin: pros and cons.* Endocrine, 2017.
74. Maahs, D.M., et al., *Effect of acetaminophen on CGM glucose in an outpatient setting.* Diabetes Care, 2015. **38**(10): p. e158-9.
75. Basu, A., et al., *Continuous Glucose Monitor Interference With Commonly Prescribed Medications: A Pilot Study.* J Diabetes Sci Technol, 2017. **11**(5): p. 936-941.
76. Calhoun, P., et al., *Resistance to Acetaminophen Interference in a Novel Continuous Glucose Monitoring System.* J Diabetes Sci Technol, 2018. **12**(2): p. 393-396.
77. Steil, G.M., et al., *Interstitial fluid glucose dynamics during insulin-induced hypoglycaemia.* Diabetologia, 2005. **48**(9): p. 1833-40.
78. Anhalt, H., *Limitations of Continuous Glucose Monitor Usage.* Diabetes Technol Ther, 2016. **18**(3): p. 115-7.
79. Langendam, M., et al., *Continuous glucose monitoring systems for type 1 diabetes mellitus.* Cochrane Database Syst Rev, 2012. **1**: p. CD008101.
80. Group, J.D.R.F.C.G.M.S., *Effectiveness of continuous glucose monitoring in a clinical care environment: evidence from the Juvenile Diabetes Research Foundation continuous glucose monitoring (JDRF-CGM) trial.* Diabetes Care, 2010. **33**(1): p. 17-22.
81. McGill, J.B. and A. Ahmann, *Continuous Glucose Monitoring with Multiple Daily Insulin Treatment: Outcome Studies.* Diabetes Technol Ther, 2017. **19**(S3): p. S3-S12.
82. Davidson, M.B., *Continuous Glucose Monitoring in Patients With Type 1 Diabetes Taking Insulin Injections.* JAMA, 2017. **317**(4): p. 363-364.
83. Beck, R.W., et al., *Effect of Continuous Glucose Monitoring on Glycemic Control in Adults With Type 1 Diabetes Using Insulin Injections: The DIAMOND Randomized Clinical Trial.* JAMA, 2017. **317**(4): p. 371-378.
84. Lind, M., et al., *Continuous Glucose Monitoring vs Conventional Therapy for Glycemic Control in Adults With Type 1 Diabetes Treated With Multiple Daily Insulin Injections: The GOLD Randomized Clinical Trial.* JAMA, 2017. **317**(4): p. 379-387.

85. Feig, D.S., et al., *Continuous glucose monitoring in pregnant women with type 1 diabetes (CONCEPTT): a multicentre international randomised controlled trial.* Lancet, 2017. **390**(10110): p. 2347-2359.
86. Kovatchev, B.P., et al., *Assessing sensor accuracy for non-adjunct use of continuous glucose monitoring.* Diabetes Technol Ther, 2015. **17**(3): p. 177-86.
87. *FDA Advisory Panel Votes to Recommend Non-Adjunctive Use of Dexcom G5 Mobile CGM.* Diabetes Technol Ther, 2016. **18**(8): p. 512-6.
88. Shapiro, A.R., *FDA Approval of Nonadjunctive Use of Continuous Glucose Monitors for Insulin Dosing: A Potentially Risky Decision.* JAMA, 2017. **318**(16): p. 1541-1542.
89. Ajjan, R.A., et al., *Accuracy of flash glucose monitoring and continuous glucose monitoring technologies: Implications for clinical practice.* Diab Vasc Dis Res, 2018. **15**(3): p. 175-184.
90. Welsh, J.B., et al., *Accuracy, Utilization, and Effectiveness Comparisons of Different Continuous Glucose Monitoring Systems.* Diabetes Technol Ther, 2019. **21**(3): p. 128-132.
91. Shah, V.N., et al., *Performance of a Factory-Calibrated Real-Time Continuous Glucose Monitoring System Utilizing an Automated Sensor Applicator.* Diabetes Technol Ther, 2018. **20**(6): p. 428-433.
92. Welsh, J.B., et al., *Performance of a Factory-Calibrated, Real-Time Continuous Glucose Monitoring System in Pediatric Participants With Type 1 Diabetes.* J Diabetes Sci Technol, 2019. **13**(2): p. 254-258.
93. Bolinder, J., et al., *Novel glucose-sensing technology and hypoglycaemia in type 1 diabetes: a multicentre, non-masked, randomised controlled trial.* Lancet, 2016. **388**(10057): p. 2254-2263.
94. Bonora, B., et al., *Head-to-head comparison between flash and continuous glucose monitoring systems in outpatients with type 1 diabetes.* J Endocrinol Invest, 2016. **39**(12): p. 1391-1399.
95. Bailey, T., et al., *The Performance and Usability of a Factory-Calibrated Flash Glucose Monitoring System.* Diabetes Technol Ther, 2015. **17**(11): p. 787-94.
96. Aberer, F., et al., *Evaluation of subcutaneous glucose monitoring systems under routine environmental conditions in patients with type 1 diabetes.* Diabetes Obes Metab, 2017. **19**(7): p. 1051-1055.
97. Slattery, D. and P. Choudhary, *Clinical Use of Continuous Glucose Monitoring in Adults with Type 1 Diabetes.* Diabetes Technol Ther, 2017. **19**(S2): p. S55-S61.
98. Blum, A., *Freestyle Libre Glucose Monitoring System.* Clin Diabetes, 2018. **36**(2): p. 203-204.
99. *FreeStyle® Libre 2 includes optional real-time alarms*. 2018 [cited 2019; The new version of the FreeStyle® Libre system can alert users in real-time of hypoglycaemia and hyperglycaemia, Abbott has announced].
100. Mittal, S. and S.C. Gough, *Pancreas transplantation: a treatment option for people with diabetes.* Diabet Med, 2014. **31**(5): p. 512-21.
101. de Kort, H., et al., *Islet transplantation in type 1 diabetes.* BMJ, 2011. **342**: p. d217.
102. Sharples, E.J., S.M. Mittal, and P.J. Friend, *Challenges in pancreas transplantation.* Acta Diabetol, 2016. **53**(6): p. 871-878.

103. Gerber, P.A., et al., *Islet transplantation as safe and efficacious method to restore glycemic control and to avoid severe hypoglycemia after donor organ failure in pancreas transplantation.* Clin Transplant, 2017.
104. Wisel, S.A., H.J. Braun, and P.G. Stock, *Current outcomes in islet versus solid organ pancreas transplant for β-cell replacement in type 1 diabetes.* Curr Opin Organ Transplant, 2016. **21**(4): p. 399-404.
105. Cryer, P.E., *Hypoglycaemia: the limiting factor in the glycaemic management of Type I and Type II diabetes.* Diabetologia, 2002. **45**(7): p. 937-48.
106. Cryer, P.E., S.N. Davis, and H. Shamoon, *Hypoglycemia in diabetes.* Diabetes Care, 2003. **26**(6): p. 1902-12.
107. Little, S.A., et al., *Severe hypoglycaemia in type 1 diabetes mellitus: underlying drivers and potential strategies for successful prevention.* Diabetes Metab Res Rev, 2014. **30**(3): p. 175-90.
108. Swinnen, S.G., et al., *Changing the glucose cut-off values that define hypoglycaemia has a major effect on reported frequencies of hypoglycaemia.* Diabetologia, 2009. **52**(1): p. 38-41.
109. Frier, B.M., *Defining hypoglycaemia: what level has clinical relevance?* Diabetologia, 2009. **52**(1): p. 31-4.
110. Workgroup on Hypoglycemia, A.e.D.A., *Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia.* Diabetes Care, 2005. **28**(5): p. 1245-9.
111. Group, I.H.S., *Glucose Concentrations of Less Than 3.0 mmol/L (54 mg/dL) Should Be Reported in Clinical Trials: A Joint Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes.* Diabetes Care, 2017. **40**(1): p. 155-157.
112. *Hypoglycemia in the Diabetes Control and Complications Trial. The Diabetes Control and Complications Trial Research Group.* Diabetes, 1997. **46**(2): p. 271-86.
113. Zatz, R., et al., *Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy.* Proc Natl Acad Sci U S A, 1985. **82**(17): p. 5963-7.
114. ter Braak, E.W., et al., *Clinical characteristics of type 1 diabetic patients with and without severe hypoglycemia.* Diabetes Care, 2000. **23**(10): p. 1467-71.
115. McCrimmon, R.J. and R.S. Sherwin, *Hypoglycemia in type 1 diabetes.* Diabetes, 2010. **59**(10): p. 2333-9.
116. Pedersen-Bjergaard, U., et al., *Severe hypoglycaemia in 1076 adult patients with type 1 diabetes: influence of risk markers and selection.* Diabetes Metab Res Rev, 2004. **20**(6): p. 479-86.
117. Cryer, P.E., *Glycemic goals in diabetes: trade-off between glycemic control and iatrogenic hypoglycemia.* Diabetes, 2014. **63**(7): p. 2188-95.
118. Cryer, P.E., *Hypoglycemia in type 1 diabetes mellitus.* Endocrinol Metab Clin North Am, 2010. **39**(3): p. 641-54.
119. Secrest, A.M., et al., *Characterizing sudden death and dead-in-bed syndrome in Type 1 diabetes: analysis from two childhood-onset Type 1 diabetes registries.* Diabet Med, 2011. **28**(3): p. 293-300.

120. Gonder-Frederick, L.A., et al., *Psychometric properties of the hypoglycemia fear survey-ii for adults with type 1 diabetes.* Diabetes Care, 2011. **34**(4): p. 801-6.
121. Pedersen-Bjergaard, U., T. Hoi-Hansen, and B. Thorsteinsson, *An evaluation of methods of assessing impaired awareness of hypoglycemia in type 1 diabetes: response to Geddes et al.* Diabetes Care, 2007. **30**(11): p. e112; author reply e113.
122. Geddes, J., et al., *An evaluation of methods of assessing impaired awareness of hypoglycemia in type 1 diabetes.* Diabetes Care, 2007. **30**(7): p. 1868-70.
123. Bolli, G.B., et al., *A reliable and reproducible test for adequate glucose counterregulation in type I diabetes mellitus.* Diabetes, 1984. **33**(8): p. 732-7.
124. Falkowska, A., et al., *Energy Metabolism of the Brain, Including the Cooperation between Astrocytes and Neurons, Especially in the Context of Glycogen Metabolism.* Int J Mol Sci, 2015. **16**(11): p. 25959-81.
125. Cryer, P.E., *Glucose counterregulation: prevention and correction of hypoglycemia in humans.* Am J Physiol, 1993. **264**(2 Pt 1): p. E149-55.
126. Cryer, P.E., *Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes.* Diabetes, 2005. **54**(12): p. 3592-601.
127. Cryer, P.E., *Diverse causes of hypoglycemia-associated autonomic failure in diabetes.* N Engl J Med, 2004. **350**(22): p. 2272-9.
128. Cryer, P.E., *Hierarchy of physiological responses to hypoglycemia: relevance to clinical hypoglycemia in type I (insulin dependent) diabetes mellitus.* Horm Metab Res, 1997. **29**(3): p. 92-6.
129. Vanessa J. Briscoe , P.a.S.N.D., MD, *Hypoglycemia in Type 1 and Type 2 Diabetes: Physiology, Pathophysiology, and Management.* Clinical Diabetes, 2006. **24 (3)**: p. 115 - 121.
130. Diedrich, L., D. Sandoval, and S.N. Davis, *Hypoglycemia associated autonomic failure.* Clin Auton Res, 2002. **12**(5): p. 358-65.
131. Heller, S.H.x.S., S.f.a.b.t. author, and E.C. , *Hypoglycaemia in diabetes*. December 2014, Elsevier Inc: http://www.medicinejournal.co.uk. p. 727–731.
132. Amiel, S.A., *Hypoglycemia: from the laboratory to the clinic.* Diabetes Care, 2009. **32**(8): p. 1364-71.
133. Dagogo-Jack, S.E., S. Craft, and P.E. Cryer, *Hypoglycemia-associated autonomic failure in insulin-dependent diabetes mellitus. Recent antecedent hypoglycemia reduces autonomic responses to, symptoms of, and defense against subsequent hypoglycemia.* J Clin Invest, 1993. **91**(3): p. 819-28.
134. SUSSMAN, K.E., J.R. CROUT, and A. MARBLE, *Failure of warning in insulin-induced hypoglycemic reactions.* Diabetes, 1963. **12**: p. 38-45.
135. Gold, A.E., K.M. MacLeod, and B.M. Frier, *Frequency of severe hypoglycemia in patients with type I diabetes with impaired awareness of hypoglycemia.* Diabetes Care, 1994. **17**(7): p. 697-703.
136. Cryer, P.E., *Iatrogenic hypoglycemia as a cause of hypoglycemia-associated autonomic failure in IDDM. A vicious cycle.* Diabetes, 1992. **41**(3): p. 255-60.

137. Heller, S.R. and P.E. Cryer, *Reduced neuroendocrine and symptomatic responses to subsequent hypoglycemia after 1 episode of hypoglycemia in nondiabetic humans.* Diabetes, 1991. **40**(2): p. 223-6.
138. Cranston, I., et al., *Restoration of hypoglycaemia awareness in patients with long-duration insulin-dependent diabetes.* Lancet, 1994. **344**(8918): p. 283-7.
139. Martín-Timón, I. and F.J. Del Cañizo-Gómez, *Mechanisms of hypoglycemia unawareness and implications in diabetic patients.* World J Diabetes, 2015. **6**(7): p. 912-26.
140. Dunn, J.T., et al., *Attenuation of amydgala and frontal cortical responses to low blood glucose concentration in asymptomatic hypoglycemia in type 1 diabetes: a new player in hypoglycemia unawareness?* Diabetes, 2007. **56**(11): p. 2766-73.
141. Cranston, I., et al., *Changes in regional brain (18)F-fluorodeoxyglucose uptake at hypoglycemia in type 1 diabetic men associated with hypoglycemia unawareness and counter-regulatory failure.* Diabetes, 2001. **50**(10): p. 2329-36.
142. Mangia, S., et al., *Hypoglycemia-induced increases in thalamic cerebral blood flow are blunted in subjects with type 1 diabetes and hypoglycemia unawareness.* J Cereb Blood Flow Metab, 2012. **32**(11): p. 2084-90.
143. Oz, G., et al., *Human brain glycogen metabolism during and after hypoglycemia.* Diabetes, 2009. **58**(9): p. 1978-85.
144. Canada, S.E., et al., *Brain glycogen supercompensation in the mouse after recovery from insulin-induced hypoglycemia.* J Neurosci Res, 2011. **89**(4): p. 585-91.
145. Öz, G., et al., *Brain glycogen content and metabolism in subjects with type 1 diabetes and hypoglycemia unawareness.* J Cereb Blood Flow Metab, 2012. **32**(2): p. 256-63.
146. De Feyter, H.M., et al., *Increased brain lactate concentrations without increased lactate oxidation during hypoglycemia in type 1 diabetic individuals.* Diabetes, 2013. **62**(9): p. 3075-80.
147. Moheet, A., et al., *Initial experience with seven tesla magnetic resonance spectroscopy of hypothalamic GABA during hyperinsulinemic euglycemia and hypoglycemia in healthy humans.* Magn Reson Med, 2014. **71**(1): p. 12-8.
148. Chan, O., et al., *Increased GABAergic tone in the ventromedial hypothalamus contributes to suppression of counterregulatory responses after antecedent hypoglycemia.* Diabetes, 2008. **57**(5): p. 1363-70.
149. Arbelaez, A.M., et al., *Attenuation of counterregulatory responses to recurrent hypoglycemia by active thalamic inhibition: a mechanism for hypoglycemia-associated autonomic failure.* Diabetes, 2008. **57**(2): p. 470-5.
150. Teh, M.M., et al., *Evolution and resolution of human brain perfusion responses to the stress of induced hypoglycemia.* Neuroimage, 2010. **53**(2): p. 584-92.
151. Bingham, E.M., et al., *Differential changes in brain glucose metabolism during hypoglycaemia accompany loss of hypoglycaemia awareness in men with type 1 diabetes mellitus. An [11C]-3-O-methyl-D-glucose PET study.* Diabetologia, 2005. **48**(10): p. 2080-9.

152. Choudhary, P. and S.A. Amiel, *Hypoglycaemia in type 1 diabetes: technological treatments, their limitations and the place of psychology.* Diabetologia, 2018. **61**(4): p. 761-769.
153. Anderbro, T., et al., *Fear of hypoglycaemia in adults with Type 1 diabetes.* Diabet Med, 2010. **27**(10): p. 1151-8.
154. Nwokolo, M., et al., *Hypoglycemic thalamic activation in type 1 diabetes is associated with preserved symptoms despite reduced epinephrine.* J Cereb Blood Flow Metab, 2019: p. 271678X19842680.
155. Dunn, J.T., et al., *The impact of hypoglycaemia awareness status on regional brain responses to acute hypoglycaemia in men with type 1 diabetes.* Diabetologia, 2018. **61**(7): p. 1676-1687.
156. Tu, E., S.M. Twigg, and C. Semsarian, *Sudden death in type 1 diabetes: the mystery of the 'dead in bed' syndrome.* Int J Cardiol, 2010. **138**(1): p. 91-3.
157. Tattersall, R.B. and G.V. Gill, *Unexplained deaths of type 1 diabetic patients.* Diabet Med, 1991. **8**(1): p. 49-58.
158. Novodvorsky, P., et al., *Diurnal Differences in Risk of Cardiac Arrhythmias During Spontaneous Hypoglycemia in Young People With Type 1 Diabetes.* Diabetes Care, 2017. **40**(5): p. 655-662.
159. Gill, G.V., et al., *Cardiac arrhythmia and nocturnal hypoglycaemia in type 1 diabetes--the 'dead in bed' syndrome revisited.* Diabetologia, 2009. **52**(1): p. 42-5.
160. Robinson, R.T., et al., *Changes in cardiac repolarization during clinical episodes of nocturnal hypoglycaemia in adults with Type 1 diabetes.* Diabetologia, 2004. **47**(2): p. 312-5.
161. Marques, J.L., et al., *Altered ventricular repolarization during hypoglycaemia in patients with diabetes.* Diabet Med, 1997. **14**(8): p. 648-54.
162. Adler, G.K., et al., *Antecedent hypoglycemia impairs autonomic cardiovascular function: implications for rigorous glycemic control.* Diabetes, 2009. **58**(2): p. 360-6.
163. Serhiyenko, V.A. and A.A. Serhiyenko, *Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment.* World J Diabetes, 2018. **9**(1): p. 1-24.
164. Pop-Busui, R., *Cardiac autonomic neuropathy in diabetes: a clinical perspective.* Diabetes Care, 2010. **33**(2): p. 434-41.
165. Olsen, S.E., et al., *Impaired Awareness of Hypoglycemia in Adults With Type 1 Diabetes Is Not Associated With Autonomic Dysfunction or Peripheral Neuropathy.* Diabetes Care, 2016. **39**(3): p. 426-33.
166. Meyer, C., et al., *Effects of autonomic neuropathy on counterregulation and awareness of hypoglycemia in type 1 diabetic patients.* Diabetes Care, 1998. **21**(11): p. 1960-6.
167. Bottini, P., et al., *Contribution of autonomic neuropathy to reduced plasma adrenaline responses to hypoglycemia in IDDM: evidence for a nonselective defect.* Diabetes, 1997. **46**(5): p. 814-23.
168. Fanelli, C., et al., *Long-term intensive therapy of IDDM patients with clinically overt autonomic neuropathy: effects on hypoglycemia awareness and counterregulation.* Diabetes, 1997. **46**(7): p. 1172-81.

169. Ryder, R.E., et al., *Unawareness of hypoglycaemia and inadequate hypoglycaemic counterregulation: no causal relation with diabetic autonomic neuropathy.* BMJ, 1990. **301**(6755): p. 783-7.
170. Yeoh, E., et al., *Interventions That Restore Awareness of Hypoglycemia in Adults With Type 1 Diabetes: A Systematic Review and Meta-analysis.* Diabetes Care, 2015. **38**(8): p. 1592-609.
171. Beck, J., et al., *2017 National Standards for Diabetes Self-Management Education and Support.* Diabetes Spectr, 2017. **30**(4): p. 301-314.
172. Gabriely, I. and H. Shamoon, *Hypoglycemia in diabetes: common, often unrecognized.* Cleve Clin J Med, 2004. **71**(4): p. 335-42.
173. Hermanns, N., et al., *Long-term effect of an education program (HyPOS) on the incidence of severe hypoglycemia in patients with type 1 diabetes.* Diabetes Care, 2010. **33**(3): p. e36.
174. Hermanns, N., et al., *The effect of an education programme (HyPOS) to treat hypoglycaemia problems in patients with type 1 diabetes.* Diabetes Metab Res Rev, 2007. **23**(7): p. 528-38.
175. Schachinger, H., et al., *Randomized controlled clinical trial of Blood Glucose Awareness Training (BGAT III) in Switzerland and Germany.* J Behav Med, 2005. **28**(6): p. 587-94.
176. Cox, D.J., et al., *Long-term follow-up evaluation of blood glucose awareness training.* Diabetes Care, 1994. **17**(1): p. 1-5.
177. Cox, D., et al., *A multicenter evaluation of blood glucose awareness training-II.* Diabetes Care, 1995. **18**(4): p. 523-8.
178. Broers, S., et al., *Blood glucose awareness training in Dutch type 1 diabetes patients: one-year follow-up.* Neth J Med, 2005. **63**(5): p. 164-9.
179. de Zoysa, N., et al., *A psychoeducational program to restore hypoglycemia awareness: the DAFNE-HART pilot study.* Diabetes Care, 2014. **37**(3): p. 863-6.
180. Shuttlewood, E., et al., *A qualitative evaluation of DAFNE-HART: A psychoeducational programme to restore hypoglycaemia awareness.* Diabetes Res Clin Pract, 2015. **109**(2): p. 347-54.
181. Cox, D.J., et al., *Hypoglycemia anticipation, awareness and treatment training (HAATT) reduces occurrence of severe hypoglycemia among adults with type 1 diabetes mellitus.* Int J Behav Med, 2004. **11**(4): p. 212-8.
182. https://www.nice.org.uk/guidance/ng18. *Diabetes (type 1 and type 2) in children and young people: diagnosis and management NICE guideline [NG18]*. August 2015 November 2016 [cited 2018 31 January 2018].
183. Jacobsen, I.B., et al., *Evidence-based insulin treatment in type 1 diabetes mellitus.* Diabetes Res Clin Pract, 2009. **86**(1): p. 1-10.
184. van Beers, C.A., et al., *Continuous glucose monitoring for patients with type 1 diabetes and impaired awareness of hypoglycaemia (IN CONTROL): a randomised, open-label, crossover trial.* Lancet Diabetes Endocrinol, 2016. **4**(11): p. 893-902.
185. Sato, J., T. Hirose, and H. Watada, *Continuous glucose monitoring system: Is it really accurate, safe and clinically useful?* J Diabetes Investig, 2012. **3**(3): p. 225-30.
186. Buckingham, B., et al., *Response to nocturnal alarms using a real-time glucose sensor.* Diabetes Technol Ther, 2005. **7**(3): p. 440-7.

187. Choudhary, P., et al., *Insulin pump therapy with automated insulin suspension in response to hypoglycemia: reduction in nocturnal hypoglycemia in those at greatest risk.* Diabetes Care, 2011. **34**(9): p. 2023-5.
188. Raccah, D., et al., *Incremental value of continuous glucose monitoring when starting pump therapy in patients with poorly controlled type 1 diabetes: the RealTrend study.* Diabetes Care, 2009. **32**(12): p. 2245-50.
189. Garg, S.K., et al., *Use of continuous glucose monitoring in subjects with type 1 diabetes on multiple daily injections versus continuous subcutaneous insulin infusion therapy: a prospective 6-month study.* Diabetes Care, 2011. **34**(3): p. 574-9.
190. Heinemann, L., et al., *Real-time continuous glucose monitoring in adults with type 1 diabetes and impaired hypoglycaemia awareness or severe hypoglycaemia treated with multiple daily insulin injections (HypoDE): a multicentre, randomised controlled trial.* Lancet, 2018. **391**(10128): p. 1367-1377.
191. Reddy, M., et al., *A randomized controlled pilot study of continuous glucose monitoring and flash glucose monitoring in people with Type 1 diabetes and impaired awareness of hypoglycaemia.* Diabet Med, 2018. **35**(4): p. 483-490.
192. Bergenstal, R.M., et al., *Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes.* N Engl J Med, 2010. **363**(4): p. 311-20.
193. Hermanides, J., et al., *Sensor-augmented pump therapy lowers HbA(1c) in suboptimally controlled Type 1 diabetes; a randomized controlled trial.* Diabet Med, 2011. **28**(10): p. 1158-67.
194. Lucidi, P., et al., *Prevention and Management of Severe Hypoglycemia and Hypoglycemia Unawareness: Incorporating Sensor Technology.* Curr Diab Rep, 2018. **18**(10): p. 83.
195. Bergenstal, R.M., J.B. Welsh, and J.J. Shin, *Threshold insulin-pump interruption to reduce hypoglycemia.* N Engl J Med, 2013. **369**(15): p. 1474.
196. Danne, T., et al., *Prevention of hypoglycemia by using low glucose suspend function in sensor-augmented pump therapy.* Diabetes Technol Ther, 2011. **13**(11): p. 1129-34.
197. Villafuerte Quispe, B., et al., *Effectiveness of MiniMed 640G with SmartGuard® System for prevention of hypoglycemia in pediatric patients with type 1 diabetes mellitus.* Endocrinol Diabetes Nutr, 2017. **64**(4): p. 198-203.
198. Abraham, M.B., et al., *Reduction in Hypoglycemia With the Predictive Low-Glucose Management System: A Long-Term Randomized Controlled Trial in Adolescents With Type 1 Diabetes.* Diabetes Care, 2017.
199. Ly, T.T., et al., *Analysis of glucose responses to automated insulin suspension with sensor-augmented pump therapy.* Diabetes Care, 2012. **35**(7): p. 1462-5.
200. Forlenza, G.P., et al., *Predictive Low-Glucose Suspend Reduces Hypoglycemia in Adults, Adolescents, and Children With Type 1 Diabetes in an At-Home Randomized Crossover Study: Results of the PROLOG Trial.* Diabetes Care, 2018. **41**(10): p. 2155-2161.

201. Thabit, H. and R. Hovorka, *Bringing closed-loop home: recent advances in closed-loop insulin delivery.* Curr Opin Endocrinol Diabetes Obes, 2014. **21**(2): p. 95-101.
202. Bally, L., et al., *Day-and-night glycaemic control with closed-loop insulin delivery versus conventional insulin pump therapy in free-living adults with well controlled type 1 diabetes: an open-label, randomised, crossover study.* Lancet Diabetes Endocrinol, 2017. **5**(4): p. 261-270.
203. Elleri, D., D.B. Dunger, and R. Hovorka, *Closed-loop insulin delivery for treatment of type 1 diabetes.* BMC Med, 2011. **9**: p. 120.
204. Thabit, H. and R. Hovorka, *Coming of age: the artificial pancreas for type 1 diabetes.* Diabetologia, 2016. **59**(9): p. 1795-805.
205. Hovorka, R., *Artificial Pancreas Project at Cambridge 2013.* Diabet Med, 2015. **32**(8): p. 987-92.
206. Sutherland, D.E., et al., *Lessons learned from more than 1,000 pancreas transplants at a single institution.* Ann Surg, 2001. **233**(4): p. 463-501.
207. Gruessner, A.C., *2011 update on pancreas transplantation: comprehensive trend analysis of 25,000 cases followed up over the course of twenty-four years at the International Pancreas Transplant Registry (IPTR).* Rev Diabet Stud, 2011. **8**(1): p. 6-16.
208. Byrne, M.L., et al., *Outcomes for adults with type 1 diabetes referred with severe hypoglycaemia and/or referred for islet transplantation to a specialist hypoglycaemia service.* Horm Metab Res, 2015. **47**(1): p. 9-15.
209. Ryan, E.A., et al., *Risks and side effects of islet transplantation.* Curr Diab Rep, 2004. **4**(4): p. 304-9.
210. Niclauss, N., et al., *Beta-Cell Replacement: Pancreas and Islet Cell Transplantation.* Endocr Dev, 2016. **31**: p. 146-62.
211. Robertson, R.P., et al., *Pancreas and islet transplantation in type 1 diabetes.* Diabetes Care, 2006. **29**(4): p. 935.
212. Shapiro, A.M., C. Ricordi, and B. Hering, *Edmonton's islet success has indeed been replicated elsewhere.* Lancet, 2003. **362**(9391): p. 1242.
213. Shapiro, A.M., et al., *International trial of the Edmonton protocol for islet transplantation.* N Engl J Med, 2006. **355**(13): p. 1318-30.
214. Rickels, M.R., et al., *Long-Term Improvement in Glucose Control and Counterregulation by Islet Transplantation for Type 1 Diabetes.* J Clin Endocrinol Metab, 2016. **101**(11): p. 4421-4430.
215. Rickels, M.R., et al., *Restoration of Glucose Counterregulation by Islet Transplantation in Long-standing Type 1 Diabetes.* Diabetes, 2015. **64**(5): p. 1713-8.
216. Rickels, M.R., et al., *Glycemic thresholds for activation of counterregulatory hormone and symptom responses in islet transplant recipients.* J Clin Endocrinol Metab, 2007. **92**(3): p. 873-9.
217. Ang, M., et al., *Magnitude and mechanisms of glucose counterregulation following islet transplantation in patients with type 1 diabetes suffering from severe hypoglycaemic episodes.* Diabetologia, 2014. **57**(3): p. 623-32.
218. Agarwal, A. and K.L. Brayman, *Update on islet cell transplantation for type 1 diabetes.* Semin Intervent Radiol, 2012. **29**(2): p. 90-8.

219. Chang, C.A., M.C. Lawrence, and B. Naziruddin, *Current issues in allogeneic islet transplantation.* Curr Opin Organ Transplant, 2017. **22**(5): p. 437-443.
220. McCoy, R.G., et al., *Increased mortality of patients with diabetes reporting severe hypoglycemia.* Diabetes Care, 2012. **35**(9): p. 1897-901.
221. Pacaud, D., et al., *Risk of recurrent severe hypoglycemia remains associated with a past history of severe hypoglycemia up to 4 years: Results from a large prospective contemporary pediatric cohort of the DPV initiative.* Pediatr Diabetes, 2017.
222. Frier, B.M., *Hypoglycaemia in diabetes mellitus: epidemiology and clinical implications.* Nat Rev Endocrinol, 2014. **10**(12): p. 711-22.
223. Liu, J., et al., *The burden of severe hypoglycemia in type 1 diabetes.* Curr Med Res Opin, 2018. **34**(1): p. 171-177.
224. Nordfeldt, S. and D. Jonsson, *Short-term effects of severe hypoglycaemia in children and adolescents with type 1 diabetes. A cost-of-illness study.* Acta Paediatr, 2001. **90**(2): p. 137-42.
225. Little, S.A., et al., *Recovery of hypoglycemia awareness in long-standing type 1 diabetes: a multicenter 2 × 2 factorial randomized controlled trial comparing insulin pump with multiple daily injections and continuous with conventional glucose self-monitoring (HypoCOMPaSS).* Diabetes Care, 2014. **37**(8): p. 2114-22.
226. Pedersen-Bjergaard, U., et al., *Activity of angiotensin-converting enzyme and risk of severe hypoglycaemia in type 1 diabetes mellitus.* Lancet, 2001. **357**(9264): p. 1248-53.
227. Pedersen-Bjergaard, U., et al., *Prediction of severe hypoglycaemia by angiotensin-converting enzyme activity and genotype in type 1 diabetes.* Diabetologia, 2003. **46**(1): p. 89-96.
228. Pedersen-Bjergaard, U., et al., *Genetic variation and activity of the renin-angiotensin system and severe hypoglycemia in type 1 diabetes.* Am J Med, 2008. **121**(3): p. 246.e1-8.
229. Bulsara, M.K., et al., *The relationship between ACE genotype and risk of severe hypoglycaemia in a large population-based cohort of children and adolescents with type 1 diabetes.* Diabetologia, 2007. **50**(5): p. 965-71.
230. Zammitt, N.N., et al., *Serum angiotensin-converting enzyme and frequency of severe hypoglycaemia in Type 1 diabetes: does a relationship exist?* Diabet Med, 2007. **24**(12): p. 1449-54.
231. Association, A.D., *Standards of medical care in diabetes--2014.* Diabetes Care, 2014. **37 Suppl 1**: p. S14-80.
232. Karter, A.J., et al., *Self-monitoring of blood glucose: language and financial barriers in a managed care population with diabetes.* Diabetes Care, 2000. **23**(4): p. 477-83.
233. Goldstein, D.E., et al., *Tests of glycemia in diabetes.* Diabetes Care, 1995. **18**(6): p. 896-909.
234. Galassetti, P.R., et al., *Breath ethanol and acetone as indicators of serum glucose levels: an initial report.* Diabetes Technol Ther, 2005. **7**(1): p. 115-23.
235. Lee, J., et al., *Improved predictive models for plasma glucose estimation from multi-linear regression analysis of exhaled volatile organic compounds.* J Appl Physiol (1985), 2009. **107**(1): p. 155-60.

236. Minh Tdo, C., D.R. Blake, and P.R. Galassetti, *The clinical potential of exhaled breath analysis for diabetes mellitus.* Diabetes Res Clin Pract, 2012. **97**(2): p. 195-205.
237. Padmanabhan, S., M. McLean, and N.W. Cheung, *Falling insulin requirements are associated with adverse obstetric outcomes in women with preexisting diabetes.* Diabetes Care, 2014. **37**(10): p. 2685-92.
238. Magnotti, M.A. and E.J. Rayfield, *An analysis of the HumaPen Luxura HD pen: what is the role of 0.5-unit insulin dosing?* J Diabetes Sci Technol, 2010. **4**(2): p. 357-8.
239. Hardy, K.J., et al., *A treatable cause of recurrent severe hypoglycemia.* Diabetes Care, 1994. **17**(7): p. 722-4.
240. Laimer, M., et al., *Variability of Basal Rate Profiles in Insulin Pump Therapy and Association with Complications in Type 1 Diabetes Mellitus.* PLoS One, 2016. **11**(3): p. e0150604.
241. Little, S., et al., *Comparison of Optimised MDI versus Pumps with or without Sensors in Severe Hypoglycaemia (the Hypo COMPaSS trial).* BMC Endocr Disord, 2012. **12**: p. 33.
242. Leelarathna, L., et al., *Restoration of self-awareness of hypoglycemia in adults with long-standing type 1 diabetes: hyperinsulinemic-hypoglycemic clamp substudy results from the HypoCOMPaSS trial.* Diabetes Care, 2013. **36**(12): p. 4063-70.
243. Rosenthal, J.M., et al., *The effect of acute hypoglycemia on brain function and activation: a functional magnetic resonance imaging study.* Diabetes, 2001. **50**(7): p. 1618-26.
244. Rosenthal, M.J., et al., *Caffeine restores regional brain activation in acute hypoglycaemia in healthy volunteers.* Diabet Med, 2007. **24**(7): p. 720-7.
245. Maran, A., et al., *Brain function rescue effect of lactate following hypoglycaemia is not an adaptation process in both normal and type I diabetic subjects.* Diabetologia, 2000. **43**(6): p. 733-41.
246. Scarpina, F. and S. Tagini, *The Stroop Color and Word Test.* Front Psychol, 2017. **8**: p. 557.
247. *Cognition and Acquired Language Disorders: An Information Processing Approach*. https://www.sciencedirect.com/book/9780323072014: ScienceDirect ®.
248. Cryer, P.E., *Minireview: Glucagon in the pathogenesis of hypoglycemia and hyperglycemia in diabetes.* Endocrinology, 2012. **153**(3): p. 1039-48.
249. Bolli, G., et al., *Abnormal glucose counterregulation in insulin-dependent diabetes mellitus. Interaction of anti-insulin antibodies and impaired glucagon and epinephrine secretion.* Diabetes, 1983. **32**(2): p. 134-41.
250. *Epidemiology of severe hypoglycemia in the diabetes control and complications trial. The DCCT Research Group.* Am J Med, 1991. **90**(4): p. 450-9.
251. Cryer, P.E., *Hypoglycemia-associated autonomic failure in diabetes.* Handb Clin Neurol, 2013. **117**: p. 295-307.
252. Amiel , S., *Hypoglycaemia Awareness Restoration Programme (HARPdoc)*, in *NCT02940873*. 2017: https://clinicaltrials.gov.
253. Pedersen-Bjergaard, U., et al., *Angiotensin-converting enzyme and angiotensin II receptor subtype 2 genotypes in type 1 diabetes and*

severe hypoglycaemia requiring emergency treatment: a case cohort study. Pharmacogenet Genomics, 2009. **19**(11): p. 864-8.
254. Nordfeldt, S. and U. Samuelsson, *Serum ACE predicts severe hypoglycemia in children and adolescents with type 1 diabetes.* Diabetes Care, 2003. **26**(2): p. 274-8.
255. Pedersen-Bjergaard, U., *RAS and severe hypoglycemia in type 1 diabetes*, B. Thorsteinsson, Editor. 2008: Expert Review of Endocrinology & Metabolism. p. 32-37.
256. Sparks, M.A., et al., *Classical Renin-Angiotensin system in kidney physiology.* Compr Physiol, 2014. **4**(3): p. 1201-28.
257. Klabunde, R.E. *Renin-Angiotensin-Aldosterone System*. Cardiovascular Physiology Concepts [cited 2016 09/2016]; *Revised 12/8/16*:[
258. Cheng, J., et al., *Effect of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on all-cause mortality, cardiovascular deaths, and cardiovascular events in patients with diabetes mellitus: a meta-analysis.* JAMA Intern Med, 2014. **174**(5): p. 773-85.
259. Demurtas, P., et al., *Association between the ACE insertion/deletion polymorphism and pterygium in Sardinian patients: a population based case-control study.* BMJ Open, 2014. **4**(10): p. e005627.
260. Rigat, B., et al., *An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels.* J Clin Invest, 1990. **86**(4): p. 1343-6.
261. Ruiter, R., et al., *The ACE insertion/deletion polymorphism and risk of cancer, a review and meta-analysis of the literature.* Curr Cancer Drug Targets, 2011. **11**(4): p. 421-30.
262. Gard, P.R., *Implications of the angiotensin converting enzyme gene insertion/deletion polymorphism in health and disease: a snapshot review.* Int J Mol Epidemiol Genet, 2010. **1**(2): p. 145-57.
263. Woods, D.R., D. Brull, and H.E. Montgomery, *Endurance and the ACE I/D polymorphism.* Sci Prog, 2000. **83**(Pt 4): p. 317-36.
264. Dzau, V.J., *Circulating versus local renin-angiotensin system in cardiovascular homeostasis.* Circulation, 1988. **77**(6 Pt 2): p. I4-13.
265. Llorens-Cortes, C. and F.A. Mendelsohn, *Organisation and functional role of the brain angiotensin system.* J Renin Angiotensin Aldosterone Syst, 2002. **3 Suppl 1**: p. S39-48.
266. Persu, A., et al., *A novel splice-site mutation in angiotensin I-converting enzyme (ACE) gene, c.3691+1G>A (IVS25+1G>A), causes a dramatic increase in circulating ACE through deletion of the transmembrane anchor.* PLoS One, 2013. **8**(4): p. e59537.
267. Kramers, C., et al., *Point mutation in the stalk of angiotensin-converting enzyme causes a dramatic increase in serum angiotensin-converting enzyme but no cardiovascular disease.* Circulation, 2001. **104**(11): p. 1236-40.
268. Nesterovitch, A.B., et al., *Angiotensin I-converting enzyme mutation (Trp1197Stop) causes a dramatic increase in blood ACE.* PLoS One, 2009. **4**(12): p. e8282.
269. Shanmugam, V., K.W. Sell, and B.K. Saha, *Mistyping ACE heterozygotes.* PCR Methods Appl, 1993. **3**(2): p. 120-1.

270. L Faerch, B.T., L Tarnow, J Holst, U Pedersen-Bjergaard, *The Effect of RAS Blockade on Cognitive Function, Hormonal Counter-Regulation and Symptom Responses during Hypoglycemia in Patients with Type 1 Diabetes*. 2013: American Diabetes Association73rd Scientific Sessions June 21 - 25, 2013 , Chicago, Illinois.
271. Harsimran Singh, L.G.-F., Karen Schmidt, Derek Ford, Karen A Vajda, Jeffrey Hawley, Daniel J Cox, *Assessing hyperglycemia avoidance in people with Type 1 diabetes*. https://www.openaccessjournals.com. p. 263–271.
272. Speight, J., et al., *Characterizing problematic hypoglycaemia: iterative design and preliminary psychometric validation of the Hypoglycaemia Awareness Questionnaire (HypoA-Q).* Diabet Med, 2016. **33**(3): p. 376-85.
273. Morris, A.D., et al., *ACE inhibitor use is associated with hospitalization for severe hypoglycemia in patients with diabetes. DARTS/MEMO Collaboration. Diabetes Audit and Research in Tayside, Scotland. Medicines Monitoring Unit.* Diabetes Care, 1997. **20**(9): p. 1363-7.
274. O'Caoimh, R., et al., *Effects of centrally acting angiotensin converting enzyme inhibitors on functional decline in patients with Alzheimer's disease.* J Alzheimers Dis, 2014. **40**(3): p. 595-603.
275. *Randomised placebo-controlled trial of lisinopril in normotensive patients with insulin-dependent diabetes and normoalbuminuria or microalbuminuria. The EUCLID Study Group.* Lancet, 1997. **349**(9068): p. 1787-92.
276. Uehara, M., et al., *Effect on insulin sensitivity of angiotensin converting enzyme inhibitors with or without a sulphydryl group: bradykinin may improve insulin resistance in dogs and humans.* Diabetologia, 1994. **37**(3): p. 300-7.
277. Pickup, J.C., S.C. Freeman, and A.J. Sutton, *Glycaemic control in type 1 diabetes during real time continuous glucose monitoring compared with self monitoring of blood glucose: meta-analysis of randomised controlled trials using individual patient data.* BMJ, 2011. **343**: p. d3805.
278. Englert, K., et al., *Skin and adhesive issues with continuous glucose monitors: a sticky situation.* J Diabetes Sci Technol, 2014. **8**(4): p. 745-51.
279. Mortellaro M, D.A., *Performance characterization of an abiotic and fluorescent-based continuous glucose monitoring system in patients with type 1 diabetes.* J Diabetes Sci Technol., 2014. **2015 Jul 29;9(5)**: p. 951-6.
280. Dehennis, A., M.A. Mortellaro, and S. Ioacara, *Multisite Study of an Implanted Continuous Glucose Sensor Over 90 Days in Patients With Diabetes Mellitus.* J Diabetes Sci Technol, 2015. **9**(5): p. 951-6.
281. Huyett, L.M., et al., *Preliminary Evaluation of a Long-Term Intraperitoneal Glucose Sensor With Flushing Mechanism.* J Diabetes Sci Technol, 2016. **10**(5): p. 1192-4.
282. Lorenz, C., W. Sandoval, and M. Mortellaro, *Interference Assessment of Various Endogenous and Exogenous Substances on the Performance of the Eversense Long-Term Implantable Continuous Glucose Monitoring System.* Diabetes Technol Ther, 2018. **20**(5): p. 344-352.

283. Kropff, J., et al., *Accuracy and Longevity of an Implantable Continuous Glucose Sensor in the PRECISE Study: A 180-Day, Prospective, Multicenter, Pivotal Trial.* Diabetes Care, 2017. **40**(1): p. 63-68.
284. Levey, A.S., et al., *A new equation to estimate glomerular filtration rate.* Ann Intern Med, 2009. **150**(9): p. 604-12.
285. https://www.trialregister.nl/trial/4328. *A Prospective, Multi-Center Evaluation of the Accuracy of a Novel Continuous Implanted Glucose Sensor*. 2014.
286. Wang, Y.P. and C. Gorenstein, *Psychometric properties of the Beck Depression Inventory-II: a comprehensive review.* Rev Bras Psiquiatr, 2013. **35**(4): p. 416-31.
287. Jacobson, A.M., M. de Groot, and J.A. Samson, *The evaluation of two measures of quality of life in patients with type I and type II diabetes.* Diabetes Care, 1994. **17**(4): p. 267-74.
288. Polonsky, W.H., et al., *Development of a New Measure for Assessing Glucose Monitoring Device-Related Treatment Satisfaction and Quality of Life.* Diabetes Technol Ther, 2015. **17**(9): p. 657-63.
289. Barnard, K.D., et al., *Acceptability of Implantable Continuous Glucose Monitoring Sensor.* J Diabetes Sci Technol, 2017: p. 1932296817735123.
290. Taylor, R.L., S.K. Grebe, and R.J. Singh, *Quantitative, highly sensitive liquid chromatography-tandem mass spectrometry method for detection of synthetic corticosteroids.* Clin Chem, 2004. **50**(12): p. 2345-52.
291. Clarke, W.L., et al., *Evaluating clinical accuracy of systems for self-monitoring of blood glucose.* Diabetes Care, 1987. **10**(5): p. 622-8.
292. Zhu, Z., et al., *A critical review of glucose biosensors based on carbon nanomaterials: carbon nanotubes and graphene.* Sensors (Basel), 2012. **12**(5): p. 5996-6022.
293. Wang, Y., et al., *Foreign Body Reaction to Implantable Biosensors: Effects of Tissue Trauma and Implant Size.* J Diabetes Sci Technol, 2015. **9**(5): p. 966-77.
294. Gerritsen, M., *Problems associated with subcutaneously implanted glucose sensors.* Diabetes Care, 2000. **23**(2): p. 143-5.
295. Renard, *Implantable glucose sensors for diabetes monitoring.* Minim Invasive Ther Allied Technol, 2004. **13**(2): p. 78-86.
296. Gerritsen, M., et al., *A percutaneous device as model to study the in vivo performance of implantable amperometric glucose sensors.* J Mater Sci Mater Med, 2001. **12**(2): p. 129-34.
297. Klonoff, D.C., et al., *The surveillance error grid.* J Diabetes Sci Technol, 2014. **8**(4): p. 658-72.
298. Vaddiraju, S., et al., *Technologies for continuous glucose monitoring: current problems and future promises.* J Diabetes Sci Technol, 2010. **4**(6): p. 1540-62.
299. Pfützner, A., et al., *Technical aspects of the Parkes error grid.* J Diabetes Sci Technol, 2013. **7**(5): p. 1275-81.
300. Parkes, J.L., et al., *A new consensus error grid to evaluate the clinical significance of inaccuracies in the measurement of blood glucose.* Diabetes Care, 2000. **23**(8): p. 1143-8.

301. Dowlaty, N., A. Yoon, and P. Galassetti, *Monitoring states of altered carbohydrate metabolism via breath analysis: are times ripe for transition from potential to reality?* Curr Opin Clin Nutr Metab Care, 2013. **16**(4): p. 466-72.
302. Ginsberg, B.H., *An overview of minimally invasive technologies.* Clin Chem, 1992. **38**(9): p. 1596-600.
303. Hansen, M.V., et al., *Frequency and motives of blood glucose self-monitoring in type 1 diabetes.* Diabetes Res Clin Pract, 2009. **85**(2): p. 183-8.
304. Bui, H., K. Perlman, and D. Daneman, *Self-monitoring of blood glucose in children and teens with diabetes.* Pediatr Diabetes, 2005. **6**(1): p. 50-62.
305. *Executive summary: standards of medical care in diabetes--2014.* Diabetes Care, 2014. **37 Suppl 1**: p. S5-13.
306. Ciudin, A., C. Hernandez, and R. Simo, *Non-invasive methods of glucose measurement: current status and future perspectives.* Curr Diabetes Rev, 2012. **8**(1): p. 48-54.
307. Vashist, S.K., *Non-invasive glucose monitoring technology in diabetes management: a review.* Anal Chim Acta, 2012. **750**: p. 16-27.
308. Cooke, D., et al., *Randomized controlled trial to assess the impact of continuous glucose monitoring on HbA(1c) in insulin-treated diabetes (MITRE Study).* Diabet Med, 2009. **26**(5): p. 540-7.
309. Wentholt, I.M., et al., *Pendra goes Dutch: lessons for the CE mark in Europe.* Diabetologia, 2005. **48**(6): p. 1055-8.
310. Rossetti, P., et al., *Estimating plasma glucose from interstitial glucose: the issue of calibration algorithms in commercial continuous glucose monitoring devices.* Sensors (Basel), 2010. **10**(12): p. 10936-52.
311. DeSalvo, D. and B. Buckingham, *Continuous glucose monitoring: current use and future directions.* Curr Diab Rep, 2013. **13**(5): p. 657-62.
312. https://www.nice.org.uk/guidance/dg21. *Integrated sensor-augmented pump therapy systems for managing blood glucose levels in type 1 diabetes (the MiniMed Paradigm Veo system and the Vibe and G4 PLATINUM CGM system)*. February 2016 [cited 2017 December].
313. https://www.nice.org.uk/guidance/ta151, *Continuous subcutaneous insulin infusion for the treatment of diabetes mellitus*. 2008, NICE technology appraisal guidance [TA151]: https://www.nice.org.uk.
314. Goodarzi, M. and W. Saeys, *Selection of the most informative near infrared spectroscopy wavebands for continuous glucose monitoring in human serum.* Talanta, 2016. **146**: p. 155-65.
315. Astrand, I., J. Engström, and P. Ovrum, *Exposure to xylene and ethylbenzene. I. Uptake, distribution and elimination in man.* Scand J Work Environ Health, 1978. **4**(3): p. 185-94.
316. Turner, C., et al., *Breath acetone concentration decreases with blood glucose concentration in type I diabetes mellitus patients during hypoglycaemic clamps.* J Breath Res, 2009. **3**(4): p. 046004.
317. van den Borst, B., et al., *Pulmonary function in diabetes: a metaanalysis.* Chest, 2010. **138**(2): p. 393-406.

318. Jandeleit-Dahm, K., A. Watson, and A. Soro-Paavonen, *The AGE/RAGE axis in diabetes-accelerated atherosclerosis.* Clin Exp Pharmacol Physiol, 2008. **35**(3): p. 329-34.
319. Weynand, B., et al., *Diabetes mellitus induces a thickening of the pulmonary basal lamina.* Respiration, 1999. **66**(1): p. 14-9.
320. Saraheimo, M., et al., *Increased levels of alpha-defensin (-1, -2 and -3) in type 1 diabetic patients with nephropathy.* Nephrol Dial Transplant, 2008. **23**(3): p. 914-8.
321. Minh, T.o.C., et al., *Noninvasive measurement of plasma triglycerides and free fatty acids from exhaled breath.* J Diabetes Sci Technol, 2012. **6**(1): p. 86-101.
322. Bennett, L., et al., *A chemometric study on human breath mass spectra for biomarker identification in cystic fibrosis.* J Breath Res, 2009. **3**(4): p. 046002.
323. Baker, E.H., et al., *Hyperglycemia and cystic fibrosis alter respiratory fluid glucose concentrations estimated by breath condensate analysis Increased breath biomarkers of oxidative stress in diabetes mellitus*, in *J Appl Physiol*. 2007, 2004 Elsevier B.V.: United States Netherlands. p. 1969-75.
324. Leopold, J.H., et al., *Glucose prediction by analysis of exhaled metabolites - a systematic review.* BMC Anesthesiol, 2014. **14**: p. 46.
325. Phillips, M., et al., *Increased breath biomarkers of oxidative stress in diabetes mellitus.* Clin Chim Acta, 2004. **344**(1-2): p. 189-94.
326. Turner, C., et al., *An exploratory comparative study of volatile compounds in exhaled breath and emitted by skin using selected ion flow tube mass spectrometry.* Rapid Commun Mass Spectrom, 2008. **22**(4): p. 526-32.
327. Kulikov, V.I.u., et al., *[Concentration of light hydrocarbons in exhaled air depending on the risk factors of metabolic disorder].* Fiziol Cheloveka, 2011. **37**(3): p. 70-5.
328. Novak, B.J., et al., *Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes.* Proc Natl Acad Sci U S A, 2007. **104**(40): p. 15613-8.
329. Aschmann, S.M., W.D. Long, and R. Atkinson, *Pressure dependence of pentyl nitrate formation from the OH Radical-initiated reaction of n-pentane in the presence of NO.* J Phys Chem A, 2006. **110**(21): p. 6617-22.
330. Laffel, L., *Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes.* Diabetes Metab Res Rev, 1999. **15**(6): p. 412-26.
331. Spaněl, P., K. Dryahina, and D. Smith, *Acetone, ammonia and hydrogen cyanide in exhaled breath of several volunteers aged 4-83 years.* J Breath Res, 2007. **1**(1): p. 011001.
332. Storer, M., et al., *Measurement of breath acetone concentrations by selected ion flow tube mass spectrometry in type 2 diabetes Toward portable breath acetone analysis for diabetes detection*, in *J Breath Res*. 2011: England. p. 046011.
333. Salerno-Kennedy, R. and K.D. Cashman, *Potential applications of breath isoprene as a biomarker in modern medicine: a concise overview.* Wien Klin Wochenschr, 2005. **117**(5-6): p. 180-6.

334. Gelmont, D., R.A. Stein, and J.F. Mead, *Isoprene-the main hydrocarbon in human breath.* Biochem Biophys Res Commun, 1981. **99**(4): p. 1456-60.
335. King, J., et al., *Physiological modeling of isoprene dynamics in exhaled breath.* J Theor Biol, 2010. **267**(4): p. 626-37.
336. Deneris, E.S., R.A. Stein, and J.F. Mead, *In vitro biosynthesis of isoprene from mevalonate utilizing a rat liver cytosolic fraction.* Biochem Biophys Res Commun, 1984. **123**(2): p. 691-6.
337. Stone, B.G., et al., *Effect of regulating cholesterol biosynthesis on breath isoprene excretion in men.* Lipids, 1993. **28**(8): p. 705-8.
338. Fritz, G., *Targeting the mevalonate pathway for improved anticancer therapy.* Curr Cancer Drug Targets, 2009. **9**(5): p. 626-38.
339. Wells, D.L., S.W. Lawson, and A.N. Siriwardena, *Canine responses to hypoglycemia in patients with type 1 diabetes.* J Altern Complement Med, 2008. **14**(10): p. 1235-41.
340. Blaikie, T.P., et al., *Comparison of breath gases, including acetone, with blood glucose and blood ketones in children and adolescents with type 1 diabetes.* J Breath Res, 2014. **8**(4): p. 046010.
341. Yamashita, H., et al., *A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver.* Proc Natl Acad Sci U S A, 2001. **98**(16): p. 9116-21.
342. Iizuka, K., et al., *Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis.* Proc Natl Acad Sci U S A, 2004. **101**(19): p. 7281-6.
343. Karl, T., et al., *Human breath isoprene and its relation to blood cholesterol levels: new measurements and modeling.* J Appl Physiol (1985), 2001. **91**(2): p. 762-70.
344. Minh, T.D., et al., *Noninvasive measurement of plasma glucose from exhaled breath in healthy and type 1 diabetic subjects.* Am J Physiol Endocrinol Metab, 2011. **300**(6): p. E1166-75.
345. Keith, K., D. Nicholson, and D. Rogers, *Accuracy and precision of low-dose insulin administration using syringes, pen injectors, and a pump.* Clin Pediatr (Phila), 2004. **43**(1): p. 69-74.
346. McCoy, E.K. and B.M. Wright, *A review of insulin pen devices.* Postgrad Med, 2010. **122**(3): p. 81-8.
347. Wright, B.M., J.M. Bellone, and E.K. McCoy, *A review of insulin pen devices and use in the elderly diabetic population.* Clin Med Insights Endocrinol Diabetes, 2010. **3**: p. 53-63.
348. Silverstein, J., et al., *Care of children and adolescents with type 1 diabetes: a statement of the American Diabetes Association.* Diabetes Care, 2005. **28**(1): p. 186-212.
349. Mortensen, H.B. and P. Hougaard, *International perspectives in childhood and adolescent diabetes: a review. The Hvidøre Study Group on Childhood Diabetes.* J Pediatr Endocrinol Metab, 1997. **10**(3): p. 261-4.
350. Mortensen, H.B. and P. Hougaard, *Comparison of metabolic control in a cross-sectional study of 2,873 children and adolescents with IDDM from 18 countries. The Hvidøre Study Group on Childhood Diabetes.* Diabetes Care, 1997. **20**(5): p. 714-20.

351. Klonoff, D.C., et al., *Half-Unit Insulin Pens: Disease Management in Patients With Diabetes Who Are Sensitive to Insulin.* J Diabetes Sci Technol, 2017. **11**(3): p. 623-630.
352. Abul-Ainine, S.A. and A.A. Abul-Ainine, *Toddlers' choice: Yo-Yoing diabetes control or deci-unit insulin dosing?* World J Diabetes, 2012. **3**(2): p. 35-7.
353. Oyer, D., M. Niemeyer, and A. Moses, *Empowering people with diabetes: improving perceptions and outcomes with technical advances in insulin pens.* Postgrad Med, 2012. **124**(5): p. 110-20.
354. Bariya, M. and I. Nayberg, *Analysis of "Dose Accuracy, Injection Force, and Usability Assessment of a New Half-Unit, Prefilled Insulin Pen".* J Diabetes Sci Technol, 2018. **12**(2): p. 373-375.
355. Clark, P.E., C.R. Okenfuss, and M. Campbell, *Half-unit dose accuracy with HumaPen Luxura HD: an insulin pen for patients who need precise dosing.* J Diabetes Sci Technol, 2010. **4**(2): p. 353-6.
356. Service, N.S.P., *Comparative table of insulin pumps 2018*. London and South East Regional Medicines Information: https://www.sps.nhs.uk/articles/comparative-table-of-insulin-pumps-2/.
357. Freckmann, G., et al., *Accuracy of Bolus and Basal Rate Delivery of Different Insulin Pump Systems.* Diabetes Technol Ther, 2019. **21**(4): p. 201-208.
358. Kerr, D., et al., *Stability and performance of rapid-acting insulin analogs used for continuous subcutaneous insulin infusion: a systematic review.* J Diabetes Sci Technol, 2013. **7**(6): p. 1595-606.
359. Ruan, Y., et al., *Pharmacokinetics of diluted (U20) insulin aspart compared with standard (U100) in children aged 3-6 years with type 1 diabetes during closed-loop insulin delivery: a randomised clinical trial.* Diabetologia, 2015. **58**(4): p. 687-90.
360. A/S, N.N. and D. 2880 Bagsværd, *Insulin Diluting Medium for NovoRapid® (insulin aspart) and Levemir® (insulin determir)*, N.N. A/S, Editor. 1995/2008. p. 1.
361. (eMC), T.e.M.C. *NovoRapid 100 units/ml in a vial*. 06/2017 [cited 2017 October 2017].
362. Senstius, J., E. Harboe, and H. Westermann, *In vitro stability of insulin aspart in simulated continuous subcutaneous insulin infusion using a MiniMed 508 insulin pump.* Diabetes Technol Ther, 2007. **9**(1): p. 75-9.
363. Senstius, J., C. Poulsen, and A. Hvass, *Comparison of in vitro stability for insulin aspart and insulin glulisine during simulated use in insulin pumps.* Diabetes Technol Ther, 2007. **9**(6): p. 517-21.
364. Kerr, D., et al., *Laboratory-based non-clinical comparison of occlusion rates using three rapid-acting insulin analogs in continuous subcutaneous insulin infusion catheters using low flow rates.* J Diabetes Sci Technol, 2008. **2**(3): p. 450-5.
365. Castle, J.R., J.H. DeVries, and B. Kovatchev, *Future of Automated Insulin Delivery Systems.* Diabetes Technol Ther, 2017. **19**(S3): p. S67-S72.
366. Peters, A.L., et al., *Advances in Glucose Monitoring and Automated Insulin Delivery: Supplement to Endocrine Society Clinical Practice Guidelines.* J Endocr Soc, 2018. **2**(11): p. 1214-1225.

367. Reiterer, F., et al., *Analyzing the Potential of Advanced Insulin Dosing Strategies in Patients With Type 2 Diabetes: Results From a Hybrid In Silico Study.* J Diabetes Sci Technol, 2018. **12**(5): p. 1029-1040.
368. Kumareswaran, K., et al., *Feasibility of closed-loop insulin delivery in type 2 diabetes: a randomized controlled study.* Diabetes Care, 2014. **37**(5): p. 1198-203.
369. Los, E.A., et al., *Reliability of Trained Dogs to Alert to Hypoglycemia in Patients With Type 1 Diabetes.* J Diabetes Sci Technol, 2017. **11**(3): p. 506-512.
370. Gonder-Frederick, L.A., J.H. Grabman, and J.A. Shepard, *Diabetes Alert Dogs (DADs): An assessment of accuracy and implications.* Diabetes Res Clin Pract, 2017. **134**: p. 121-130.
371. Maran, A., et al., *Lack of preservation of higher brain function during hypoglycaemia in patients with intensively-treated IDDM.* Diabetologia, 1995. **38**(12): p. 1412-8.
372. *Abstracts of 51st EASD Annual Meeting.* Diabetologia, 2015. **58 Suppl 1**: p. 1-607.
373. *Abstracts of the Diabetes UK Professional Conference 2014, Arena and Convention Centre, Liverpool, UK, 5-7 March 2014.* Diabet Med, 2014. **31 Suppl 1**: p. 1-184.
374. Lam, S., *American Diabetes Association - 76th Scientific Sessions (June 10-14, 2016 - New Orleans, Louisiana, USA).* Drugs Today (Barc), 2016. **52**(6): p. 361-6.