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Continuous glucose monitoring in a cohort of prepubertal children with Type 1 diabetes mellitus: A comparison of glycaemic outcomes to clinical and biochemical markers of diabetes control.

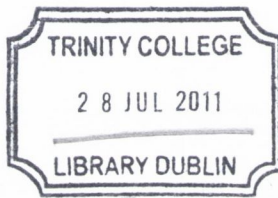
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## Summary

In type 1 diabetes, monitoring of glycaemic status is considered a cornerstone of diabetes care and vital in the prevention of diabetes complications. Intermittent daily self monitoring of blood glucose levels provides a limited view of a patient's glycaemic control whereas continuous glucose monitoring provides an ambulatory record of glucose levels which more accurately reflects glycaemic control. Available data on continuous glucose monitoring in children is limited, difficult to replicate and restricted to basic comparative analysis.

This project aimed to devise a novel algorithm that would comparatively analyse CGMS data expanding on previously published methods to include quantitation of inter- and intra-day glycaemic variation. The CGMS was repeated on four occasions to ascertain if education and repeated CGMS use improved glycaemic variation or HbA1c and reduced hypoglycaemia. Finally, short-term outcomes of glycaemia were evaluated to ascertain if twice daily insulin regimens or behaviour are directly impacted by glycaemia.

A cohort of children were studied over a twelve month period wearing the continuous glucose monitor on four occasions (0,3,6,9 months after recruitment). Participants were less than 10 years old with type 1 diabetes for at least two years. Clinical and demographic data was collected at each clinic visit on height, weight, HbA1c and insulin regimen. At the three and six month visits, parents were asked to complete the Behavioral Assessment System for Children (BASC).

Fifty two of ninety nine eligible children consented to the study with forty four children completing all study parameters. The study group were representative of the diabetes clinic population when compared for age, HbA1c and duration of diabetes. The algorithm devised included six glycaemic outcomes to summarise the data provided by CGMS. Outcomes included mean CGMS glucose, percent time in the low [ $<4\text{mmol/L}$ ], normal [ $4\text{-}12\text{mmol/L}$ ], and high [ $>12\text{mmol/L}$ ], CGMS range, inter-day [MODD] and intra-day glycaemic variation [CONGA] measured at 1, 2 and 4 hourly intervals. MODD is the mean of daily differences used to calculate the consistency between the days of the trace. CONGA represents continuous overall net glycaemic action which numerically summarises the extent of intra-day glycaemic variation and was innovatively devised for this project.

The analysis of CGMS data in this cohort showed a high level of inter- and intra-day glycaemic variation reflecting a wide pattern of glycaemic control among the cohort of children with diabetes. Repeated continuous glucose monitoring at three monthly intervals resulted in a





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significant decrease in CONGA1 ( $p=0.03$ ) CONGA2 ( $p=0.05$ ) and CONGA4 ( $p=0.03$ ), the measures of glycaemic variation over this period.

No direct relationship was found between glycaemic variation and HbA1c although mean blood glucose was positively correlated with HbA1c at three of the four timepoints [ $r=0.35, 0.36, 0.18, 0.56$ ]. A significant rise in group mean HbA1c over the 12 month period was noted within the study group (8.1 vs. 8.5,  $p=0.01$ ). This was reflected by a significant increase ( $p=0.05$ ) in percent time spent in the high glycaemic range. Over the twelve months of the study there was a negative correlation between HbA1c and percent time in the low glycaemic range [ $r=-0.49, p=0.002$ ] and percent time in the normal glycaemic range [ $r=-0.41, p=0.01$ ] balanced by a positive correlation between HbA1c and percent time in the high glycaemic range [ $r=0.63, p<0.001$ ]. No relationship was found between adjustments in twice daily insulin dosage and metabolic or glycaemic outcomes calculated from CGMS.

Comparison of types of behaviour to glycaemic outcomes from CGMS, showed a strong relationship between rising levels of glycaemia and externalising behaviours (e.g. aggression) in the child. Mean blood glucose was significantly associated with the externalizing behaviour score [regression co-efficient =1.7 (95% C.I. 0.6-2.8) adjusted  $r^2 = 0.088$ ]. This was reflected by a negative association between percentage time in the normal glycaemic range [regression co-efficient = - 0.2 (95% CI -0.3 to -0.5) adjusted  $r^2 = 0.068$ ] and a positive association with percent time in the high glycaemic range with mean externalizing behaviour score [regression co-efficient= 0.2 (95% C.I. 0.07-0.3) adjusted  $r^2=0.089$ ].

This methodology of CGMS analysis provides further perspective to the glycaemic control of an individual than is evident from self monitoring or HbA1c. The CONGA measure is a novel measure that is suitable for use in ambulant continuous glucose monitoring systems and should be an integral component in the assessment of CGMS data. Insulin regimens do not appear to be the major determinant of metabolic outcome. Short term measures of glycaemic control are associated with clinically significant externalising behaviour.

Longitudinal studies with larger cohorts and appropriate lifestyle measures (including nutrition and exercise) may further delineate the role of glycaemic variation in the management of type 1 diabetes. Further studies are required to establish whether behavioural symptoms are reduced with improved metabolic control.



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## Glossary

BASC	Behavioural Assessment System for Children
BMI	Body Mass Index
CG-EGA	Continuous glucose - error grid analysis
CGMS	Continuous glucose monitoring system
CONGA	Continuous overall net glycaemic action
CSII	Continuous subcutaneous insulin infusion
CV	Co-efficient of variation
DCCT	Diabetes Complications and Control Trial
EDIC	Epidemiology of Diabetes Interventions and Complications Study
EEG	Electroencephalogram
HAAF	Hypoglycaemia associated autonomic failure
ISO	International Standardization Authority
ISPAD	International Society of Paediatric and Adolescent Diabetes
LI	Lability index
MAGE	Mean amplitude of glycaemic excursions
MBG	Mean blood glucose
MDI	Multiple daily injections
MODD	Mean of daily differences
NPH	Neutral protamine Hagedorn
Sd	Standard deviation
SDIS	Stockholm Disease and Intervention Study
UKPDS	United Kingdom Prospective Diabetes Study



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## Introduction

Continuous glucose monitoring has dramatically refocused the approach to glycaemic control in diabetes in the twenty-first century. The introduction of an ambulatory record of glucose levels has given an accurate and unexpected reflection of the glycaemic control of a person with diabetes. Undetected prolonged episodes of hypoglycaemia (especially nocturnal) and responses to daily diet and exercise have provided invaluable information in managing glycaemic control. Much of the literature on continuous monitoring to date concentrates on general information based on the percentage time in hypoglycaemia or the area under the curve. Only cursory information exists on the impact of short periods of continuous glucose monitoring upon long term glycaemic control. In the late twentieth Century, HbA1c provided the gold standard of metabolic control and is the marker for development of long term complications. Significant questions in this area remained unanswered especially for young adults and children with type 1 diabetes. Do prolonged periods of hyperglycaemia or hypoglycaemia adversely affect health? Are dramatic swings in glycaemic control reflected in a child's behaviour? Does repetitive continuous glucose monitoring have a role consistent with the three monthly clinic attendance scheduled for children with diabetes? Is there a quantifiable relationship between proportions of free mixing insulin and glycaemic variation? This study followed a prepubertal group of children through twelve months of diabetes management monitoring growth, HbA1c, insulin requirements and levels of glycaemia using continuous glucose monitoring at clinic visits. This dissertation addresses the questions posed above by examining the relationship between CGMS and metabolic control and determining the role of glycaemic variation in routine monitoring of diabetes. In addition, information derived from continuous glucose monitoring is used to determine the impact of insulin on glycaemia and effects of glycaemia on the behaviour of the child.

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# 1 Background

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## 1.1 Type 1 Diabetes Mellitus – a brief history

*"Diabetes is a wonderful affection, not very frequent among men, being a melting down of the flesh and limbs into urine"*

Areteus the Cappadocian

Greece ~150 AD

Diabetes mellitus was first identified by Egyptians over 3,500 years ago. It was originally known as simply *diabetes* (the greek word meaning siphon). Cases continued to be described across the centuries and the belief was widely held that diabetes was a disease of the kidneys and bladder. Willis in 1674 was the first observer of diabetes to relate the disease to a disturbance in the blood (1). Then, the experiments of Dobson in 1776 demonstrated that a sugary substance was eliminated into urine (1). This discovery led to the delineation between two types of chamber pot dropsy namely diabetes mellitus (honey urine) and diabetes insipidus (limpid urine) by Cullen in 1769 (1). Isolation of the pancreatic islets by Langerhans in 1869 (2) was followed by the work of Minkowski in the late 19<sup>th</sup> century which proved that removal of the pancreas in dogs led to fatal diabetes (3). Many groups set about to identify the substance insulin (after the Greek for island) believed to be produced from the islets of the pancreas. Although the work of Panlescu was thought to be the first to isolate insulin, the subsequent work of Banting, Best, Collip and McLeod led to the discovery and purification of the first insulin trialled on a human (a 12 year old patient named Leo Thompson) in 1922 (4).

---

## 1.2

### Definition of diabetes mellitus

According to the 2007 Diagnosis and Classification of Diabetes Mellitus by the American Diabetes Association, diabetes mellitus is classified as a group of metabolic diseases characterised by hyperglycaemia resulting from defects in insulin secretion, insulin action or both (5). Type 1 diabetes mellitus or immunogenic diabetes accounts for 5-10% of those with diabetes and results from a cellular mediated autoimmune destruction of the beta-cells (islet cells) of the pancreas (5). Diagnosis of diabetes is made on the presence of symptoms of diabetes (polyuria, polydipsia, and unexplained weight loss) with a random plasma glucose concentration  $>11.1\text{mmol/L}$  ( $200\text{mg/dl}$ ) or a fasting plasma glucose  $>7.0\text{mmol/L}$  ( $126\text{mg/dl}$ ) (5). Individuals with type 1 diabetes are dependent on insulin for survival.

---

## 1.3

### Insulin therapy

Shortly after the introduction of insulin as a therapy by Banting and colleagues, widescale production of insulin was instituted by Connaught Antitoxin Laboratories and subsequently Eli Lilly (6). This was achieved through isoelectric precipitation of insulin which allowed production of larger quantities of higher potency insulin from animal sources adequate for commercial supply (7; 8). In 1926, J.J. Abel demonstrated that crystallized amorphous insulin had increased stability in comparison to the original purified extracts (7; 8). In the search to develop an insulin of longer duration, scientists started to combine insulin with other substances. Protamine insulinate was introduced by Hagedorn et al in 1936 (6-8). Addition of a fish protein (protamine) to purified animal insulin led to the formation of a protamine insulin complex which was poorly soluble at physiological pH and thus slowly absorbed from the subcutis (9). During the same period, investigators in Toronto were developing an insulin whose action was prolonged through the addition of zinc (8). The combination of protamine



and zinc with insulin resulted in the development of neutral protamine Hagedorn (NPH) (8; 9) which had a peak onset of action at 6-10 hours and a duration of action up to 24 hours (9). These developments led to a wider variety of insulins on the market and the ability to use different combinations of long and regular acting insulin to suit patient needs. The availability of insulins of varying duration of action enabled the user to more accurately reflect physiological insulin action within their everyday routine. The ongoing goals of insulin therapy are to replace the hormone in a manner which reproduces physiologic insulin secretion as closely as possible, ultimately restoring normal plasma glucose concentrations, suppressing ketogenesis and maintaining the vitality and health of patients affected with diabetes (9).

### **1.3.1 Animal insulin**

Porcine and Bovine insulin were the main sources of insulin used in people with diabetes until the 1980s when synthetic human insulin was introduced. Porcine and bovine insulin differ from endogenous human insulin by one and two amino acids, respectively (7; 9). Although use of purified animal insulin extracts treated glycosuria, polyuria and polydipsia successfully, there were limitations due to allergic reactions to impurities in the insulin formulations or the insulin molecule itself (9). With the delineation of the structure of insulin by Sanger and his co-workers between 1951 and 1955 (10-15) and the discovery of proinsulin in 1967 (16), research was directed towards development of biosynthetic human insulin.

### **1.3.2 Recombinant human insulin**

In 1982, Eli Lilly introduced recombinant DNA insulin (7). The recombinant DNA technology produced the alpha and beta chains of the insulin molecule separately through transfection of *Escherichia coli* with requisite genetic fragments (9). These chains were then biochemically combined to yield the intact insulin molecule (9). In the same year, Novo Industri A/S developed enzymatic conversion of the porcine insulin sequence to the human insulin sequence



(7) where the alanine residue at position B29 of porcine insulin was replaced with a threonine residue to yield human insulin (9). These developments led to the availability of synthetic regular insulin which complemented longer duration insulins with a quicker onset, peak and shorter duration than the intermediate acting isophane insulins on the market. These human insulins which remain in current use have a quicker onset and earlier peak of action in comparison to their animal counterparts probably explained by the increased hydrophilic nature of human insulin leading to faster absorption (9).

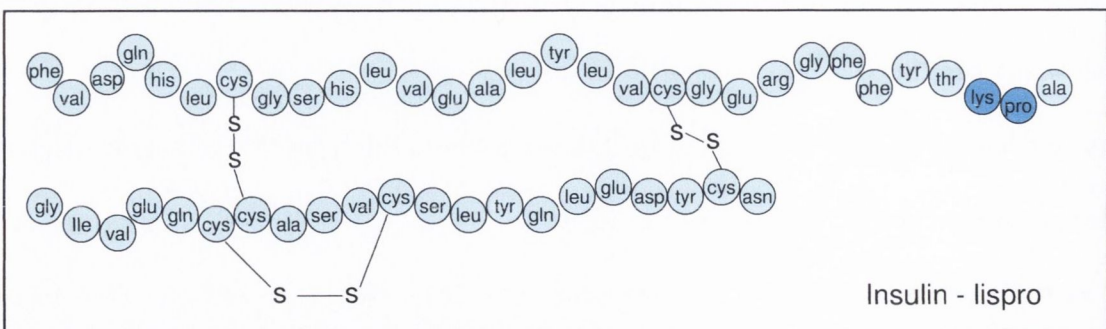
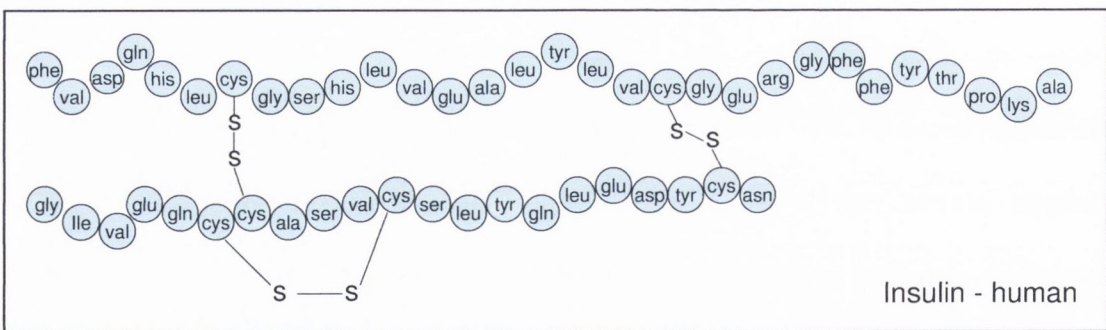
### **1.3.3 Short acting insulin analogues**

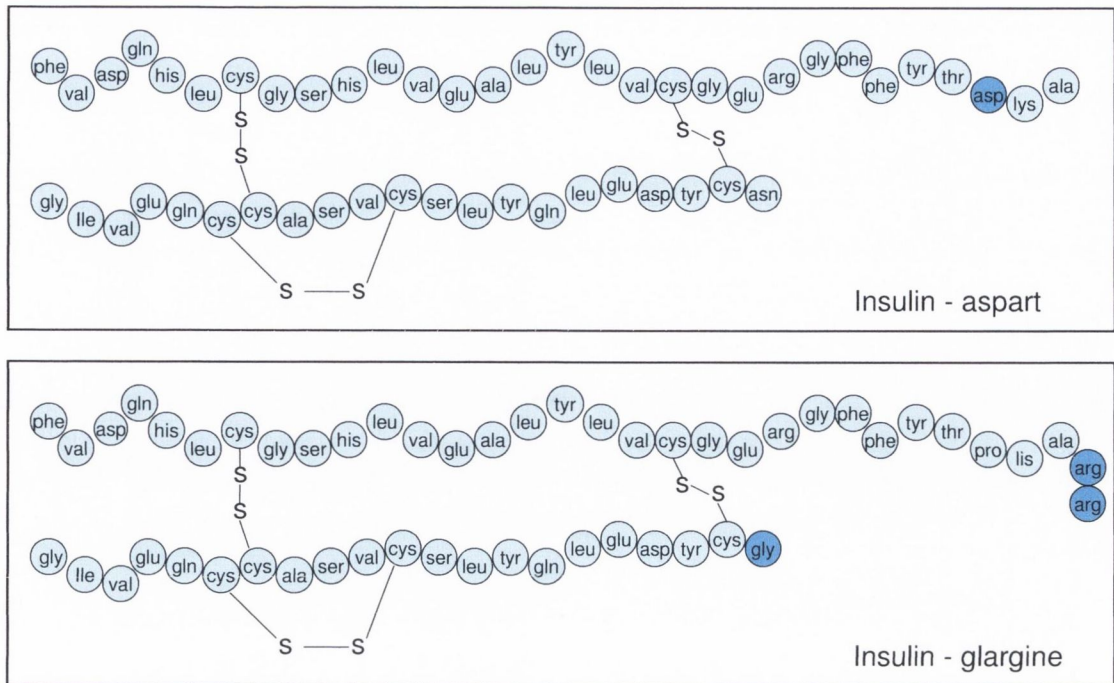
One approach to improving the efficiency of insulin therapy was to maximise the efficiency of synthetic insulins to simulate normal physiologic insulin secretion (9; 17). This has resulted in the development of novel synthetic insulins referred to as insulin analogues. Insulin analogues are peptides that differ from human insulin in amino acid sequence but bind to insulin receptors and act in a similar fashion to human insulin (9). Early attempts to develop rapidly absorbed insulin analogues were successful with improved absorption after subcutaneous injection but these products had to be withdrawn after a dose dependent increase resulted in the occurrence of mammary carcinoma in mice (9; 18).

Rapid-acting insulin in the form of Insulin lispro (18; 19) and Insulin aspart (20) were introduced at the turn of the 21st Century. Both analogues arise from amino acid substitutions, reversing of proline and lysine at positions 29 and 30 of the beta-chain (lispro) or substitution of aspartic acid for proline at position 28 of the beta-chain (aspart), which result in increased insulin absorption without altering the metabolic actions of insulin (19-21). These insulins work within minutes of injection, peak within thirty minutes and have a duration of less than four hours. This profile has led to their widespread use in basal bolus regimens and as the insulin type of choice in insulin pumps (22-25). Changes in molecular structure of human insulin (15) to insulin lispro, aspart and glargine are illustrated in figure 1-1.

### 1.3.4 Long acting insulin analogues

The first long-acting analogue insulin, Insulin glargine, became available in Germany in 2000. This is an analogue of human insulin with C-terminal elongation of the B-chain by two arginines and substitution of asparagine in position 21 of the  $\alpha$ -chain with glycine [21<sup>A</sup>-Gly-30<sup>B</sup>a-L-Arg-30<sup>B</sup>b-L-Arg human insulin] (21). This modification shifts the isoelectric point for the molecule to a slightly acidic pH with the subsequent slow dissolution of the microprecipitates formed following subcutaneous injection, markedly delaying absorption into the systemic circulation (9; 21). Pharmacokinetic and pharmacodynamic studies have demonstrated a flat and prolonged time- action profile compared to its long acting predecessors Protaphane and Humulin NPH (26; 27). Insulin Glargine when given at the same time once daily has an onset of approximately thirty minutes to reach a plateau level and a basal profile lasting approximately 24 hours (28; 29). Meal boluses with rapid-acting insulin injections are required to supplement this background level, which is a refinement of the traditional basal bolus system (28; 29).





**Figure 1-1: Human insulin and structural changes in insulin analogues.**

The above figure illustrates the changes between human insulin (box 1), insulin lispro (box 2 with lysine and proline exchanging positions 28 and 29 of the beta chain), insulin aspart (box 3 with a substitution of aspartate for proline at position 28 of the beta chain) and insulin glargine (box 4 with a substitution of glycine for asparagine at position 21 of the alpha chain and the addition of two arginine molecules onto position 30 of the beta chain).

Insulin detemir, with a similar profile to insulin glargine, was introduced to the market in 2004. Insulin detemir is an acylated derivative of human insulin with protracted action [Lys<sup>B29</sup>(N<sup>B</sup>-tetradecanoyl) des (B30) human insulin] (30). By binding to albumin via the fatty acid chain, only a small fraction of insulin detemir is released in a free and unbound form at steady state (30). The advantage shown in early research with insulin detemir is the introduction of improved glycaemic control with lower risk of hypoglycaemia and no concomitant weight increase (30-32). In comparison with NPH insulin, detemir has been associated with more predictable glycaemic control in patients with Type 1 diabetes on a basal bolus regimen (30). Initial clinical experience suggests that patients on insulin detemir require a twice daily regimen compared to once daily injections using insulin glargine. However, the acidic nature of insulin glargine limits the ability to mix with other insulins, a problem not encountered when using insulin detemir (30).



The newest insulin to become available is insulin glulisine which was released in 2005. This is another synthetic analogue of regular human insulin. Insulin glulisine [3(B)-Lys, 29(B)-Glu-human insulin] is a human insulin analogue designed to control mealtime blood sugar with a faster absorption and onset of action and shorter duration than that of human insulin (33). The modifications of the amino acid sequence at positions 3 and 29 in the B chain of human insulin simultaneously provide stability to the molecular structure and render the insulin glulisine molecule less likely to self-associate compared with human insulin (33). Research in children and adolescents shows that, compared to regular human insulin, it has a quicker time to maximum insulin concentration (54 vs. 66min) and a lower mean residence time ( 88 vs. 137 mins,  $p < 0.05$ ) (34). Studies to date have shown that insulin glulisine is well tolerated (34).

### **1.3.5 Adverse effects of synthetic insulin**

Changes in insulin structure may result in alterations in both metabolic and mitogenic activity. Multiple factors such as residence time on the receptor, dissociation rate, rate of receptor internalization and the degree of phosphorylation of signalling proteins can affect the mitogenic potencies of insulin analogues (35). Changes in the structure of the insulin have raised concern about the safety of the insulin analogues. For example, *Glargine* insulin binds to IGF-1 receptors with up to six-fold higher affinity than human insulin, raising concerns about possible mitogenic and angiogenic effects of the peptide (21). One paper queried the relationship between the use of insulin lispro and the progression of diabetic retinopathy in pregnancy (36) however this data remains independently unsubstantiated. At the time of writing, there was no evidence in the literature to suggest that any adverse outcomes had arisen from the use of any of the synthetic insulins listed above.



### **1.3.6 Insulin Administration**

Insulin has been administered by injection since the purification of the first pancreatic extracts in 1922. Injections were initially intramuscular and often produced a painful abscess. It was soon discovered that subcutaneous injection was as effective and less painful (37). Subcutaneous injections are now administered using microfine needles with either syringes or pen shaped devices which administer a selected dosage of insulin by depressing a plunger after the needle is injected (37). An alternative mode of injected therapy involves the Mediject [Minneapolis, MN, USA], an air powered injector which forces insulin through the skin in an aerosolized burst (38). This has only been cautiously endorsed in selected cases (39).

### **1.3.7 Continuous subcutaneous insulin infusion (CSII)**

Since 1976, work has progressed on the refinement of continuous subcutaneous insulin infusion [CSII], using a portable electromechanical pump to help mimic nondiabetic insulin delivery. The system infuses rapid-acting insulin into the subcutaneous tissue at preselected rates with patient activated boosts at mealtimes (40). The rapid-acting insulin is infused from a small housing through a catheter into a transcutaneous needle which delivers insulin to the same subcutaneous site for 3 days (37; 41). The overall size of such insulin delivery systems has been progressively reduced, now approximating the size of a pager (37) increasing their appeal and convenience. Use of CSII in children and young adults less than 20 years of age constituted less than five percent of the total use by 1996 (42). Subsequent clinical trials are showing sustained value of CSII in maintaining or improving glycaemic control when compared to multiple daily injections [MDI] over a 12 month period (40; 42; 43).

### **1.3.8 Non injectable insulin administration**

Further work in insulin therapy has focused on non-injectable methods of insulin. Alternative routes of insulin administration (transdermal, oral, nasal, intestinal and rectal) have been

extensively investigated since as early as 1924 (44). Methods currently being trialled include oral, nasal and inhaled insulin (21). Inhaled insulin has progressed through clinical trials and studies are now available in children and adolescents (21; 45). In the past five years, studies have been published on the efficacy of inhaled insulin in both type 1 (46-49) and type 2 (50-54) diabetes. A recent Cochrane review analysed results from six clinical trials incorporating 1191 adult patients and found that inhaled insulin administration resulted in similar levels of glycaemic control, similar incidence of hypoglycaemia and better quality of life in comparison to injected insulin (55). The inhaled insulin is administered in place of short-acting insulin as adjustable premeal boluses while still requiring the concomitant use of a basal injection of insulin (55; 56). Inhaler devices include the AERx (Novo Nordisk/Aradigm) which uses a liquid formulation and Exubera (Pfizer/Inhale Therapeutic systems) which is based on a dry powder formulation (55). Patient satisfaction with a non-injectable form of insulin has been shown to be higher with regard to ease of administration, comfort, convenience, time and flexibility of eating schedule when compared with subcutaneous insulin (57-59).

As with the development of injectable synthetic insulins, concerns remain regarding the safety profile of inhaled insulin, especially the effect of long term administration on pulmonary and alveolar function (9). Inhaled insulin is an inefficient method of taking insulin with much higher doses required to achieve an effect similar to soluble insulin (44). Use of inhaled insulin has been cautioned in smokers and asthmatics (55; 60; 61). Different smoking patterns between and within individuals could theoretically influence dose requirements of inhaled insulin (61). The recent clinical study by Henry et al showed that when non diabetic patients with asthma [n=17] were compared to healthy non diabetic controls [n=28], they absorbed significantly less insulin [p=0.013] and had a lower reduction of their blood glucose [p=0.007] after inhalation of insulin using the AERx system (60). Presence of an upper respiratory tract infection in people without diabetes did not alter the pharmacokinetic

profile of inhaled insulin absorption however this has yet to be shown in subjects with diabetes (62).

The use of an inhaled molecule in patients with a known autoimmune disease must be cautiously monitored as the immune response of the body to inhaled insulin has been shown to differ between type 1 and type 2 diabetes. Fineberg et al examined the immune response to insulin in subjects participating in phase II and III clinical trials. At the end of the initial studies, the median percentage antibody binding in type 1 diabetes patients increased to 29.0% (mean 31.0%; SD 20.3) in the inhaled insulin group, but did not increase significantly in the subcutaneous insulin group (63) indicating a production of antibodies in response to inhaled insulin. At study end point, the median percentage insulin antibody binding in type 2 patients increased to 6.0% (mean 13.1; SD 18.2) in the inhaled insulin group and remained unchanged in the subcutaneous insulin group (63). Overall, there was a clear difference in antibody responses between patients with type 1 diabetes versus insulin-using patients with type 2 diabetes (63). Skyler et al showed an increase in insulin antibody serum binding without associated clinical manifestations in a group taking inhaled insulin compared to a group on basal bolus injections with comparable pulmonary function between the groups (56). Further clinical trials and long term safety surveillance are required to ascertain whether such an immune response results in adverse events in these patients. The Inhaled Human Insulin Type 1 Diabetes Study Group recently published two year data on safety and efficacy which showed a decline in forced expiratory volume in one second (FEV1) in the first three months of inhaled insulin therapy in comparison to subcutaneous insulin which was not progressive thereafter (64). Despite this information, in October 2007, Pfizer announced their intention to withdraw their inhaled insulin product Exubera® from the market due to the occurrence of isolated cases of bronchial carcinoma.



### **1.3.9 Insulin adjuncts**

The modality of choice for children and adolescents with type 1 diabetes continues to be subcutaneous injections. Alternative strategies designed to reduce the need for subcutaneous injections include the use of dipeptidyl peptidase 4 inhibition for islet enhancement as a treatment strategy for prediabetes (65) and the use of pramlintide (a synthetic amylin analogue) which is used with mealtime insulin to decrease glucagon release, slow gastric emptying and reduce food intake (66). However, these methods are very much at a preliminary stage of research, especially with regard to children.

### **1.3.10 Insulin balance**

In children with subcutaneous insulin regimens, attempts to supply physiological needs for insulin continue in boluses of one to four injections daily. Many primary school-aged children, and in some countries the majority, with type 1 diabetes receive their insulin in a twice-daily regimen of free-mixed insulins (67-69). A combination of regular and isophane insulins has traditionally been the most common regime with analogue insulins being more recently incorporated. Diabetes care manuals and textbooks have suggested proportions of short to intermediate-acting insulins within these admixtures (70; 71) with the aim being to avoid peaks and troughs of circulating insulin levels and to optimize glycaemic stability. Most texts recommend that the majority of insulin is given in the morning and that the majority of this should be given as intermediate-acting insulin (70-72). Large swings in glucose readings post meal ingestion (post-prandial excursions) occur because of the limitations of subcutaneous insulin injections in mimicking endogenous portal delivery of insulin (73; 74). Postprandial excursions complicate the management of diabetes where tight metabolic control is associated with fewer long-term complications (75) although no clinical trial data currently addresses the issue of whether postprandial glucose independently plays a unique role in the pathogenesis of complications. Following the DCCT and EDIC studies [to be discussed in detail in section 1.5]

some clinicians have suggested that all children should be commenced on multiple daily injections from diagnosis. This would provide a physiological, flexible insulin regimen rather than a fixed insulin regimen which could protect against the risk of hypoglycaemia in relation to food ingestion, physical exercise and sleep (76). Ludvigsson and Bolli also advocate that appropriate education should be delivered at diabetes onset to the child and parents in order to commence intensified insulin therapy as early as possible (76). Holl et al studied adolescents on twice daily injections and those on multiple daily injections and found that metabolic control was unsatisfactory in many adolescents with type 1 diabetes irrespective of the insulin regimen (77). Subsequently, metabolic control in this cohort deteriorated further after more adolescents were switched over to MDI regimes (77). Danne and Becker concluded that advanced insulin formulations, particularly insulin analogues, tailored insulin regimens and delivery systems needed to be delivered in combination with age-appropriate education, patient/carer involvement and ongoing support from the wider diabetes team to assist in the effective management of diabetes among children and adolescents (78). Despite the push for increasing use of MDI in children and adolescents [for which the evidence base is not established] (79) many centres are still using a twice-daily regimen. In twice-daily regimens the textbooks recommend a balance between short and intermediate-acting insulins to avoid major swings in glycaemia, however the evidence base for this has never been properly tested. The advent of continuous glucose monitoring allows the promise of improved glycaemic stability with such regimes to be tested.

---

## **1.4 Methods for monitoring glucose levels**

Although, the use of insulin was widespread from the 1920s, the first methods of self assessment of blood glucose were not developed until the 1960s. Before then, tests were based on urine glucose testing for the presence of glycosuria (presence of glucose in urine).

Home testing of glycosuria became possible in the United States around 1925 using Benedict's solution. The substance to be tested is heated with Benedict's solution and formation of a brick-red precipitate indicates presence of the aldehyde functional group, -CHO. Since simple sugars (e.g., glucose) give a positive test, the solution was utilised to test for the presence of glucose in urine. However, glycosuria is the net result of two processes, glomerular filtration and tubular reabsorption of glucose so differences can exist between patients (80). The rationale behind urine glucose testing was that urine glucose reflected blood glucose levels during the time of urine production (81). In later decades, urine glucose was measured as a "spot test" with a dipstick containing a colour-sensitive pad. This pad was saturated with specific chemicals which reacted with glucose to give a colour indicative of glucose concentration (82). Most current commercial strips utilise a glucose oxidase reaction (82). This is a semi-quantitative technique with minimal ability to detect glucose once the blood level falls below 10mmol/L (180mg/dl), the renal threshold for glucose (81). Concentration of urine can also affect urine glucose concentrations (82). Therefore, the current recommendation on glucose testing is that urine glucose testing should only be encouraged for patients who cannot or will not perform self monitoring of blood glucose levels (81-83).

#### **1.4.1 Self monitoring of blood glucose**

It took a further decade after the development of urine glucose testing before home blood glucose monitoring became a reality. Anton Hubert Clemens received the first patent for a blood glucose meter called the *Ames Reflectance Meter* on September 14, 1971. Richard K. Bernstein, an insulin dependent physician with diabetes, was one of the first patients to monitor his blood glucose at home using a glucose meter (84). While originally intended for physician use, this method of glucose monitoring quickly became viable for patient use at home. The introduction of patient self monitoring of blood glucose called attention to the



failure of commonly used regimens to normalise blood glucose (17). Monitoring of glycaemic status has since become considered a cornerstone of diabetes care (85; 86). Results of monitoring are used to assess the efficacy of therapy and to make adjustments in diet, exercise and medications in order to achieve the best possible blood glucose control (86).

At least 25 different meters are currently commercially available. All calculate the level of glucose in a whole blood sample determined by capillary sampling usually through finger prick monitoring using a sterile lancet. Meters differ in regard to volume of blood required, test speed, size, memory and cost of consumables (86). Enzymatic methods utilizing glucose dehydrogenase, hexokinase or glucose oxidase in conjunction with colorimetric, photometric or electrochemical detection devices form the basis for both the highly accurate glucose analyzers and small inexpensive hand-held meters (87).

However, to obtain multiple readings, multiple blood tests must be done. Blood glucose testing in this manner can be time consuming and invasive to the person with diabetes. Although self monitoring of blood glucose is recommended a minimum of three times daily in people with type 1 diabetes (88; 89) levels of compliance have been recorded at 40% in adults with type 1 diabetes mellitus (90). A study of adults with type 1 Diabetes from Tayside, Scotland showed that only 1% of the cohort obtained enough glucose strips to test their blood sugar four times daily in the test period (91). Barriers to successful self monitoring of blood glucose include user error, inadequate sampling and reduced compliance due to discomfort and inconvenience. Factors relating to compliance and the inability or unwillingness of patients to make the necessary quality-of-life sacrifices undoubtedly constitute the major impediments to normalization of blood glucose in most patients (17). Conflict around blood glucose monitoring may also serve to promote negative feelings in children and adolescents directed at diabetes management tasks specifically blood glucose monitoring (92).

#### 1.4.2 Alternative site testing

In order to alleviate issues of site discomfort, alternative site testing was introduced. The use of alternative sampling sites including forearm, upper arm, abdomen, thigh and calf can be advantageous as these sites are less densely innervated than the fingertip with consequent reduction in pain (93). At the time of introduction, it was assumed that capillary blood glucose measurements do not differ from those taken at the fingertip. Lock et al examined 50 non fasting subjects and found no difference between glucose measurements of capillary blood sampled from the forearm or fingertip (94). However, Ellison et al did demonstrate lower glucose readings during rapid fluctuations of glycaemia (e.g. postprandially) in 42 adults with diabetes when samples were compared between the fingertip, forearm and thigh (95). A similar delay was noted by Jungheim et al who noted a delay between the fingertip and forearm in both the rise (11.5 vs 8.4mmol/L  $p<0.001$ ) and fall (15.0 vs 12.1mmol/L  $p<0.001$ ) of laboratory controlled blood glucose using 3 different alternative site monitors in 17 adults with type 1 diabetes mellitus (93). These findings have been supported by two further studies which also showed that the rate of hypoglycaemia detection differs among blood glucose monitoring sites particularly the forearm (96; 97).

As a result, blood glucose meters are considered the most accurate method of assessing short term ambulatory blood glucose control. However, each measurement only represents a glimpse of the glycaemic milieu. Technical advances in testing and meters now result in sophisticated printouts of data which require further study to determine their full usefulness (86). Finally, the accuracy and precision of these meters remain limited by issues concerning calibration and individual techniques. Other methods of glycaemic monitoring are warranted to oversee and improve patient care. The development of continuous monitoring techniques like CGMS and microdialysis are discussed in section 1.10.

In the latter part of the twentieth century, methods of measuring metabolic control as a summary of blood glucose control over a six week to three month period were developed.

### 1.5.1 Glycosylated haemoglobin [HbA<sub>1c</sub>]

Glycosylated haemoglobin was first suggested as a marker of metabolic control of diabetes in the 1970s (98). Glycosylated haemoglobin occurs when a stable ketoamine linkage forms during the attachment of glucose to the terminal amino acid of the beta-chain of the haemoglobin A molecule. Although the HbA molecule has four components (HbA<sub>1a1</sub>, HbA<sub>1a2</sub>, HbA<sub>1b</sub> and HbA<sub>1c</sub>), HbA<sub>1c</sub> is quantitatively the most important, as the biosynthesis of HbA<sub>1c</sub> proceeds at a constant rate throughout the life span of the erythrocyte (red blood cell) in direct relation to the prevailing glycaemia to which the erythrocyte is exposed (80). HbA<sub>1c</sub> was identified as a minor fraction of normal adult haemoglobin by ion exchange chromatography over four decades ago (99; 100). In 1971, Trivelli reported that HbA<sub>1c</sub> comprised 6-12% of circulating haemoglobin in many diabetics compared to 3-6% in normal subjects (101). Koenig, in 1976, demonstrated that improved control resulted in a return of HbA<sub>1c</sub> to near normal levels (98). HbA<sub>1c</sub> gained prominence when it was used as the chief determinant of metabolic control in two landmark, prospective, randomised studies; the Diabetes Complications and Control Trial [DCCT] (102-104) and the United Kingdom Prospective Diabetes Study [UKPDS] (105-107), which are discussed in more detail in section 1.6. HbA<sub>1c</sub> was shown in the feasibility studies for the DCCT to show a strong positive correlation with the mean of all quarterly blood glucose profiles, regardless of treatment group with a correlation co-efficient of  $r=0.8$  (104). Since then, HbA<sub>1c</sub> has become a standard assessment of glycaemia and a routine part of diabetes management (108) and is now regarded



as the gold standard of metabolic control and the surrogate marker for subsequent development of complications (109).

Current technology involves the calculation of HbA<sub>1c</sub> by various methods including immunoassay and high performance liquid chromatography using microlitres of blood and reporting in less than five minutes allowing point of care use. The method employed must ideally have a total imprecision [coefficient of variation (CV)] of  $\leq 4\%$  and the 95% C.I. of the differences must fall within the clinically significant limits of  $\pm 1\%$  HbA<sub>1c</sub> (86). Further studies are recommended to determine whether more [or less] frequent testing of glycosylated haemoglobin is clinically useful than the current frequency of every three months (86).

### 1.5.2 Fructosamine

An alternative short-term marker of metabolic control is fructosamine. Fructosamine is a condensation product of glucose and proteins which acts as an intermediate marker of glycaemic control changing over a four to six week period (80). The fructosamine assay is based on the ability of glycated proteins to act as reducing agents in alkaline solution, with continuing debate on whether fructosamine assays need to be corrected for serum protein or albumin concentrations (81). Fructosamine and other measures of intermediate glycaemic control were not used in the landmark complications studies listed above and therefore less information is evident about the ability of tests such as fructosamine to predict the risk of diabetes complications as efficiently as HbA<sub>1c</sub> (100). Alternative methods of routine monitoring of glycaemic control in patients with diabetes include total glycated serum proteins and glycated serum albumin. These methods correlate well with one another and with glycated haemoglobin and have been suggested as alternative methods for routine monitoring of glycaemic control in patients with diabetes (81).

HbA<sub>1c</sub> does not appear to take account of glycaemic variability, whose role as an independent risk factor in type 1 diabetes remains undefined (109). Although glycation of haemoglobin

occurs over the life span of the erythrocyte, theoretical models and clinical studies suggest that a patient in stable control will have 50% of their HbA<sub>1c</sub> formed in the month before sampling, 25% in the month before that and the remaining 25% in the two months previous (100). In theory, a patient with periods of wildly fluctuating concentrations could have the same HbA<sub>1c</sub> value as one whose glucose varies little throughout the day. Rohlfing et al defined the relationship between HbA<sub>1c</sub> and plasma glucose levels in patients with type 1 diabetes mellitus using data from the DCCT (110). Quarterly HbA<sub>1c</sub> measurements [n=37,058] and corresponding blood glucose profiles were obtained from 1,439 subjects averaging 18 measurements and profiles per patient (110). Mean plasma glucose was estimated by adding 11% to mean blood glucose estimates (110). There was a strong correlation between HbA<sub>1c</sub> values and mean plasma glucose [r=0.82] and the change in mean plasma glucose for each 1% increase in HbA<sub>1c</sub> was 1.98mmol/L [35.6g/dl] (110). Individual readings for breakfast [r=0.69], lunch [r=0.72] and dinner [r=0.75] had a lower correlation with HbA<sub>1c</sub> compared to the seven point profile [mean plasma glucose] (110). The authors have used this information to set day to day glycaemic targets for patients to enable them to achieve target HbA<sub>1c</sub> values (110).

Derr et al investigated whether glycaemic variation affected HbA<sub>1c</sub> by assessing statistically the contribution of the standard deviation [sd] of blood glucose to HbA<sub>1c</sub> controlled for mean blood glucose, in a group of people with diabetes who had performed self monitored blood glucose over the months before the assessment of HbA<sub>1c</sub> (108). Each of the 136 different subjects had their 250 most recent meter readings taken within the last 90 days, downloaded and compared to HbA<sub>1c</sub> taken on the day of the download (108). The results showed that HbA<sub>1c</sub> correlated strongly with mean blood glucose [r=0.62, p<0.001] (108). Multiple regression analysis carried out after addition of controlling factors for mean blood glucose indicated the standard deviation of blood glucose had an insignificant effect on HbA<sub>1c</sub>. This is attributed to the strong correlation between standard deviation of blood glucose and mean of

blood glucose (108). Through mean blood glucose, HbA1c is therefore linked to self monitored blood glucose readings. This important link has enabled physicians to illustrate to patients how high blood glucose readings (hyperglycaemia) is associated with an increased risk of adverse outcomes and the possible development of diabetic complications (108).

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## 1.6 Hyperglycaemia

The introduction of insulin therapy on a commercial basis in 1923 by Eli Lilly and Company (Indianapolis) resulted in increased longevity for those with diabetes. As people with type 1 diabetes began to live longer they experienced complications, not previously seen (1). Elliot P. Joslin noted in 1934 that although mortality from diabetic coma had fallen from 60% to 5%, deaths from diabetic gangrene of the feet had risen significantly (1). Joslin proposed in the 1930s that physicians should be more aggressive with treatment yet it was not until 1970-80 that better measurements of glycaemic control became available enabling improved control. Retrospective human studies as well as a number of animal studies carried out at this time suggested that elevated blood glucose levels caused or contributed to the development of microvascular complications (104).

The development of home glucose monitoring and glycosylated haemoglobin in the late 1970s, the increase in computerised technology in the 1980s and the publication of two landmark studies in the 1990s have altered perspectives on diabetes control and corresponding insulin therapy (75; 106).

### 1.6.1 The Diabetes Complications and Control trial [DCCT]

The Diabetes Complications and Control trial [DCCT] (74; 75; 111; 112) confirmed findings from the Stockholm Disease Intervention Study [SDIS] (113-116) which showed that improved metabolic control and a reduction in hyperglycaemia resulted in a reduced incidence



of longterm complications in large cohorts with type 1 diabetes. In the DCCT, 1441 patients were recruited from 29 centers in the United States and followed for an average of 6.5 years [range 3-9 years]. Intensive therapy [multiple insulin injections >3 daily or continuous subcutaneous insulin injections] had been shown in the feasibility study to result in an absolute difference in mean HbA<sub>1c</sub> of 1.87 for all subjects in comparison to standard therapy [one to two insulin injections per day with advice on diet and exercise] (104). At 12 months, the mean blood glucose was also significantly different between the intensive and standard therapy groups ( $p<0.0001$ ) and was positively correlated [ $r=0.80$ ] with the HbA<sub>1c</sub> (104). This difference was maintained throughout the study and resulted in a reduction of complications in the intensive group compared to the standard therapy group for retinopathy [23 vs 91 patients], nephropathy [2 vs 5 patients] and neuropathy [43 vs 144 patients] (75). Intensive therapy resulted in significant reductions in adjusted mean risk of retinopathy [63% (95% C.I. 52-71%)], nephropathy [54% (95% C.I. 19-74%)], neuropathy [60% (95% C.I. 38-74%)] and a large but non- significant reduction in the risk of macrovascular disease [41% (95% C.I. -10-68%)] (74; 75; 111; 112).

### 1.6.2 United Kingdom Prospective Diabetes Study [UKPDS]

The United Kingdom Prospective Diabetes Study [UKPDS] showed similar findings in the treatment of type 2 diabetes where 5,102 patients with newly diagnosed type 2 diabetes in 23 centers were followed between 1977 and 1991 (105-107). The overall microvascular complication rate decreased by 25% in the intensive group [median HbA<sub>1c</sub> 7.0%] compared to the conventional group [median HbA<sub>1c</sub> 7.9%] (106). Epidemiological analysis of the UKPDS data showed a continuous relationship between the risks of microvascular complications and glycaemia, such that for every percentage point decrease in HbA<sub>1c</sub> there was a 35% reduction in the risk of complications (105; 107).

The two groups involved in the DCCT were subsequently followed up for eight years. The Epidemiology of Diabetes Interventions and Complications study [EDIC] provided the opportunity to ask how long the effects of intensive vs conventional treatment of type 1 diabetes mellitus are sustained (117). During the DCCT the two groups were exposed to two different levels of glycaemic control over an average of 6.5 years (117). Once the DCCT study ended however, the conventional therapy group were encouraged to initiate intensive therapy and within eighteen months the mean difference in HbA1c between the intensive and conventional groups was eliminated (118). Despite this finding, persistent beneficial effects on albumin excretion and reduced incidence of hypertension suggested that previous intensive treatment had an extended benefit in delaying progression of diabetic nephropathy (117). In particular, the benefits of intensive therapy persisted in the former adolescent cohort with 74% less retinopathy, 48% less microalbuminuria and 85% less albuminuria (117; 118).

### **1.6.3 Pathogenesis of diabetes complications**

In parallel to these findings, work has continued on elucidating the pathogenesis of complications of diabetes. Brownlee has proposed a unifying mechanism to explain how overproduction of superoxide by the mitochondrial electron transport chain is the single process which initiates a large number of hyperglycaemic-induced mechanisms resulting in the pathogenesis of diabetes complications (119). This increased production of reactive oxygen species (the product of oxidative stress) can be triggered by hyperglycaemia, but Monnier et al recently illustrated how glycaemic variability can also lead to increased oxidative stress (120). Therefore the possibility that glycaemic variability is an independent risk factor of diabetes complications remains and requires further investigation. Two groups have examined this possibility using data from the DCCT and both reached the conclusion that glycaemic variability was not a risk factor for the development of microangiopathic complications (121; 122). However, the data from the DCCT uses seven point blood glucose profiles which are a

suboptimal measure of glycaemic variability and further studies are required utilising continuous glucose data (123).

#### **1.6.4 Early prevention of diabetes complications**

Since the DCCT, the emphasis of diabetes management has focussed on maintaining good control, avoidance of hyperglycaemia and early intervention of complications (118; 124). Similar management has been advocated in children (125). According to the International Society of Paediatric & Adolescent Diabetes (ISPAD) consensus guidelines, the optimal blood sugar level in children and adolescents should be 4.0-7.0mmol/L preprandially and 5.0-11.0mmol/L postprandially (126). In order to achieve these goals, older children and adolescents are encouraged to use multiple daily injections [MDI] or continuous subcutaneous insulin infusion [CSII] therapy with increasing evidence suggesting that children on MDI or CSII have a greater chance of achieving these metabolic goals as opposed to twice daily insulin regimens (127). Either method requires multiple blood tests daily, both pre and post prandially, to ensure targets are being met. The limiting factor of such intensive therapy with resultant reduction in HbA1C is the inversely increased rate of severe hypoglycaemia as demonstrated in adolescents and adults by the DCCT (75) and in children by the Hvidore Study Group (128).

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### **1.7 Hypoglycaemia**

Hypoglycaemia remains a critical limiting factor in the management of type 1 diabetes (129; 130). Under normal physiological circumstances, the body has a hierarchy of responses to a falling blood glucose level, which become deficient in the presence of diabetes. In hypoglycaemia due to iatrogenic hyperinsulinism, as glucose levels fall, the concentration of blood insulin does not decrease appropriately (131). The predisposition to hypoglycaemia in



the patient with type 1 diabetes is also a result of defects in the counter-regulatory mechanisms that normally protect against reductions in circulating glucose (131). The release of glucagon from the pancreatic alpha-cell in response to hypoglycaemia is usually impaired in patients with established type 1 diabetes and may occur as early as the first twelve months after diagnosis (131; 132). With the loss of the glucagon hormonal response, patients with type 1 diabetes have increased dependency on the sympathoadrenal responses to hypoglycaemia but these responses to falling glucose concentrations may also become attenuated (133). Symptoms of hypoglycaemia vary from individual to individual and can be autonomic or neuroglycopenic in nature (133). Autonomic symptoms of hypoglycaemia include hunger, trembling, anxiety, palpitations, pallor and sweating (133). Neuroglycopenic symptoms and signs include impaired thinking, confusion, clumsiness, behavioural changes and temper tantrums (133). Cognitive function is progressively impaired below 2.8mmol/L and without intervention may result in convulsions, coma or death (134) increasing parental concern regarding the immediate risk of hypoglycaemia compared to the long term risks of hyperglycaemia.

### **1.7.1 Classification of hypoglycaemia**

Hypoglycaemia can be defined as all episodes of abnormally low plasma glucose concentrations that expose the individual to potential harm (134). The classification of hypoglycaemic events consists of (126; 134):

Severe hypoglycaemia: - any event where the assistance of another person to actively administer carbohydrate, glucagon or other resuscitative actions is required.

Documented symptomatic hypoglycaemia: - any event where typical symptoms of hypoglycaemia are accompanied by a measured blood glucose level <4.0mmol/L.

Asymptomatic or biochemical hypoglycaemia: - any event where the measured blood glucose level is <4.0mmol/L.

The value 4.0mmol/L is based on the critical level at which the glycaemic threshold for activation of glucagon and epinephrine secretion is reached as glucose levels decline with the subsequent appearance of hypoglycaemic symptoms in nondiabetic, nonpregnant individuals (135). Not all individuals with type 1 diabetes will present with autonomic or neuroglycopenic symptoms until well below this level.

### **1.7.2 Importance of hypoglycaemia in diabetes therapy**

In the DCCT, hypoglycaemia was the commonest adverse effect of intensive diabetes management (135). Overall, adolescents had a significantly higher rate of hypoglycaemia in both the conventional and intensive treatment groups when compared to adults, with no difference in the relative risks (136). A prospective population study carried out in Sweden between 1992-4 surveyed 146 children with IDDM less than 19 years of age, found that the incidence of severe hypoglycaemia ranged from 27-34% per year for episodes where the child did not lose consciousness and 10-16% per year for episodes where the child did lose consciousness (137). Bulsara et al recently reviewed the impact of changing treatments on the incidence of severe hypoglycaemia in a cohort of children attending Princess Margaret Hospital in Perth, Australia and found that over the previous 10 years the incidence of severe hypoglycaemia had increased by 29% per year for the first five years but had appeared to plateau over the last 5 years (137). Of the available modalities of treatment [twice daily regimes, multiple daily injection and continuous subcutaneous insulin infusion] only CSII therapy was found to be associated with a reduced rate of severe hypoglycaemia (126; 129; 134; 135; 138).

### 1.7.3 Factors causing hypoglycaemia

Many predisposing factors to hypoglycaemia have been identified. These include errors in insulin dosage, reduced food intake, increased physical activity, lower HbA1c, antecedent hypoglycaemia, alcohol ingestion and hypoglycaemia unawareness (129).

### 1.7.4 Hypoglycaemia unawareness

Hypoglycaemia unawareness results from a vicious circle of preceding hypoglycaemia which reduces the autonomic, symptomatic and counterregulatory hormonal response to subsequent hypoglycaemia (139). It has been described in the literature as early as 1941 when RD Lawrence described hypoglycaemia with reduced awareness (140). Not all patients experience total loss of symptoms and most describe attenuation or loss of autonomic cues (141). Affected patients are prone to more frequent and serious episodes of severe hypoglycaemia. Hypoglycaemia associated autonomic failure (HAAF) proposes that recent antecedent iatrogenic hypoglycaemia causes both defective glucose counterregulation and hypoglycaemia unawareness (129). In type 1 diabetes, hypoglycaemia occurs most frequently during sleep, where episodes range from asymptomatic to severe and are potentially fatal if left untreated (138). Glucose clamp techniques have illustrated how epinephrine responses are reduced during sleep which may contribute to the increased susceptibility to nocturnal hypoglycaemia observed in type 1 diabetes (142). Scrupulous avoidance of iatrogenic hypoglycaemia over as little as two to three weeks in affected patients can however lead to reversal of hypoglycaemia unawareness and improvements in the epinephrine component of glucose counterregulation (129).



Since the 1970s, the concept of glycaemic variation has been evident in the literature, questioning whether actual glucose values [i.e. hypoglycaemia and hyperglycaemia] or swings in glycaemia have the most impact on diabetes control and subsequent development of complications (143-145). The introduction of patient self-monitoring of blood glucose and laboratory measurement of glycosylated haemoglobin called attention to the failure of commonly used regimens to normalize blood glucose. The ability to accurately measure glucose levels on a day to day basis was identified as an integral part of maintaining good control. The DCCT showed that there were varying rates of microvascular complication rates within each mean HbA1c grouping (75). The DCCT concluded that HbA1c is not the most complete expression of the degree of glycaemia and other features of diabetic glucose control may add to or modify the risk of complications (74). This raised the issue of the potential importance of shorter term glycaemic control (such as postprandial glycaemic excursions) as a secondary determinant of complication risk. In vitro studies by Brownlee et al have illustrated that microvascular complications result from intracellular hyperglycaemia which induces through various mechanisms the overproduction of the reactive free radical molecule superoxide (119). Quagliaro et al subsequently showed that the exposure of endothelial cells to both stable and intermittent hyperglycaemia also stimulates reactive oxygen species overproduction (146). Hirsch and Brownlee have speculated that if hyperglycemia-induced oxidative stress is the chief underlying mechanism of glucose-mediated vascular damage, then glycaemic excursions may be of greater frequency and magnitude among conventionally treated patients, who received fewer insulin injections (147). They support the hypothesis that glucose variability, considered in combination with HbA1c, may be a more reliable indicator of blood glucose control and the risk for long-term complications than mean HbA1c alone (147).

Daily self-monitoring of blood glucose levels provides a limited view of a patient's glycaemic control, while glycosylated haemoglobin (HbA1c) is a reliable marker which only reflects average glucose control over a two to three month period (98). For any glycosylated haemoglobin concentration, the associated mean level of glycaemia that can be predicted within a 95% certainty lies within a wide range of glucose values (80). In addition, measures of mean glycaemia fail to provide any information about glucose oscillations (80). With the advent of desktop computers in the 1980s came the ability to analyse and display large volumes of data. Computer programs then allowed the diabetic patient to store self-monitored blood glucose readings with the potential to identify trends in glucose regulation (148). This was followed by further development of blood glucose meters which enabled the storage and subsequent download to a computer of patient tests by glucose value and time (149; 150). However, in order to examine trends multiple tests are required. The concept of continuous glucose monitoring was devised in part to address these shortcomings. Initial attempts at continuous glucose monitoring had many limitations, as it could only be performed in an inpatient setting and required continuous venous access (80). However, further development of continuous blood glucose monitoring has improved our ability to study glucose instability in patients with type 1 diabetes under ambulatory conditions and subsequently allowed complete quantitative characterization of blood glucose dynamics (144). The aim of diabetes therapy is to maintain blood glucose levels as close to normal as possible without compromising patient safety due to hypoglycaemia (151). The importance of understanding the degree of variation/lability in glycaemic control in childhood and adolescence is yet to be fully established. Glycaemic variation should incorporate the frequency of glycaemic excursions, including postprandial glycaemia - a phenomenon increasingly recognized to be of importance in overall metabolic control (152).

Continuous glucose monitoring has demonstrated the wide degree of glycaemia occurring in children with type 1 diabetes even those with excellent HbA1c levels (153-155). Early continuous glucose monitoring studies have also shown that asymptomatic hypoglycaemia in childhood diabetes is a relatively common event (154; 155). Reliance only upon HbA1c and intermittent finger-prick testing may lead to a limited picture of overall metabolic control with little or no appreciation of glycaemic excursions or variation (156). Prior to continuous glucose monitoring, investigators recognised the importance of trying to measure glycaemic variation and had devised algorithms for use in both daily and postprandial experimental models (143-145; 157). With the advent of continuous glucose monitoring sensors, some of these methods were translated for use in continuous glucose monitoring with minimal critical review.

### 1.8.1 The M-value

The M-value [see table 1.1] is a measure of the stability of the glucose metabolism in comparison to an ideal glucose value (156). The M-value was first described in an attempt at a quantitative analysis of postprandial blood glucose variation (157). The M-value was originally designed against a subjective standard of nine investigators' assessments of 72-hour profiles from 20 patients with diabetes [6 blood glucose measures per 24-hour period] and was subsequently extrapolated for use in CGMS with little critical review (158). The M-value is 0 in healthy controls, rising with increasing glycaemic variation. In the context of diabetes, results are categorised as good [0-18], fair [19-31] or poor [ $>32$ ] control (157). For healthy individuals the M-value falls in the range of  $1 \leq M \leq 4$  (157). Arbitrary cut-offs are required for administration of the formula with logarithmic transformation being required to increase the impact of hypoglycaemic events on the index (157). The original formula was later modified [Adjusted M-value; see table 1.1] for a higher arbitrary comparative glucose value when it was found that in some cases the M-value was lower than that of a reference group of healthy



individuals (156). When originally devised, the authors acknowledged that the M value was limited by the number of glucose values measured (157). To adjust for this, subsequent authors added an appendix [Mw( table 1.1)] to the formula for calculations of 24 readings or fewer (145). Current continuous glucose monitoring system traces produce up to 288 readings in a day so this appendix is no longer applicable. As mentioned above, the M-value relies on selection of arbitrary glycaemic reference points by the investigators. This introduces a bias effect which impedes the M-value's use in comparing separate studies that may use varying reference points. Further to this, hypoglycaemia has a greater impact on the M-value than hyperglycaemia, which limits the M value's usefulness as a true descriptor of glycaemic variation.

### 1.8.2 The J-index

In view of these disadvantages, Wojcicki et al proposed the "J"-index as an alternative formula for calculation of glycaemic variation (159). The aim of this new index was to incorporate mean level and variability of glycaemia utilising one variable. The calculation [see table 1.1] can apply to glucose readings in either mmol/L or mg/dl through use of the appropriate version of the formula. Comparisons of five exemplary profiles were used to evaluate the formula using eight point glucose profiles (159). A J-index between 10-20 would represent ideal glucose control, 20-30 indicates good glucose control, 30-40 suggests poor glucose control while an index >40 would indicate lack of glucose control (159). This index has not been validated in continuous glucose monitoring and excludes hypoglycaemia alarm states defined as severe hypoglycaemia (<1.67mmol/L) and continuous hypoglycaemia (3 measurements <2.78mmol/L).

### 1.8.3 Mean Amplitude of Glycaemic Excursions [MAGE]

The Mean Amplitude of Glycaemic Excursions [MAGE (see table 1.1)] algorithm was suggested in 1970 to quantitate glycaemic excursion under controlled dietary/exercise conditions (145). The MAGE algorithm was designed to calculate the peaks and nadirs encountered in a day, generating a value for the variation around a mean glucose value. MAGE values differ from M-values in that the reference point is a mean of the glucose values rather than an arbitrarily chosen cut-off. The degree of variation is calculated according to the standard deviation of postprandial glycaemic excursion. Definition of glycaemic peaks and nadirs is arbitrary or subjective (145), this being the main factor limiting its use in ambulatory, non-controlled CGMS analyses. MAGE uses the pooled results of arbitrarily designated glycaemic peaks [chosen by the investigators in a non-reproducible fashion] and ignores blood glucose swings which are designated as insignificant by the person interpreting the data. When MAGE was first proposed hourly blood glucose measures were assessed. By way of comparison, CGMS records 12 measures per hour each an average of five minutes of readings (160). In continuous monitoring, the distinction between peaks and nadirs is unclear compared to the original hourly measurements used when MAGE was devised. MAGE analysis ignores a large percentage of CGMS data. Notwithstanding this, MAGE has recently been used in conjunction with CGMS in a diet and exercise-controlled cohort with diabetes (161). In that study, the authors defined the standard deviation according to the mean CGMS glucose value and defined CGMS glucose peaks and nadirs manually in a way that is difficult to independently reproduce. In addition, the diet and activities of participants in the CGMS study by Alemzadeh et al (161) were controlled. The necessity for such restrictions limits the use of MAGE in non-controlled, ambulatory CGMS use.

#### **1.8.4 The Mean of Daily Differences [MODD]**

The Mean of Daily Differences [MODD] value was derived by Molnar et al in 1972 (143). This value [see table 2.1] was designed to illustrate inter-day variation of blood glucose levels. Care has to be taken in adapting this formula for use with continuous monitoring. A high MODD score is indicative of a large glycaemic difference between days. MODD values have utility in that the degree of consistency in a CGMS trace can be assessed and thus the degree to which observed daily patterns are ongoing and representative can be assessed.

#### **1.8.5 The Lability Index [LI]**

The Lability Index [LI] was published by Ryan et al in 2004. This index [see table 2.1] utilised four weeks of glucose records to calculate the change in glucose levels over time and compare this to a clinical assessment of glycaemic lability (162). The Lability index was used in conjunction with a HYPO score which is a composite score devised based on the frequency, severity and degree of unawareness of hypoglycaemia (162). According to the authors, the LI and HYPO score provide measures of the extent of problems with hypoglycaemic and glycaemic lability and complement clinical assessment (162). The algorithm was not designed for use with continuous glucose monitoring systems. Also, the LI score is a summative assessment of glucose readings which means that the longer the time interval incorporated and the more measurements involved the greater the result achieved.

#### **1.8.6 Average Daily Risk Range [ADRR]**

Kovatchev and colleagues have been involved in a number of different mathematical methods for assessing glycaemia variability including the Low blood glucose index [LBGI], high blood glucose index [HBGI] and a measure for variability called the blood glucose rate of change which are all derived from continuous glucose monitoring data (163). They have also designed an algorithm for use with self-monitored blood glucose data called the Average Daily Risk



Range (ADRR) which was shown by the authors to be the best predictor of both hypoglycaemia and hyperglycaemia in a cohort of adults with type 1 and type 2 diabetes in comparison to mean blood glucose, M-values, LI and MAGE (164).

#### 1.8.7 Glycaemic Risk Assessment Diabetic Equation [GRADE]

Hill et al designed a similar formula called GRADE [Glycaemic Risk Assessment Diabetic Equation] (165). Fifty diabetes healthcare professionals were asked to assign subjective risk values using a visual analogue scale to a range of 40 blood glucose concentrations. The median responses for each blood glucose value were used to develop a function relating risk to glycaemia. The mean approximation of these risk values is termed GRADE (165). The authors suggest that the method encapsulates the totality of hypo-/hyper- glycaemic risk to a patient based on their glycaemic profile and is suggested as an adjunct to the HbA1c to report the degree of risk associated with glycaemic variability. The method has not been validated in clinical studies outside of the methodology paper which was published in December 2007. The formulae for the methods described above are included in Table 1.1 below.

Table 1-1: Definition of the formulae used to calculate measures of glycaemia

Formula	Derivation	
Adjusted M-value [M]	$M_{GR} + M_W$ <p>where</p> $M_{GR} = \frac{\left  \frac{1}{n} \sum_{t=1}^{t_k} 10x \log \frac{GR_t}{IGV} \right ^3}{n}$ <p>and</p> $M_W = \frac{G_{max} - G_{min}}{20}$	<p>k = number of observations (number of glucose readings for a given individual)</p> <p><math>M_{GR}</math> = M-value for glucose readings</p> <p><math>M_W</math> = Correction factor for <math>n &lt; 24</math></p> <p>IGV = ideal glucose value (arbitrary number)</p> <p><math>G_{max}</math> = maximum glucose reading</p> <p><math>G_{min}</math> = minimum glucose reading</p>
MODD [Md]	$Md = \frac{\sum_{t=1}^{t_{k^*}}  GR_t - GR_{t-1440} }{k^*}$ <p>[<math>GR_t</math> glucose reading at time <math>t</math> = min after start and <math>t_i</math> = time in minutes after start of observations of the <math>i^{th}</math> observation]</p>	<p><math>k^*</math> = Number of observations where there is an observation at the same time 24 hours ago</p>

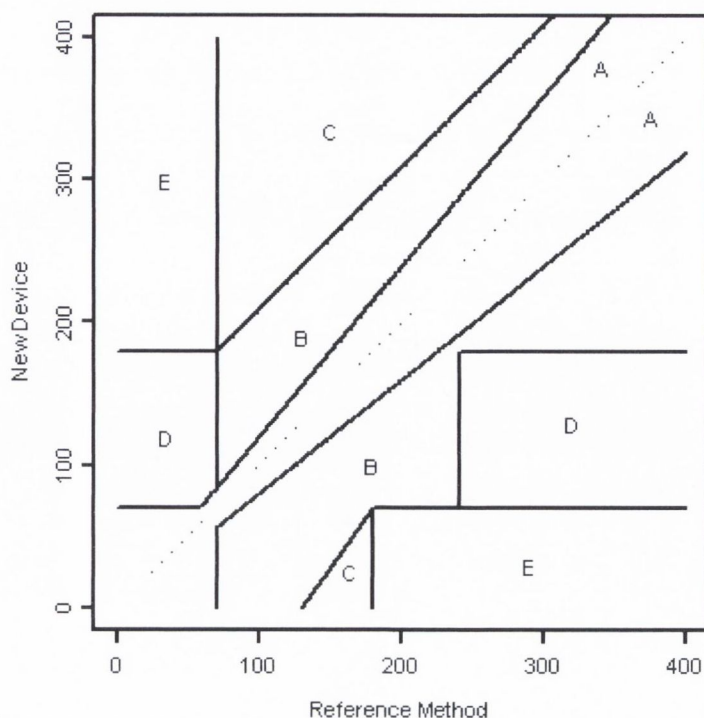
<p>MAGE [Mg]</p>	$Mg = \sum \frac{\lambda}{x}$ <p>If <math>\lambda &gt; v</math></p>	<p><math>\lambda</math> = each blood glucose decrease from peak to nadir</p> <p>x = number of valid observations</p> <p>v = 1SD of mean glucose for 24-hour period</p>
<p>J-index [J]</p>	$J = 0.324x(MBG + SD)^2$	<p>0.324 = multiplier used for blood glucose measurements in mmol/L</p> <p>MBG = mean blood glucose</p> <p>Sd = standard deviation</p>
<p>Lability Index [LI]</p>	$LI \text{ mmol}^2/\text{h} \cdot \text{week}^{-1} = \sum_{n=1}^N \frac{(Gluc_n - Gluc_{n+1})^2}{(h_{n+1} - h_n)}$	<p>Gluc<sub>n</sub> mmol/l) is the nth reading of the week taken at time h<sub>n</sub> (rounded to the nearest hour). N is the total number of readings in a week.</p>



<p>Average daily risk range [ADRR]</p>	$ADRR = \frac{1}{M} \sum_{i=1}^M [LR^i + HR^i]$	<p> <math>LR^i = \max [rl(x_1^i), \dots, rl(x_n^i)]</math>  <math>rl(BG) = r(BG)</math> if <math>f(BG) &lt; 0</math>  <math>HR^i = \max [rh(x_1^i), \dots, rh(x_n^i)]</math>  <math>rh(BG) = r(BG)</math> if <math>f(BG) &gt; 0</math>            For day <math>i: i=1,2,\dots,M</math>            BG - blood glucose  <math>r</math> = risk values <math>h</math>=high <math>l</math>=low  <math>f</math>= data transformation that normalises glucose scale.         </p>
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## 1.9 Continuous glucose monitoring

Innovative methods have now been developed to assess blood glucose control using continuous glucose monitoring systems. A successful method should allow frequent measurements of glucose control resulting in quality readings in the least invasive manner possible on a regular basis without interfering with the normal routine of the person involved. Many of the results are summarised using the Clarke error grid analysis first published in 1987 (166). This method evaluates the correspondence and discrepancy between blood glucose values and sensor readings at isolated static points in time. A sample of this grid is shown in figure 1-2 to assist understanding of the results discussed in subsequent paragraphs.



**Figure 1-2: Clarke error grid**

The graph above illustrates acceptable levels of blood glucose measurements (mg/dl). Zone A is considered accurate, Zone B acceptable, Zone C overcorrected, Zone D indicates dangerous failure and Zone E erroneous treatment.

### 1.9.1 GlucoWatch

The first system to be approved for patient use outside a hospital setting was the GlucoWatch which was developed by Cygnus (formerly of Redwood, CA, now defunct) and bought by Animas® in March 2005. The GlucoWatch G2 Biographer (Animas® Corporation) is worn on the arm like a wristwatch and is based on the principle of reverse iontophoresis. Reverse iontophoresis is a process in which an electrical potential is applied across the skin surface causing anions to flow towards attached electrodes from the epidermis (167). These anions migrate to gel collection discs where they react with an enzyme, glucose oxidase, in the gel to form hydrogen peroxide (168). A biosensor in contact with each gel collection disc detects the hydrogen peroxide generating a current (168). This current is integrated, producing a signal in units of electric charge (168). The biographer uses a calibration value

entered by the patient to convert the signal into a glucose measurement (168). The single point calibration is performed with a traditional blood glucose meter after a 3 hour warm-up period (168).

The GlucoWatch provides real-time measurements of interstitial glucose concentrations at 10 minute intervals (169). However, the GlucoWatch measures interstitial glucose values which are shifted in time due to physiological and instrumental lag (168). A cross-correlation analysis has shown an average total lag of  $17.2 \pm 7.2$  minutes for 51 unique subjects with type 1 [n=32] or type 2 [n=19] diabetes mellitus. Instrumental lag has been averaged at -13.5 minutes due to the production of a glucose value every 20 minutes by averaging two 10-minute values (168). Therefore, physiological lag has been estimated at approximately 5 minutes (168). As the GlucoWatch biographer contains an alarm for hypoglycaemia and pending hypoglycaemia, such evidence raises queries over the accuracy of the alarm. The Diabetes in Research in Children Network studied ninety-one children with type 1 diabetes ranging in age from 3.5-17.7 years who wore a GlucoWatch biographer for a twenty-four hour period during a research centre admission (169). For a hypoglycaemia alarm setting of 3.3mmol/L (60mg/dl) the sensitivity of the biographer in detection of an actual serum glucose level of 3.3mmol/L (60mg/dl) was 23%, indicating that 51% of all alarms would be false (169). The GlucoWatch has been shown to be accurate at normal - high blood glucose levels (170) but concerns remain regarding accuracy in the hypoglycaemic range (169). The unit has been noted to cause some degree of local skin irritation and cannot be used during periods of increased sweating (171). However, features of this device include real-time readings, an alarm for out-of-range values and a needle free attachment (172).

### 1.9.2 Near infra-red spectroscopy

Other non-invasive sensors were in the clinical phase of experimental trials when this project was planned. These included near infra-red spectroscopy, photoacoustic spectroscopy and



light scattering techniques. Near infra-red spectroscopy involves the passage of radiation of 70-100nm which can identify changes in glucose specific absorption peaks. Preliminary studies have shown the standard error in glucose measurement to be approximately 1.1mmol/L making noninvasive glucose determinations a viable analytical method (173; 174). Cohen et al examined the accuracy of occlusion near infra-red spectroscopy using a stepped hyperinsulinaemic hypoglycaemic clamp in five adults with type 1 diabetes mellitus (175). Linear correlation showed 94.3% of measurements to fall in zones A&B of the Clarke error grid when compared to intravenous and interstitial glucose levels (175). The Pendra device (Pendragon Medical, Zurich, Switzerland), a continuous glucose monitor utilising impedance spectroscopy, has since been released. This device is worn on the wrist, takes one hour to warm up and has a sensor lifespan of three months. Unlike, the GlucoWatch, the Pendra does not harvest fluid from the skin or cause local skin irritation, while also containing a real-time alarm and real-time glucose display (172).

### 1.9.3 Ultrasound sensors

Chuang et al have recently published clinical evaluation of a continuous minimally invasive glucose flux sensor placed over ultrasonically permeated skin in ten patients with type 1 diabetes (176). Sonoprep™ [Sontra Medical, Franklin, MA, USA] is an ultrasonic skin permeation device that works with a glucose flux biosensor and a meter that uses the application of low-frequency ultrasound to render the skin transiently permeable which improves glucose flux extractions through increased skin conductivity (176). Ten patients [three with type 1 diabetes, seven with type 2 diabetes] each wore two biosensors. Using a Clarke error grid, 92% of the glucose estimations using this method fell within the clinically relevant zones A & B [ $r=0.84$ ] (177). Treated skin developed slight erythema in all sites with minimal swelling in 11/20 biosensor sites immediately after removal (177). The lag time using this method averaged 21 minutes [range 0-50min] (177).

#### 1.9.4 Continuous Glucose Monitoring System [CGMS]

At the time of this thesis design, the most recent continuous monitoring device approved for use in type 1 diabetes was the Continuous Glucose Monitoring System [CGMS] devised by Minimed [Medtronic Ltd., CA, USA] (160). The device consists of a subcutaneous sensor connected by cable to a recording device. The subcutaneous sensor measures interstitial glucose using a glucose oxidase reaction to produce hydrogen peroxide [ $H_2O_2$ ] that diffuses to a platinum electrode poised at 0.535V. The  $H_2O_2$  is oxidised to produce the sensor current (160). Current values are converted via an algorithm to glucose values which are recorded every thirty seconds and a five minute average recorded. Unlike the GlucoWatch biographer, the values are not displayed in real-time and are downloaded after the device is worn for a 72-96 hour period. Analysis of the data is carried out by the Minimed Solutions Software. In February 2003, the most up-to-date version of the software package available was 1.7A. Subsequently, this has now been updated to Version 3.0. Solutions 3.0 has been clinically shown to give similar analysis results to earlier versions in children with type 1 diabetes (178). The Minimed CGMS remains the most popular system worldwide for continuous glucose monitoring.

#### 1.9.5 Interstitial glucose vs. blood glucose values

The relationship between interstitial glucose values and blood glucose values has been an area of ongoing debate since the development of subcutaneous monitors. If the capillary represents a diffusional barrier to glucose between blood and the interstitium, then, interstitial fluid glucose and plasma glucose could be expected to have different dynamic responses and under some conditions, different steady state concentrations (87). Experimental work comparing blood glucose to hindlymph (interstitium) in dogs has shown a sensor delay time between five and twelve minutes (87). Rebrin and Steil concluded that interstitial glucose can replace blood glucose measurements with the delay in interstitial fluid

glucose equilibration most likely less than ten minutes and the error between blood and interstitial glucose measurements no more than five or six percent (87). Boyne et al studied fourteen patients with type 1 diabetes who wore two subcutaneous sensors in the abdomen while having simultaneous blood sampling every five minutes for eight hours during which two liquid meals were given (179). Statistically significant lag times occurred in the rise [10.1+10.1min,  $p<0.001$ ], the fall [6.9+8.5min,  $p<0.017$ ] and the nadir [9.4+7.7min,  $p<0.001$ ] of all measurements with the blood glucose preceding the interstitial glucose at all timepoints (179). In order to accommodate such potential dynamic lag between blood and interstitial glucose, the meter values in the Continuous Glucose Monitoring System are automatically shifted ten minutes later in time by the calibration software within the CGMS monitor (160).

While some of these methods of glucose sensing are still under development, the continuous glucose monitoring system has been applied for use in management of children with hypoglycaemic disorders (180), evaluation of new therapies to control postprandial glucose elevations (181) and as an adjunct for adjustments to pump therapy in children and adults with type 1 diabetes (181-185).

#### **1.9.6 Implantable, real-time continuous glucose sensors**

A range of implantable, real-time continuous glucose sensors are also in development (186). Most involve the wireless transmission of glucose readings from an subcutaneous sensor to an external receiver. The impetus behind these advances is based on the belief that an increase in glucose monitoring can lead to a reduction in HbA1c, thus delaying the onset of diabetic complications. One of the most important potential uses for in-vivo glucose monitoring is a detector or alarm for hypoglycaemia (167). Hypoglycaemia, especially nocturnal hypoglycaemia is the commonest complication of diabetes (167) as reflected in the findings of the continuous glucose monitoring system discussed in the last section (187). However, if subcutaneous



glucose does not reflect blood glucose in the very low range then subcutaneous sensors cannot be used as an alarm .

The microdialysis method has been used in an integrated system to allow continuous glucose monitoring in the interstitial fluid for up to 120 hours (177). The sensor unit and Data Manager in the SCGM1 system (Roche Diagnostics, Mannheim, Germany) exchange glucose data and calibration data by radio transmission (177). In vitro testing has shown a mean relative difference of -0.6% to 3.7% and mean absolute difference from 0.2% to 3.8% when compared to fifteen standard glucose solutions (177).

In March 2004, Garg et al published promising data using an implantable sensor developed by Dexcom [San Diego, CA, USA] . Fifteen adults consented to the surgical implantation of a subcutaneous sensor in the abdominal wall after local anaesthetic (186). Average duration of use was one hundred and two days which included a blinded control period [data downloaded retrospectively], a testing period [where both self-monitoring blood glucose levels and implanted sensor levels were compared to a laboratory glucose analyser] and an unblinded study period [where sensor levels are available on real-time monitor display with alarms for hypo and hyperglycaemia] (186). Over 95% of readings obtained from the implanted sensor correlated with areas A&B of the Clarke error grid compared to the self-monitoring blood glucose [96%] and the laboratory analyser [97%] (186). After the open phase of the study, patients experienced 47% less time in the hypoglycaemia range [ $p < 0.05$ ] and 25% less time in the hyperglycaemia range [ $p < 0.05$ ] compared to the blinded phase (186). Longer studies are still required to establish that this difference was not due to a study effect associated with weekly clinic attendances. The study did not clarify whether the sensors were electively explanted or failed at the endpoint of the study. Durability of implanted sensors as well as the risk of extrusion and local infection are areas that require further investigation before implanted sensors become a reality in day to day glucose monitoring.

### 1.9.7 Accuracy of continuous glucose monitoring sensors

With the influx of new methods entering the market, standards of excellence and accuracy need to be available for comparison and reference. Kovatchev et al have designed a continuous glucose-error grid analysis (CG-EGA) to evaluate the accuracy of continuous glucose monitoring sensors (188). The grid illustrated by Therasense Freestyle Navigator data examines temporal characteristics of the continuous glucose sensor data, analysing pairs of reference and sensor readings as a process in time represented by a bidimensional time series and taking into account inherent physiological time lags (188). The International Organization of Standardization (ISO) provides standards for accuracy of point blood glucose tests. These standards require that a sensor blood glucose value be within 15mg/dl for any reference value <75mg/dl and within 20% of reference for a reference value >75mg/dl (172). All new monitors are now tested and reported according to this standard.

The DirecNet study group who previously studied both the CGMS (189) and the GlucoWatch G2 (169) have studied the accuracy of the Freestyle Navigator CGMS as a real-time continuous glucose monitor across a wide range of glucose levels (190). Using a hexokinase enzymatic method as a inpatient laboratory reference and a Freestyle glucometer as an outpatient sensor reference, the median relative absolute difference was calculated for sensor-reference and sensor-sensor pairs. Over 1,800 inpatient values and greater than 8,600 outpatients values were collected with 91% of sensors for inpatients and 81% of sensors for outpatients showing a median relative absolute difference of <20% (190). This was not adjudged to approach current acceptable levels of home monitoring accuracy (190). Weinstein et al compared the accuracy of the Freestyle Navigator system with the Yellow Springs Instrument (YSI) laboratory reference measurements of venous blood glucose and found a median absolute relative difference of 9.3% (191). Using Clarke error grid analysis, 81.7% of readings fell in Zone A and 16.7% of readings in Zone B indicating a higher degree of

accuracy (191) than that found by the DirecNet group. In addition to these findings, the sensor remained consistent when calibrated over the five days of the study with 82.5% of readings in Zone A on Day 1 compared to 80.9% of readings in zone A on Day 5 (191). Buckingham et al refers to real-time continuous glucose monitors as behaviour modification tools where improvement in diabetes control will only occur through the patient's willingness to modify their management based on the instrument provided (192).

The realisation of continuous glucose monitoring has allowed complete quantitative characterization of blood glucose dynamics as predicted by Service in 1980 (144). The next step involves the understanding of which factors impact on glycaemic variation.

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### **1.10 Clinical studies and the Continuous glucose monitoring system**

The availability of continuous glucose monitoring offered people with diabetes the opportunity to match the demands of intensive therapy with intensive monitoring (160). In a short period of time, many groups have explored the utility of continuous glucose monitoring in clinical and research applications. Initial performance evaluation of the CGMS, using Clarke error grids, showed that 96.2% of data pairs comparing continuous glucose monitoring sensor values to self monitoring blood glucose values fell between the clinically acceptable zones [Zone A 70.2%, Zone B 26%] in 135 patients who tested the monitor for home use. Independent clinical evaluation in patients with type 1 diabetes [n=18] showed 95% of paired non-calibration samples between the continuous glucose monitoring system and self-monitored blood glucose readings to fall between the clinically acceptable zones [Zones A&B] of the Clarke error grid (193). Further work by Steil et al using a stepped hyperinsulinaemic, hypoglycaemic glucose clamp in five healthy male subjects demonstrated high correlation between the subcutaneous sensor and a reference glucose analyser [ $r=0.91$ ,  $p<0.001$ ] (194).



### 1.10.1 The relationship between CGMS and HbA1c

Research initially focussed on the correlation between continuous glucose monitoring and previously accredited reference standards (195; 196). The first study in children was a pilot study by Chase et al comparing five children using the continuous glucose monitoring system against six controls, all with type 1 diabetes (195). The study group were asked to wear six sensors over a thirty day period while both groups were asked to perform a minimum of four self-monitored blood glucose readings daily (195). The group using the continuous glucose monitoring system detected a higher number of hypoglycaemic [ $<3.25\text{mmol/L}$  ( $60\text{mg/dl}$ )] events in the first month compared to the controls [ $12.8\pm 1.6$  vs  $6.7\pm 1.1$  episodes per participant,  $p=0.001$ ]. A decrease in HbA1c was observed in both groups after thirty days but was significantly greater in the continuous monitoring group [ $0.36\pm 0.7\%$   $p<0.01$ ] than the controls [ $0.2\pm 0.2\%$ ] when compared to baseline values (195). This reduction was not maintained when HbA1c was repeated three months later (195).

Kaufman et al did show a significant decrease in HbA1c measurement from time of sensor placement to three months later in twenty-seven paediatric and adolescent patients [ $p=0.03$ , mean drop in HbA1c =  $0.66\%$ ] (196). The study also showed a high occurrence [83%] of asymptomatic nocturnal hypoglycaemia in paediatric subjects [ $1.17\pm 1.3$  events per patient] not apparent with finger stick monitoring alone (196).

Ludvigsson and Hanas were the first to examine whether continuous subcutaneous glucose monitoring improved HbA<sub>1c</sub> using a controlled cross-over study format (187). They randomised thirty-two patients into an open study arm and a blinded arm (187). Each arm had eight pump users and eight patients on multiple daily injections. Participants in both study arms wore the continuous glucose monitor for three days every two weeks with instruction to complete a seven point self monitoring of blood glucose profile once weekly (187). Neither the patient or the diabetes team reviewed the trace outcomes in the blind arm. After three months, the

open and blind arms crossed over and continued for a further three months using an identical protocol. In both study arms, a decrease in HbA<sub>1c</sub> was noted in the first three month period (187). This was significant for the open arm [7.7% to 7.31%, p=0.013] but not the blinded arm [7.75% to 7.65%, no p value given] (187). After crossover, the now open arm had a further decrease in HbA<sub>1c</sub> levels whereas the now blind arm increased again (187). The return of HbA<sub>1c</sub> to baseline levels in the group that ended with the blind arm indicated a need for repeated use of the sensor (187). Again, a large number and duration of low subcutaneous readings [ $<3.0\text{mmol/L}$ ] was noted. Twenty-six of the twenty-seven participants exhibited low daytime readings [0.8 episodes/day, duration  $58\pm 29$  minutes, 5.5% of total time] while all twenty-seven participants experienced nocturnal hypoglycaemia [0.4 episodes/night, duration  $132\pm 81$  minutes, 10.1% of total time] (187). The authors concluded that changes in intensive insulin therapy based on CGMS profiles improved metabolic control in paediatric patients with type 1 diabetes. Their intention was to implement the device to complement treatment choices and as an educational tool for families.

### 1.10.2 Intermittent blood glucose readings vs. CGMS

Boland et al utilised the monitor to examine how standard self monitored blood glucose readings reflected twenty-four hour glucose excursions in youth with diabetes (153). Pre-meal blood glucose meter readings agreed with glucose sensor values obtained using continuous glucose monitoring (153). However, approximately 90% of postprandial glucose sensor values exceeded postprandial targets set by the research team of  $10\text{mmol/l}$  [ $180\text{mg/dl}$ ] (153). The mean of fifteen blood glucose readings [ $8.6\pm 2.1\text{mmol/L}$  ( $155\pm 38\text{mg/dl}$ )] was remarkably similar to the mean of  $>800$  glucose sensor values [ $8.3\pm 2.4\text{mmol/L}$  ( $150\pm 43\text{mg/dl}$ )] recorded in the same time period [ $r=0.78$ ] (153). Both values were consistent with HbA<sub>1c</sub> values, with patients mean HbA<sub>1c</sub> of 7.7% shown by normograms to be equivalent to a three month blood glucose average of  $8.3\text{-}8.9\text{mmol/L}$  [ $150\text{-}160\text{mg/dl}$ ] (153).

Zavalkoff and Polychronakos used the continuous glucose monitoring to evaluate how well the customary intermittent self monitoring of blood glucose correlates with integrated values during the surrounding time periods in ambulatory patients with type 1 diabetes mellitus (197). Eighteen patients aged between seven and twenty years participated in the study. Strong correlations were identified for the blood glucose reading at breakfast and the mean for continuous glucose monitoring for the 8 hours prior to breakfast [ $r=0.75$ ,  $p<0.00001$ ], the blood glucose reading at dinner and the mean for continuous glucose monitoring from lunch to dinner [ $r=0.75$ ,  $p<0.00003$ ], the blood glucose reading at bedtime and the mean for continuous glucose monitoring from dinner to bedtime [ $r=0.81$ ,  $p<0.0005$ ] and the blood glucose reading at bedtime and the mean for continuous glucose monitoring overnight [ $r=0.65$ ,  $p<0.0005$ ] (197). Poor, non-significant correlations were shown from breakfast to lunch [ $r=0.21$ ,  $p=0.32$ ] and for all mealtime blood glucose readings compared with the subsequent continuous glucose monitoring period to the next meal (197). Despite the strong correlation between the night-time blood glucose reading and overnight glycaemia, the bedtime test was not predictive of nocturnal hypoglycaemia (197).

### **1.10.3 Identification of nocturnal hypoglycaemia using CGMS**

Boland et al identified the usefulness of continuous glucose monitoring in identifying asymptomatic nocturnal hypoglycaemia (153). Nocturnal hypoglycaemia of prolonged duration with a median of 32 minutes [range 0-280 minutes] per night was identified using the CGMS (153). Sixty-seven percent of subjects had a recorded nadir glucose level  $<3.3\text{mmol/L}$  [ $60\text{mg/dl}$ ] during at least one night of sensing with twenty-one percent of subjects demonstrating low glucose levels on all three nights (153). The subjects who experienced nocturnal hypoglycaemia on all three nights had lower  $\text{HbA}_{1c}$  levels compared to the remainder of the cohort [ $6.9\pm 0.8\%$  vs  $7.0\pm 1.4\%$ ,  $p<0.01$ ] (153).



Kaufman et al also used the continuous glucose monitoring system to determine if blood glucose levels were associated with the occurrence of nocturnal hypoglycaemia and to try and identify a specific bedtime glucose level at which hypoglycaemia was reduced to a prevalence of <10% (198). Forty-seven children of mean age  $11.8 \pm 4.6$  years participated in the study. The mean bedtime blood glucose level was  $10.3 \pm 5.2$  mmol/l [ $185 \pm 94$  mg/dl] (198). Going to bed with a blood glucose level  $< 5.6$  mmol/L [ $< 100$  mg/dl] was significantly associated with a recording of  $\leq 2.2$  mmol/L [ $\leq 40$  mg/dl] overnight [ $p=0.15$ ] particularly in the 9pm to 1am period [ $p=0.006$ ] (198). Repeated analysis using  $\leq 8.3$  mmol/L [ $\leq 150$  mg/dl] as the cut-off point showed a similar association with a recording of  $\leq 2.8$  mmol/L [ $\leq 50$  mg/dl] [ $p=0.001$ ] overnight but not with  $\leq 2.2$  mmol/L [ $\leq 40$  mg/dl], also with particular emphasis on the 9pm to 1am time period [ $p=0.0004$ ] (198). Analysis of blood glucose values from  $6.1$  mmol/L [ $110$  mg/dl] to  $16.7$  mmol/L [ $300$  mg/dl] was carried out but the researchers were not able to demonstrate a blood glucose value that predicted an incidence of nocturnal hypoglycaemia of <10% (198).

#### 1.10.4 Analysis of CGMS using area above or below the curve quantification

Researchers dealing with CGMS data have also used area under or above the CGMS curve to describe the degree of glucose deviation above or below a certain threshold (154; 181). The method has been used to describe the incidence of hypo- and hyperglycaemia and the effect of new therapies (154; 181). Researchers using this method have analysed data using the whole area under the curve of each 24 hour glucose profile within the range of the CGMS [ $2.2$ - $22.2$  mmol/l], and have evaluated both mean value of the area for 3 days and the sum of the areas for the same 3 days (154). Similar to mean CGMS glucose values, a relationship has been shown between HbA1c and mean 3-day glucose area under the curve values (154). Another group evaluating a new therapy employed the incremental area under the curve using the trapezoidal rule to calculate postprandial glucose excursions (181). In the context of CGMS, areas under the curve analysis are problematic in that the curve is truncated at

glucose values under 2.2 mmol/l and over 22.2 mmol/l. Thus the area under the CGMS curve does not distinguish between extremes in glucose levels [e.g. values of 23mmol/l and 40mmol/l are treated equally]. Salardi et al used the area under the curve to monitor the system relationship with HbA<sub>1c</sub> in paediatric patients with type 1 diabetes mellitus (154). They aimed to ascertain if the three day glucose profile was sufficiently representative of the whole metabolic control of a patient, whether short term HbA<sub>1c</sub> levels could be reduced through the modification suggested by continuous glucose monitoring, whether it was possible to identify a particular blood glucose mean value which may contribute more to the glycation of haemoglobin and whether it was possible to identify particular glucose profiles in subjects with better or worse metabolic control (154). Twenty-eight traces from the forty-four children who completed a continuous glucose monitoring trace were analysed (154). Inclusion criteria included a high level of HbA<sub>1c</sub> for more than 1 year or a history of frequent hypoglycaemic episodes (154). Areas under the curve were measured using a digital planimeter. Mean 3-day glucose area values [ $98.6 \pm 27.1 \text{ cm}^2$ ] correlated positively with HbA<sub>1c</sub> [ $r=0.53$ ,  $p=0.002$ ] and fructosamine [ $r=0.64$ ,  $p<0.0001$ ] (154). HbA<sub>1c</sub> and fructosamine values also correlated positively with partial glucose area values except for the area encompassing the hypoglycaemic range from 2.2-5mmol/L [40-90mg/dl] (154). HbA<sub>1c</sub> levels measured in the months after wearing the continuous glucose monitoring device were significantly lower than baseline values with a reduction of  $-0.40 \pm 0.94\%$  [ $p=0.05$ ] at three months and a reduction of  $-0.43 \pm 0.87\%$  [ $p=0.03$ ] at six months. No particular glucose profile or glucose value was identified which predicted patients with various patterns of metabolic control (154). The day to day inpatient variability of the continuous glucose trace was  $>50\%$  in 14/28 subjects and  $>100\%$  in a further 7 subjects (154). Like other reports, this study identified a high proportion of asymptomatic, nocturnal hypoglycaemia with 12/28 patients demonstrating

glucose values  $<2.2\text{mmol/L}$  [ $40\text{mg/dl}$ ] during the overnight segments of the continuous trace (154).

#### **1.10.5 Analysis of CGMS using percentage time**

Amin et al used percentage time to illustrate the proportion of hypoglycaemia [defined as a sensor glucose value  $<3.5\text{mmol/L}$  or  $60\text{mg/dl}$ ] identified throughout the day in a cohort of 28 children aged  $<12$  years [median age 9.8years, range 6.9-11.8 years] who completed three days of continuous glucose monitoring (155). Of all observations, 10.1% were in the hypoglycaemic range which was equivalent to 2.6 hours per subject per day (155). When the definition of hypoglycaemia was readjusted to  $<2.5\text{mmol/L}$  or  $45\text{mg/dl}$  the percentage of time spent in the hypoglycaemic range was 6.0% equivalent to 1.4 hours per subject per day (155). Hypoglycaemia was more prevalent at night compared with daytime [18 vs 4.4%,  $p<0.001$ ] (155). A logistic regression model used to determine factors related to hypoglycaemia risk showed that an increase in hypoglycaemia risk was independently associated with decreasing age [ $p=0.001$ ], increased daily insulin dose [ $p=0.007$ ] and increased standard deviation score for weight [ $p=0.04$ ] (155). Increased nocturnal hypoglycaemia was also independently associated with decreasing age [ $p=0.005$ ], increased daily insulin dose [ $p=0.003$ ], insulin regimen [ $p=0.03$ ] and increased standard deviation score for weight [ $p=0.02$ ] (155). There was no relationship between hypoglycaemia and the other two covariates within the analysis;  $\text{HbA}_{1c}$  [ $p=0.06$ ] and duration of diabetes [ $p=0.2$ ] (155).

#### **1.10.6 Identification of hypoglycaemia using the CGMS**

Specific groups have examined the validity of the subcutaneous sensor used in the continuous glucose monitoring system during hypoglycaemia in healthy volunteers (199; 200). Monsod et al used microdialysis techniques and a stepped euglycaemic-hypoglycaemic-hyperglycaemic insulin clamp where insulin levels were raised to approximately  $360\text{-}390\text{mmol/L}$  (199). At



baseline, sensor and plasma glucose levels were similar ( $5.0 \pm 0.3$  vs  $5.2 \pm 0.3$  mmol/L) (199). During the hyperinsulinaemic-euglycaemic study, sensor glucose was significantly reduced when compared to plasma glucose [ $3.7 \pm 0.6$  vs  $4.9 \pm 0.3$  mmol/L,  $p < 0.001$ ] (199). The sensor glucose levels remained lower than plasma glucose values during mild hypoglycaemia [ $2.5 \pm 0.6$  vs  $3.1 \pm 0.3$  mmol/L,  $p < 0.01$ ] and recovery from hypoglycaemia [ $7.3 \pm 1.2$  vs  $8.6 \pm 0.6$  mmol/L,  $p < 0.01$ ] (199). However, when calibration of the continuous glucose monitor against plasma glucose levels took place before and during every step of the clamp, sensor values did not differ from plasma glucose values during hypoglycaemia (199).

Similar evidence was shown using a hypoglycaemic, hyperinsulinaemic clamp by Cheyne et al in nine healthy volunteers each wearing two subcutaneous sensors (200). Reasonable agreement was shown between arterialised blood glucose levels and sensor values at each of three plateaus, namely, euglycaemia- hypoglycaemia - euglycaemia [correlation co-efficient  $r = 0.79$ ] (200). The sensor drop was shown to closely match the drop in blood glucose although the recovery from hypoglycaemia was delayed by an average of twenty-six minutes (200). The study concluded that while the device's potential to detect hypoglycaemia is reliable, the duration of episodes may be overestimated (200).

The wealth of information on direction, magnitude, duration, frequency and causes of fluctuations in blood glucose levels obtained with continuous glucose monitoring illustrates the advantage of this technology over intermittent blood glucose monitoring (172).

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## **1.11 Integration of continuous glucose monitoring in diabetes management**

The introduction of continuous glucose monitoring has enabled the provision of ambulatory data on the glycaemic profile including pre- and postprandial glucose levels, incidence and timing of hypo- and hyperglycaemia (196). Adoption of a uniform approach to analysis of

continuous glucose monitoring has the potential to enable this research tool to be applied to important outcomes regarding the child with diabetes mellitus. While insulin dose and duration of diabetes have been analysed to define their relationship with glucose values (155), no work has been done to date to assess whether insulin balance has a definable relationship on the glycaemic profile of the child with type 1 diabetes. Good diabetes control should be reflected in good physical and mental health. The possibility exists that by correlating such outcomes with short-term glycaemic control markers using CGMS, further information on how physical and mental health affects diabetes may aid management strategies, especially in children and adolescents. To provide a comprehensive evaluation, traditional markers of diabetes control namely HbA1c, BMI z-score, age, duration of diabetes and insulin dosage need to be considered to ascertain their relationships with each of the outcome measures provided using the CGMS algorithm.

#### **1.11.1 Closing the loop using CGMS**

Continuous glucose monitors have also advanced the technology required to achieve a realistic closed loop system. In parallel with the development of synthetic insulins, advances in subcutaneous insulin infusion are aimed towards the realisation of an artificial pancreas using closed loop systems or depots of insulin which are released under electronic conditions simulating physiological control (37). Implantable insulin pumps [IIP] have been pioneered since the early 1980s (37) with the first Programmable Implantable Medication System [PIMS] implanted in a human in 1986. Expectations were that a fully implantable closed-loop system would be available within years (201). However, ongoing technical problems with the insulin used and the devices have resulted in limited uptake of the implantable pump technique with only 689 patients enrolled on the International Study Group on Innovative Insulin Delivery Registry over 10 years (201).

The potential for achieving near-normal blood glucose control comes from the likely advent of CGMS output automatically directing insulin delivery on demand (37). Havorka has systemically reviewed the various closed loop systems in evolution including s-c, s-c (subcutaneous glucose monitoring with subcutaneous insulin delivery, i-v,i-v (intravenous glucose monitoring with intravenous insulin delivery) and i-v, i-p (intravenous glucose monitoring with intraperitoneal insulin delivery)(202). Work continues on the development of control algorithms which will direct insulin delivery however the challenge lies in the independent response of a closed loop system to meals and exercise (203; 204). In contrast to physiological control, glucose sensing and insulin delivery are conceptually and spatially separated in the biomechanical artificial pancreas (205). Also, despite these advances in insulin pump techniques, glucose sensing has proven to be a difficult technology to develop with progress measured in decades rather than years (37). As Klonoff has commented, it appears likely that CGMS will eventually become a routine part of diabetes management in this data-hungry world and the associated data printouts will increasingly provide a roadmap for diabetes management in the 21<sup>st</sup> century (206).

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## 1.12 Psychological outcomes in children with diabetes

In early childhood, a time sensitive to neurological development, changes in glycaemia may have adverse effects on cognitive function, potentially as significant as the long-term microvascular complications due to hyperglycaemia (207-210). Functional and structural changes within the central nervous system have been documented in patients with type 1 diabetes mellitus using neuroimaging (208; 211). Neuropsychological deficits have particularly been demonstrated in groups with high incidence of both hypo- and hyperglycaemia (208; 211). Early onset age of type 1 diabetes (<7 years of age) has been shown to influence cerebral structure and cognitive function (212). Seventy-one young adults with long duration type 1 diabetes participated in a



cross-sectional study examining cognitive ability (neuropsychological test battery) and brain structure (magnetic resonance imaging) (212). Current intellectual ability and information processing were comparatively poorer and lateral ventricle volume and ventricular atrophy greater in the cohort with early onset age for type 1 diabetes (212). The same group had previously examined a cohort of seventy-four young people using the same methods to see if severe hypoglycaemia or the presence of microangiopathy (background retinopathy) predisposed the patient to abnormalities in brain structure or cognitive ability (213). Recurrent exposure to severe hypoglycaemia alone in young people with type 1 diabetes had no detrimental impact on brain structure or function over the duration of diabetes examined (213). Chronic hyperglycaemia (inferred by the presence of background diabetic retinopathy) was associated with focal white matter changes and reduced abilities in information processing, attention and concentration (213).

#### **1.12.1 Cognitive impairment and diabetes**

Improving glycaemic control through intensive therapy with the aim of reducing long-term complications has been shown to result in increased rates of severe hypoglycaemia (151; 214). In the DCCT, 65% of patients in the intensive therapy group reported at least one severe episode of hypoglycaemia compared to 35% of patients in the conventional therapy group (151). In the SDIS, 77% of patients in the intensive therapy group reported severe hypoglycaemia compared to 56% in the conventional therapy group (215). Separate neuropsychological analyses of the subjects included in the DCCT and in the SDIS failed to identify any deterioration in neuropsychological measures between the intensive and conventional treatment groups despite the difference in hypoglycaemia incidence (215; 216). These studies focussed predominantly on adults, when evidence suggests that is the children and adolescents with insulin dependent diabetes that have an increased risk of development of cognitive impairment .

During hypoglycaemia, muscle and liver can easily switch from oxidation of glucose to other non-glucose fuels and thus are not energy-deprived (217). In contrast, the brain is strictly dependent on continuous glucose delivery from the circulation for its metabolism and function (217). Experimentally induced hypoglycaemia in humans has been shown to cause progressive but reversible cognitive dysfunction (218). Northam et al showed that the neuropsychological profile of newly diagnosed children is similar to that of control children (207). However, follow up at two and six years post diagnosis showed a deterioration in measures of intelligence, attention, processing speed, long-term memory and executive skills (208; 211). Such deterioration is suggestive of subtle compromise of anterior and medial temporal brain regions (208). Whether hypoglycaemia or hyperglycaemia is responsible for these changes remains the focus of extensive research. Various studies have examined cognitive function under experimentally induced hypo- and hyperglycaemia with varying outcomes (219-221).

#### **1.12.2 EEG changes in children with diabetes**

From an electrophysiological viewpoint, electroencephalographic abnormalities are more frequent in both adults and children with type 1 diabetes (222). Soltesz and Ascadi reported EEG abnormalities related to a history of severe hypoglycaemia among 49% of seventy children with type 1 diabetes mellitus compared to 24% of seventy healthy control subjects (223). When the seventy children with diabetes were subanalysed, 80% of seventy children with a history of severe hypoglycaemia had EEG abnormalities compared with 30% of the forty-three children with no history of severe hypoglycaemia (223). Tupola et al compared EEG traces in thirty-six patients with type 1 diabetes who had experienced severe hypoglycaemia with age matched controls with type 1 diabetes mellitus and age matched healthy controls (222). Both groups with diabetes had baseline EEG traces from diagnosis. The authors concluded that patients with an abnormal EEG recording at the time of diagnosis of type 1 diabetes mellitus were more likely to have a subsequent coma and/or convulsion

associated with hypoglycaemia than those with a normal initial recording (odds ratio 8.0, 95% C.I. 1.1-354.7) (222).

Predictors of change in neuropsychological profiles of children with type 1 diabetes include early onset of type 1 diabetes mellitus, chronically elevated blood glucose levels and recurrent severe hypoglycaemia (224). As previously mentioned, the continuous glucose monitoring trace has identified long periods of hypoglycaemia, particularly nocturnal. Whether behaviour changes result from nocturnal hypoglycaemia remains unclear. Much of the literature that exists on the impact of behaviour on diabetes or vice versa relates to long term effects of poor metabolic control (207; 208; 211) or acute, severe episodes of hypoglycaemia (225; 226). Little data exists in the literature comparing short-term outcomes of glycaemic control to non-microvascular outcomes such as neuropsychological outcomes.

### **1.12.3 Psychiatric disorders and diabetes**

Kovacs et al investigated the longitudinal relationship between psychiatric diagnostic variables and metabolic control among youths with type 1 diabetes (227). Eighty-eight children (8-13 years old) were followed for 9 years from onset of diabetes and evaluated repeatedly using a standardised psychiatric protocol (227). The psychiatric diagnosis of noncompliance with medical treatment was significantly related to HbA1c level with a trend of association between any major psychiatric disorder, as well as nondepressive disorder and HbA1c (227). The authors concluded that psychiatric morbidity negatively affects blood glucose regulation and that its consequences are more marked the longer patients have had type 1 diabetes (227). Northam et al showed that 37% of adolescents in a ten year follow-up from diagnosis of type 1 diabetes met the criteria for a DSM-IV psychiatric disorder with females significantly more likely to receive a diagnosis (228). Adolescents with a current mood, anxiety or behaviour disorder or a history of poorly controlled diabetes had higher



externalising behaviour problem scores at diagnosis (228). They concluded that poorly controlled diabetes over the first ten years of type 1 diabetes was associated with pre-existing behaviour problems at diagnosis and there was a trend for an association with current psychiatric status (228). A comparison between children with asthma and children with diabetes showed a higher overall incidence of psychiatric problems in children with asthma than type 1 diabetes with no gender difference observed. The problems included anxiety disorders, internalizing symptoms and disruptive behaviours (229) indicating that it may be the chronicity of disease rather than abnormalities in glycaemia that play a major role in psychological dysfunction.

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## 1.13

### Summary

In the last century, science and technology have combined to prolong the lives of those affected by diabetes mellitus. However, it remains clear from this literature review that gaps persist in our knowledge regarding many aspects of diabetes care.

Increased understanding of the structure and physiological role of insulin has resulted in the development of highly specified insulin analogues. On the horizon is the possibility of insulin adjuncts and implantable insulin pumps. Yet methods of deciding insulin doses and regimens remain crude and lack evidence based studies regarding the direct dosage impact on glycaemia.

Methods of monitoring blood glucose have evolved but remain centred on multiple daily blood tests and three-monthly HbA1c tests. The DCCT and EDIC studies have demonstrated that good metabolic control will reduce the incidence and progression of complications. However, a proportion of those with such appropriate control still advance to complications without explanation. Could this be due to unidentified fluctuations in glycaemic variation? Should techniques measuring glycaemic control of diabetes be directed towards dual outcomes of

near normal HbA1c with a corresponding low level of glycaemic variation? To answer these queries, continuous glucose monitoring needs to develop the ability to accurately measure glycaemic variation in daily situations. Clinical questions posed by the advent of continuous glucose monitoring include whether CGMS data is representative of average glycaemic control within the same 3 months? What is the risk of marked glycaemic excursions? What is the lability/variation of glycaemic control?

Although many studies have been carried out examining the utility of continuous glucose monitoring with preexisting methodology there remains minimal novel use of this technology to date. There has also been limited applicability of CGMS analysis in real-life clinical situations particularly in children. For example, the relationship between poorly controlled diabetes and an increased incidence of disorders defined by DSM IV have been established in teenage girls with diabetes. The question remains whether abnormal levels of glycaemia cause the increase in such disorders or a predisposition to such disorders influences the level of glycaemic control of an individual.

This dissertation aims to address these unanswered questions while improving and expanding on techniques for the analysis of continuous glucose monitoring.

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## 2 Aims & Hypotheses

The major aim of this study was to develop a new, improved system of analysis for the continuous glucose monitoring system (CGMS) and then to examine the utility of this system in answering clinical questions in a group of prepubertal children with type 1 diabetes.

**Specific Aims** were as follows:

1. To devise an algorithm which would encompass the multiple facets of continuous glucose monitoring including a novel measurement for glycaemic variation and to apply this algorithm to subjects with and without diabetes to ascertain the utility of the algorithm in clinical diabetes.
2. To describe the outcomes of continuous glucose monitoring in a cohort of children with diabetes over repeated three day periods with emphasis on whether outcomes of continuous glucose monitoring outcomes reflect metabolic control measured by HbA1c.
3. To assess the impact of continuous glucose monitoring on metabolic and glycaemic outcomes over a twelve month period.
4. To ascertain if the insulin regimen used by the study cohort impacts on glycaemic outcomes measured by continuous glucose monitoring.
5. To ascertain whether types of behaviour are associated with glycaemic outcomes using continuous glucose monitoring.



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## 3 Methods and Materials

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### 3.1 Planning & Recruitment

Planning for this project began in October 2002. There was an initial consultative phase between the author [CMcD], local supervisor [FC] and the Clinical, Epidemiological and Biostatistical unit [CEBU] who provided statistical support throughout the study. An extensive literature review was undertaken by the author. The project design was based on the number of continuous glucose monitors available (4) and average clinic attendance (every 3 months) for each child. In order to quantify the effect of glycaemic variation, the outcomes of the CGMS were correlated with parameters of diabetes outcome. A timeline was drawn up for each participant in the study which is summarised in figure 3.1.

**Figure 3-1: Clinical assessment of each patient attending for conjoint clinical review and study visit over the twelve month duration of the study**

Baseline	3months	6months	9months
↓	↓	↓	↓
Height	Height	Height	Height
Weight	Weight	Weight	Weight
HbA1c	HbA1c	HbA1c	HbA1c
CGMS	CGMS	CGMS	CGMS
-	BASC	-	BASC

#### 3.1.1 Ethical agreement

Consent for the project was granted by the Ethics committee of the Royal Children' Hospital. The initial approval was granted in November 2002 [Approval number 22070, Appendix 1]. A

further modification allowing application of the behavioural questionnaire [BASC] was approved in April 2003 (Approval number 22070b).

### **3.1.2 Recruitment**

Any child who attended the diabetes outpatient clinic of the Royal Children's Hospital, Melbourne was eligible for this study if they were less than 10 years of age on February 1<sup>st</sup> 2003 and had diabetes for more than 2 years duration when they entered the study. Ninety nine children were eligible for the study based on a review of our clinic database. A letter explaining the project was sent by the author to each eligible family and they were approached by the author on their next routine clinic visit between February 1<sup>st</sup> and May 31<sup>st</sup> 2003 to request their participation in the study.

### **3.1.3 Clinic visits**

The diabetes outpatient clinics at the Royal Children's Hospital provides the majority of paediatric diabetes care in the greater Melbourne area. More than 1,350 children with diabetes attend the clinic annually. Each child diagnosed with insulin dependent diabetes attends clinic every three months for a multidisciplinary review including medical consultation, auxology, HbA1c measurement and access to advice from diabetes nurse educators, dieticians and social workers if required. This was adjudged to be an appropriate time interval for application of the continuous glucose monitoring system. At the first clinic, prior to admission to the study, pubertal status was assessed by the author and clinical and demographic data was recorded at each visit prior to application of the continuous glucose monitor. All demographic data collection, application and removal of the CGMS, analysis of the data and feedback to families was provided by the author.

#### **3.1.4 Height**

Each child was measured by the clinic nurse using the Harpenden Stadiometer. This is a wall mounted apparatus which measures standing height in centimetres to one decimal place

#### **3.1.5 Weight**

Each child was weighed during the three monthly clinic visit using the electronic chair scales which measures sitting weight in kilograms to one decimal place.

#### **3.1.6 BMI z-score**

Body Mass Index [BMI] was calculated using the standard formula for weight in kilograms adjusted for height in metres squared ( $\text{kg}/\text{m}^2$ ). BMI Z-score was calculated using a Stata™ computer program [Zanthro]. The reference used to calculate the Z-score in this program was provided by the British 1990 growth reference centiles for weight, height, body mass index compiled by Cole et al (230).

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### **3.2 Development of the novel algorithm for CGMS analysis**

#### **3.2.1 Continuous glucose monitoring system (CGMS)**

The monitoring system available for use in Australia at the commencement of this project was the Medtronic Minimed Continuous Glucose Monitoring System [Medtronic, Northridge, California, USA] [figure 3.2]. This system is based on an electrode contained in a sensor placed in the subcutaneous fat of the abdomen or buttock [figure 3.3 & 3.4].



Figure 3-2: The Minimed CGMS

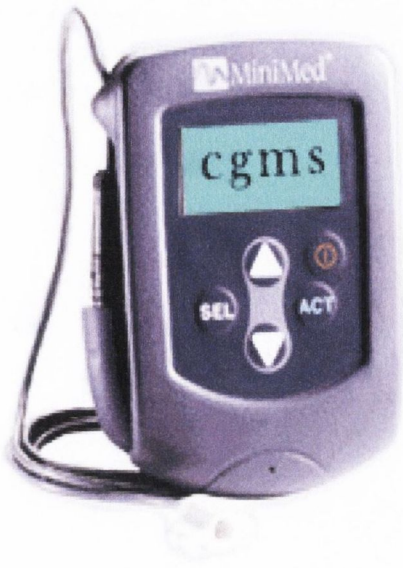


Figure 3-3: Wearing of the CGMS



Figure 3-4: Close-up of the sensor placement of the CGMS.



The electrode contains the enzyme glucose oxidase. When the enzyme comes into contact with interstitial glucose an electrochemical reaction is triggered converting glucose at the sensor surface into electronic signals. These electronic signals are continuously transmitted

from the electrode to the monitor where they are stored along with any fingerstick blood glucose measurements entered into the monitor (160).

A glucose reading is stored every 10 seconds and an average reading is calculated every 5 minutes and saved by the monitor. In a 24 hour period, 288 readings are recorded. Calibration of the monitor is carried out using the patients own glucometer readings which are manually input by the patient at least four times in each 24 hour period.

### **3.2.2 Data analysis of the CGMS**

The data obtained from each continuous glucose monitor trace was input into a computer programme called Minimed Solutions version 1.7A [provided by Minimed software] which analyses the data and produces the graphs and data utilising Microsoft excel spreadsheets. The data spreadsheet gives the date, time, current, voltage and sensor value for each five minute sample and the paired glucometer value when available. The output summary includes a daily synopsis of the monitor performance and a series of graphs illustrating the recorded data. A graph is produced for each 24 hour period [midnight to midnight] as well as a modal day comprised of an overlay of the days involved [see Appendix 3].

The author was aware prior to the commencement of the study that problems would be encountered with the wearing of the monitor. Any of the parts [sensor, monitor or cable] are liable to malfunction or damage. In prepubertal children, over-activity could lead to loss of the trace, this was noted in children who were known to be restless sleepers. Although families were clearly instructed to input at least four sensor values for calibration per day this was not always the case. Therefore, as part of the data analysis, the author decided only to use cleaned, verified, calibrated data. This was done using the following guidelines set before the project began.

1. At least four calibrated glucose readings had to be present in a 24 hour period for the trace to be valid
2. A calibrated glucose reading had to be present at least once in every eight hour period
3. Data that was deemed uncalibrated or was interrupted by loss of trace without recalibration would be deleted.

### **3.2.3 Cleaning of data generated by CGMS**

This data was cleaned using a programme generated in collaboration with the Clinical Epidemiology and Biostatistics Unit, Royal Children's Hospital. This programme identified any mismatched readings or missing sensor value over the duration of the trace. Each identified error was then manually scrutinised by the author to ascertain if the sensor reading was inaccurate. A corrected dataset was then constructed manually by the author after individual correction of each file. The corrected dataset was then run through a series of do files and ado files [computer programs specific to Stata™] designed to summarise the data. A final validated data sheet was then summarised for each individual, for use by the author in statistical analysis. This data cleaning process was repeated for each cycle of continuous glucose monitoring to maintain accuracy.

### **3.2.4 Development of the CGMS algorithm**

The approach to CGMS analysis was devised by the planning group led by the author using a series of questions to be applied to each CGMS trace. For clarity the data is summarized according to the clinical questions posed prior to the project commencement.



*1). How representative is the CGMS data of average glycaemic control within the same 3 months?*

Mean blood glucose was calculated as a measure of glycaemic control within the CGMS trace. This data was then compared to the HbA1c measured on the first day the CGMS was worn. The mean CGMS glucose value was calculated as the arithmetic mean of glucose values within a given period. The formula to calculate mean blood glucose is summarised in Table 3.1.

Consistency between days on a CGMS trace was assessed using the mean of daily differences of measured glucose [MODD]. MODD was calculated by the difference between glucose values taken on two consecutive days at the same time with the mean of the differences then calculated. The formula to calculate mean of daily differences is summarised in Table 3.1.

*2) What is the risk of marked glycaemic excursions?*

To assess the risk of marked glycaemic excursion over the duration of the trace, the percent time spent in three defined glucose ranges was calculated after data cleaning: low [CGMS value <4mmol/l], normal [CGMS value 4-12mmol/l] and high [CGMS value >12mmol/l]. Percentage time is calculated by taking the time spent in a specified glucose range and dividing by the total valid time for that range and multiplying by 100. Mean minutes per hour is calculated by taking the time spent in a specified glucose range and dividing by the total valid time for that range and multiplying by 60.

*3) What is the lability/variation of glycaemic control?*

As discussed in the literature review, the planning team led by this author identified shortcomings in all the existing formulations to assess glycaemic variation when faced with the large volumes of data provided by continuous glucose monitoring. No single available mode of measurement available at the planning stage was capable of measuring variation using continuous glucose monitoring although many were trialled and discarded. The novel measure of glycaemic variation was devised following lengthy roundtable discussion between Dr.

Cameron and the author and translated into a mathematically calculable term by Dr. Donath. This measure was termed "continuous overall net glycaemic action" [CONGA] and the methodology was peer reviewed and published by the author during the course of this dissertation [Appendix 4]. The formula to calculate Continuous overall net glycaemic action is summarised in Table 3.1.

**Table 3-1: Summary of formula used to calculated outcome variables of CGMS**

Name	FORMULA	
Mean glucose	$\frac{\sum_{t=t_1}^{t_k} GR_t}{k}$	k = no. of observations (no. of glucose readings for a given individual),
MODD	$\frac{\sum_{t=t_1}^{t_{k^*}}  GR_t - GR_{t-1440} }{k^*}$	k* = Number of observations where there is an observation at the same time 24 hours ago
CONGA	$\sqrt{\frac{\sum_{t=t_1}^{t_{k^*}} (D_t - \bar{D})^2}{k^* - 1}}$ <p>where  <math>D_t = GR_t - GR_{t-60}</math>  and  <math display="block">\bar{D} = \frac{\sum_{t=t_1}^{t_{k^*}} D_t}{k^*}</math></p>	k* = Number of observations where there is an observation 60 minutes ago

### 3.2.5 Continuous overall net glycaemic action [CONGA]

CONGA is defined as the standard deviation of the differences of a continuous trace and measures the overall intra-day variation of glucose recordings. For each observation after the first  $n$  hours of observations, the difference between the current observation and the observation  $n$  hours previous was calculated.  $CONGA_n$  was defined as the standard deviation of the differences. Higher CONGA values therefore indicate greater glycaemic variation. The choice of the time difference,  $n$ , will depend on the clinical question being addressed. Three time periods were selected as most pertinent for the age group to be studied namely  $CONGA_1$ ,  $CONGA_2$ , and  $CONGA_4$ , the time periods 1 h, 2 h, and 4 h, corresponding approximately to time between different activities in school [1 hour], time between snacks [2 hours], and time between meals [4 hours].

### 3.2.6 Summary of the novel CGMS algorithm

For every continuous glucose monitoring trace the following information is summarised

- Mean blood glucose [MBG]
- Mean of daily differences [MODD]
- Percent time spent in the low CGMS glucose range
- Percent time spent in the normal CGMS glucose range
- Percent time spent in the high CGMS glucose range
- Continuous overall net glycaemic action [CONGA]

### 3.2.7 Trial application of the CGMS algorithm

CGMS recordings were obtained over 72-hour periods from 10 children with type 1 diabetes [age range, 9.3-19.5 years] randomly chosen from the diabetes clinic at the Royal Children's Hospital [RCH], Melbourne, Victoria, Australia and from 10 healthy, adult controls without diabetes [age range, 30.0-46.5 years].



Each CGMS trace was calibrated by a minimum of four finger-prick blood glucose measurements per 24-hour period. A calibration point had to be carried out at least every 8-hours for the data to be included in the analyses. Data cleaning entailed confirmation of regular calibration, identification of errors with paired sensor values, and review of missing data points or time points and was carried out using Stata™ statistical software as previously detailed above. To establish the impact of the artificial limitations of the glucose monitor on the outcomes of the CONGA the data was further cleaned to remove all values  $\leq 2.2$  and  $\geq 22.2$  mmol/L. The analysis was then re-run on the cleaned data and the results labelled "adjusted CONGA".

The traces from the children with diabetes [diabetes group] were then compared to the people without diabetes [control group] to see if ascertainable differences were evident. This comparison was carried out prior to application of the algorithm to the study cohort.

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### 3.3 Use of CGMS in the study cohort

As described in Section 3.1 the study population consisted of a cohort of prepubertal children with diabetes followed over a twelve month period. Each child who participated in the project was asked to wear the CGMS on four occasions. Each occasion ranged from Tuesday to Friday or from Friday to Tuesday so that each child would complete the equivalent of two weeks of readings by the end of the project encompassing both weekdays and weekends. Prior to monitor attachment each child was given the option of having a local anaesthetic gel [An-gel: 4% amethocaine gel developed by the pharmacy department of the Royal Children's Hospital] topically applied to the site of insertion. An-gel numbs the epidermis if applied at least 30 minutes before the procedure.

The sensor was inserted manually with the aid of a Sen-serter [©Medtronic; spring loaded device] held at a 45° angle to the skin. All monitor visits were coordinated by the author and

all sensor insertions were completed by the author. The parent and child received a tutorial from the author on how to use the monitor prior to the sensor insertion. A written set of instructions were also given on each occasion. Parents had 24 hour contact with the author via mobile phone to correct any problems encountered throughout the duration of the monitoring. Each child returned for monitor download on the subsequent Tuesday or Friday as appropriate when they were met by the author. The monitor was removed, data downloaded and the graphs shown to the family [see Appendix 3 for sample trace] with appropriate explanation and adjustment of insulin if required.

Downloading of the monitor involved the use of an infra red port ["the com station"] which transmitted the information to a computer programme. The data was then saved under the child's study identification number to protect privacy and confidentiality.

### **3.3.1 Outcome of continuous glucose analysis at each individual study timepoint**

Clinical and demographic characteristics of the cohort included age, gender, bmi z-score and insulin dose and were expressed using mean, range and standard deviation [SD] unless otherwise indicated. Significance was set at 95%. Glycaemic outcomes from the novel algorithm included mean blood glucose, mean of daily differences, percentage time in the low, normal and high CGMS ranges and continuous overall net glycaemic action as described in Section 3.2.6.

### **3.3.2 Statistical analysis of the study cohort**

All statistics for this project were carried out using the Stata™8.0 statistics/data analysis system [Stata Corporation, TX, USA] (231). Do-files [computer programs specific to Stata™] were devised by the Clinical Epidemiological and Biostatistical unit in collaboration with the author or in some cases written by the author to calculate the outcome variables for continuous glucose monitoring.

The children participating in the study were compared to those who were eligible but did not participate using student t-tests. Significance was set at 95%.

### **3.3.3 Assessment of HbA1c**

This reading is a reflection of the percentage of glycosylated haemoglobin present in the body (98). At the Royal Children's Hospital, the analytical method employed is the bedside immunoassay using the DCA 2000 (Bayer Diagnostics Pty Ltd). For normal readings, the between run co-efficient of variation is 3.5% for a HbA1c of 5.5% (n=186). For abnormal readings, the between run coefficient of variation is 3.4% for a HbA1c of 10.3 % (n=197). This data is based on HbA1C levels measured at scheduled clinic appointments between March 2002 and April 2004, which was inclusive of the study period.

### **3.3.4 Comparison between glycaemic outcomes measured by CGMS and HbA1c:**

At each timepoint in the study the glycaemic outcomes measured by continuous glucose monitoring were compared to HbA1c to ascertain if CGMS reflected metabolic control. Bivariate regression analyses were employed with HbA1c as the independent variable and each of the continuous glucose monitor measures as the dependent variable. The relationships were described through the use of correlation coefficients, 95% confidence intervals and p values. Stepwise multiple linear regression was used to examine predictors of change in HbA1c from CGMS data including age and gender of the children involved.

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## **3.4 Continuous glucose monitor outcomes over a 12 month period**

This section of the thesis aimed to examine the changes in glycaemic outcomes from repeated continuous glucose monitoring over a twelve month period in the study cohort. Glycaemic variables were expressed using mean, range and standard deviation [SD]. Significance was set at 95%. Each glycaemic outcome (MBG, MODD, Percent time in low, normal, high ranges and



CONGA) was plotted using a longitudinal plot and a slope calculated using linear regression to determine whether the outcome had improved or deteriorated over the year. Direct correlations were carried out between the slope of glycaemic variables and independent variables of diabetes [HbA1c, BMI z-score, age and insulin dose]. Changes in slope in a positive or negative direction were used to define groups for comparison with the independent variables using unpaired student t-tests.

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### **3.5 Insulin regimen and glycaemic outcomes measured by CGMS**

All children within the study cohort who completed the twelve month study period while receiving twice-daily variable insulin regimes were eligible for inclusion in this aspect of the study. During the period of each CGMS trace, insulin doses were not adjusted. Demographic data was collated as previously described. In particular, details of insulin regimen included type of insulin, total daily dosage, proportion of intermediate and short acting insulin and total morning and evening dosages. All children were under parental supervision for insulin injections.

Data cleaning and analysis of the CGMS data was carried out as previously described. CGMS data included: mean CGMS glucose, inter-day glycaemic variation as measured by the mean of daily differences (MODD), percentage time spent within glucose ranges defined as low (<4.0 mmol/L),

normal (4.0 -12.0 mmol/L) and high (>12.0 mmol/L), intra-day glycaemic variation as measured by continuous overall net glycaemic action (CONGA) and calculated for time intervals of 1, 2 and 4 hours.

### **3.5.1 Analysis of the relationship between insulin regimens and CGMS**

Data was pooled for all subjects. Insulin dosage and glycaemic variables were expressed using mean, range and standard deviation [SD]. Proportions of insulins between short and long acting insulin types and between morning and evening dosages were described in percentages. Significance was set at 95%. Glycaemic variables were evaluated against insulin status using the correlation coefficient [r]. Linear regression analysis was used to evaluate whether a change in insulin type or dose resulted in a corresponding effect on glycaemic variation or HbA1c.

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## **3.6 Behavioural assessment and outcomes measured by CGMS**

This section of the study determined whether types of behaviour are associated with glycaemic outcomes using continuous glucose monitoring. The BASC questionnaire was applied on the second and fourth occasions that the CGMS trace was applied. Any child who continued through the study was eligible for this aspect of the study. Demographic details were collected as previously described. Behavioural questionnaires were collated and analysed by the author under the supervision of a behavioural psychologist. Glycaemic outcomes were summarised using the algorithm described in section 3.2.6 from the CGMS data supplied at the relevant timepoints.

### **3.6.1 Behavioural Assessment System for Children (BASC) questionnaire**

Parents were asked to complete the Behavioural Assessment system for children [BASC], an individual instrument (232). This is a standardized, validated, parent-report instrument that takes ~20minutes to complete on each occasion and reflects the child's typical behaviour over the previous six months. To minimize the risk that parental ratings are influenced by factors extraneous to the child's actual behaviour, parents are asked to report on the occurrence and

frequency of specific behaviours and not on their perceptions and feelings regarding the child's behaviour [e.g. "Does your child hit other children almost always? Often? Sometimes? Never?" rather than "Is your child aggressive?"]. The author spent detailed time with each family explaining the need for the questionnaire and the instructions required to complete the form. The completed questionnaire was returned when the monitor was removed (see Appendix 5 for example). Analyses of BASC data were carried out using BASC Enhanced ASSIST (version 2.0), which generates summary *T*-scores (mean of 50 and sd of 10) standardised for age and sex for externalising behaviour and internalising behaviour. Externalizing behaviour comprises hyperactivity, aggression and conduct scores, whereas internalizing behaviour comprises anxiety, depression and somatization scores. High scores indicate greater psychopathology.

### **3.6.2 Analysis of the relationship between behavioural outcomes and CGMS**

The summary scores for behavioural variables and the glycaemic outcomes calculated from CGMS were expressed using mean, range and standard deviation [SD]. Significance was set at 95%. The degree of agreement between intra-individual measurements on the two occasions that the BASC questionnaire was administered was evaluated using the correlation coefficient [*r*]. Linear regression was used to evaluate the relationship between behavioural scores and glycaemic measurements.



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## 4 Results

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### 4.1 Study population

#### 4.1.1 Participation

Ninety nine children who were attending the diabetes outpatient clinic of the Royal Children's hospital were eligible for participation in this thesis and were systematically approached during clinic attendance over a three month period. Of this group 52 agreed to participate and 47 declined. Forty of the families who declined, cited the time involved or the children's reluctance as an issue. No difference was found between participants and non-participants for age, duration of diabetes, insulin dose and HbA1c [see table 4.1].

**Table 4-1: Comparison of children eligible for this study based on participation.**

	Participants	Non-participants	t-test
Total number	52	47	
Gender Male	21	33	
Female	31	14	
Age (years)	8.0 (4.3-10.3)	7.7 (3.2-9.9)	p=0.16
Duration of diabetes (years)	3.7 (2.0-7.2)	4.0 (2.1-8.1)	p=0.38
Mean HbA1c (%) at baseline	8.2 (6.4-11.4)	8.1 (6.3-9.7)	p=0.55

#### **4.1.2 Age**

The mean age of children participating in the study was 8.0 years with a range of participants from 4.3 years to 10.3 years [sd 1.5 years]. A similar range was seen in the non participants. Although the mean age was slightly lower [8.0 vs. 7.7] this was not statistically significant [p=0.16]. See table 4.1.

#### **4.1.3 Duration of diabetes**

All children were required to have diabetes of at least two years duration prior to study commencement. The mean duration was 3.7 years with a range of 2.0 - 7.2 years [sd 1.5 years] which was similar to non-participants. Although the mean duration of diabetes was slightly lower in participants [3.7 - 4.0 years] this was not statistically significant [p=0.38]. See table 4.1.

#### **4.1.4 HbA1c**

The metabolic control of the participants vs non participants was compared using HbA1c at the start of the study. The mean HbA1c of the study cohort was 8.2% [sd 0.9%] which was representative of the spread of HbA1c values seen within the diabetes clinic and was not statistically different from the mean [8.1%] of those who chose not to participate in the study [p=0.55]. See table 4.1.

#### **4.1.5 Insulin regimen**

Fifty-one of the children were on twice daily standard insulin regimens consisting of either Protaphane or Humulin NPH in combination with regular insulin or analogues. One child received a third injection per day of regular insulin. Two children switched to MDI during the study between the second and third monitor runs with minimal change in total daily insulin

dosage. Further examination of the insulin regimen of the study cohort is carried out in section 4.5.

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## 4.2 A novel algorithm for continuous glucose monitoring analysis

The first aim of the study was to devise an algorithm which would reflect the many aspects of continuous glucose monitoring. The method behind this algorithm is discussed in section

3.2. For every continuous glucose monitoring trace the following information is accrued.

- Mean blood glucose
- Mean of daily differences
- Percent time spent in the low CGMS glucose range
- Percent time spent in the normal CGMS glucose range
- Percent time spent in the high CGMS glucose range
- Continuous overall net glycaemic action

### 4.2.1 Trial application of the CGMS algorithm to compare outcomes from people with and without diabetes.

CGMS recordings were obtained over 72-hour periods from 10 children with type 1 diabetes [age range, 9.3-19.5 years] randomly chosen from the diabetes clinic at the Royal Children's Hospital and compared to traces obtained from 10 healthy, adult controls without diabetes [age range, 30.0-46.5 years]. The data obtained was descriptively compared between the diabetes group and the control group under each of the headings listed above and is summarised in Table 4.2.

### 4.2.2 Mean blood glucose

Mean blood glucose was calculated as a measure of glycaemic control within the CGMS trace. The data in Table 4.2 shows a wide variety of mean CGMS glucose values in the cohort with



diabetes (6.0-16.2mmol/L) compared to the relatively narrow range in the cohort without diabetes (4.8 to 5.8mmol/L). This is reflected by the wide range of HbA1C values for the diabetic cohort from 6.6% to 9.9% showing them to have a wide range of metabolic control such as is evident in most diabetes clinics.

#### **4.2.3 Mean of daily differences**

Consistency between days on a CGMS trace was assessed using the mean of daily differences of measured glucose [MODD]. The group mean MODD value for the diabetic patients' was 4.3 [Range 2.9 - 8.1]. There was considerable variability in the MODD values within the diabetic group compared to those of the healthy controls whose group mean was 0.8 [Range 0.5 - 1.2]. The lower MODD values less than 1.0 corresponded with those CGMS traces with low levels of variability in which all 3 daily recordings closely overlapped each other on the modal trace [all 3 days recorded on the one graph].

#### **4.2.4 Percent time spent in the low, normal and high glycaemic range**

The risk of marked glycaemic excursion was based on the percentage time spent in the high or low glucose ranges. Table 4.2 shows that none of the healthy control traces recorded high CGMS values (>12mmol/L) and only 0-20.5% of time was spent with low CGMS values (<4mmol/l). None of the healthy controls experienced CGMS glucose levels less than 3 mmol/l or symptoms of hypoglycaemia. By contrast the traces from the patients with diabetes recorded percent time rates of 2.3 to 88.9% in the high range and 0-29.1% of time in the low range. Subjects with diabetes with similar HbA1c levels demonstrated different percentage time spent in the three glycaemic ranges e.g. subjects 13 and 17 and subjects 14 and 15. Higher HbA1c did not necessarily correspond with higher levels of inter or intra day variation.

#### 4.2.5 Continuous overall net glycaemic action

The lability of glycaemic control within a CGMS trace was assessed by calculating the CONGA value. Higher CONGA values indicate greater glycaemic variation. The choice of the time difference,  $n$ , will depend on the clinical question being addressed. Three time periods were assessed namely CONGA1, CONGA2, and CONGA4 for time periods 1 hour, 2 hour, and 4 hours apart, corresponding approximately to time between different activities [1 hour], time between snacks [2 hours], between meals [4 hours].

The group mean CONGA1 value in the group with diabetes was 2.5 (Range 1.7-3.2) compared to the healthy control group value of 0.7 (Range 0.4-1.2). None of the group with diabetes had a CONGA1 score less than 1.7 indicating a higher degree of glycaemic variation than the healthy controls. For CONGA2 and CONGA4, the differences between the two groups were even more marked with no noticeable differences in the control group for increased time intervals due to the limited variability of the normal blood sugar range. Figure 4.1 shows this relationship between CONGA value and time period use to calculate CONGA for each of the patients and controls. For the patients with diabetes CONGA generally increases as the time period increases with the mean CONGA4 value in the diabetes group reaching 4.6, compared with 1.0 in the healthy group. The CONGA values and "adjusted CONGA" values were comparable (within 0.1 of each other) in all but three of the diabetic subjects with a maximum observed difference of 0.3.

The glycaemic range of a person without diabetes is very narrow as the blood glucose level is controlled internally between 3.3-8.0mmol/L therefore the CONGA value is very low [ $<1.5$ ]. The largest increases are seen in patients with large glycaemic swings over the period of the trace. This is illustrated in Figure 4.2b, which shows the CGMS trace and CONGA values for patients with diabetes having low and high intra-day glycaemic variation, respectively. For comparison, the CGMS trace and CONGA values for a healthy control are shown in Figure 4.2a.

**Table 4-2: Comparison of traces from subjects without diabetes (1-10) to young people with diabetes (11-20).**

ID	Type 1 diabetes	MBG	MODD	CONGA1	CONGA2	CONGA4	HbA1c (%)	% time hypo	% time norm	t
1	No	4.8	1.2	1.1	1.2	1.2	-	20.5	79.5	
2	No	5.0	1.1	1.2	1.1	1.5	-	19.4	80.6	
3	No	5.2	0.9	0.6	0.9	1.2	-	15.4	84.6	
4	No	5.3	0.6	0.4	0.4	0.5	-	0.0	100.0	
5	No	5.3	0.8	0.6	0.7	1.0	-	4.2	95.8	
6	No	5.4	0.5	0.7	0.9	0.9	-	0.6	99.4	
7	No	5.6	0.6	0.7	0.9	1.0	-	0.3	99.7	
8	No	5.6	0.8	0.6	0.7	0.8	-	0.5	99.5	
9	No	5.8	0.6	0.6	0.7	0.7	-	0.0	100.0	
10	No	5.8	0.8	0.5	0.6	0.7	-	0.3	99.7	
11	Yes	6.0	3.2	2.0	2.9	4.0	6.6	29.1	68.6	
12	Yes	8.0	3.6	2.4	3.3	3.5	7.0	15.4	67.3	
13	Yes	8.5	2.9	2.5	3.3	3.5	8.2	4.5	82.5	
14	Yes	8.8	3.7	2.1	2.6	2.9	7.9	1.9	84.1	
15	Yes	9.2	8.1	2.4	4.1	6.1	7.8	20.2	49.1	
16	Yes	10.8	5.4	3.2	5.0	7.3	7.6	9.2	57.1	
17	Yes	12.6	4.3	2.9	4.4	5.4	8.4	0.8	50.1	
18	Yes	12.8	4.2	1.7	2.6	3.6	9.9	0.0	38.4	
19	Yes	13.5	3.9	2.7	3.9	4.9	9.7	0.0	41.7	
20	Yes	16.2	4.1	2.6	3.5	4.4	9.0	0.0	11.5	

**Figure 4-1: Relationship between CONGA value and CONGAn (the time difference between CONGA measurements) in children with diabetes versus normal controls.**

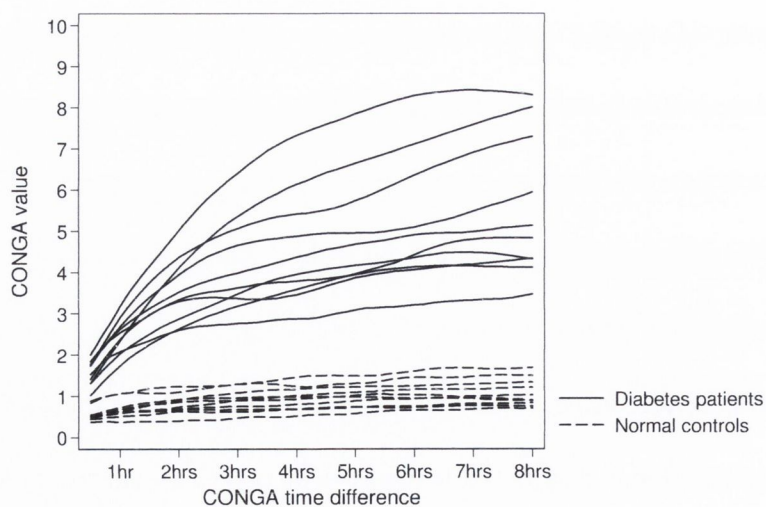




Figure 4-2: CONGA diagrams comparing data from representative controls and subjects with diabetes.

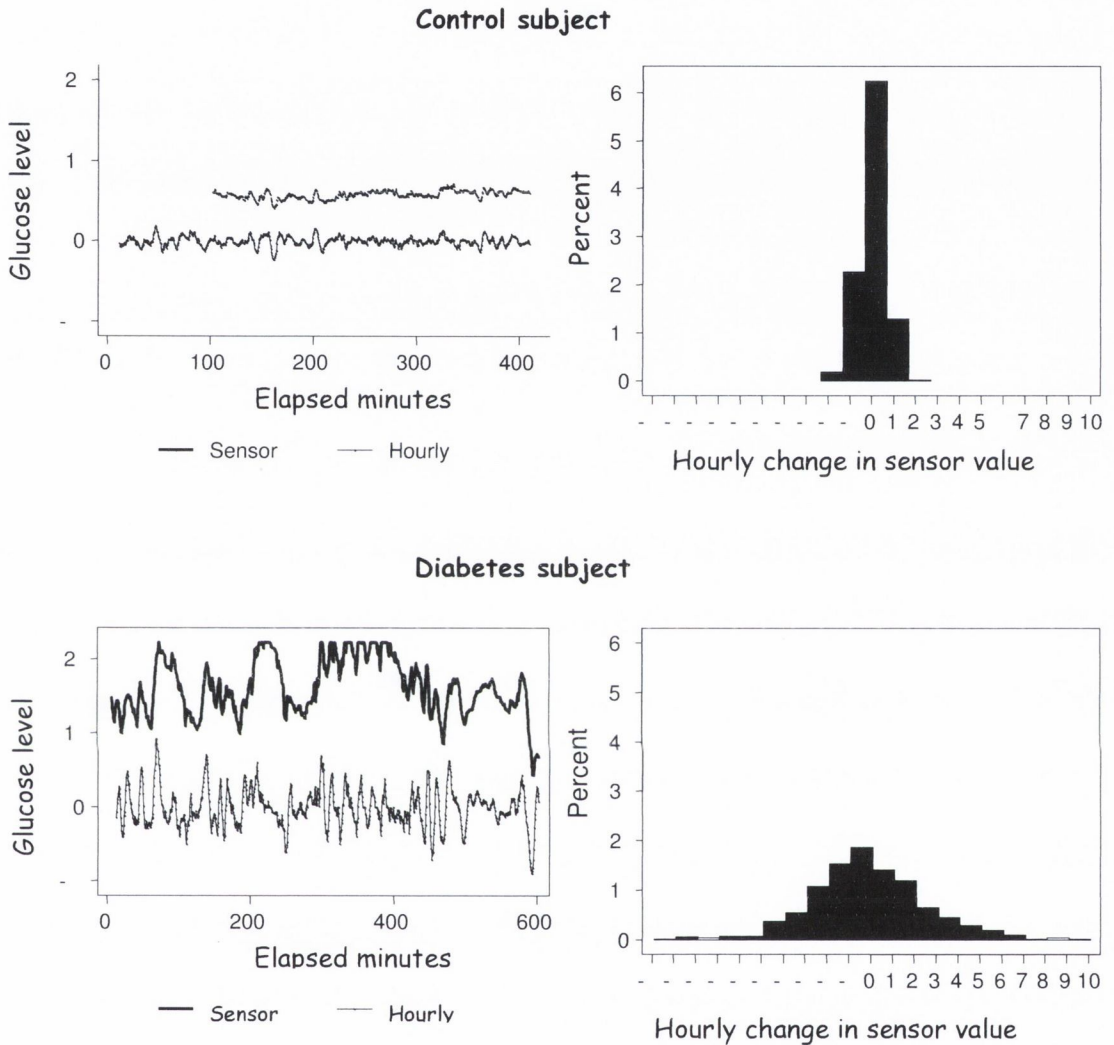


Figure 4.2a: Illustration of CONGA outcome in person without diabetes. The diagram on the left shows the actual trace and the distribution of hourly changes in glucose levels. The diagram on the right shows the standard deviation of this distribution. The trace is expressed as a percentage of the total.

Figure 4.2b: Illustration of CONGA outcome in child with diabetes. The diagram to the left shows the actual trace and the distribution of hourly changes in glucose levels to be much more erratic and more varied than the control. The diagram to the right shows the standard deviation of the distribution of hourly traces expressed as a percentage of the total with a much wider range of values illustrating a higher degree of glycaemic variation.

The data obtained in testing the *CONGA* demonstrates the dramatic difference in glycaemic variation between people with diabetes and healthy controls. In Figure 4.2 both distributions are centred around zero but a much wider range of differences is evident in the subjects with diabetes. *CONGA* analyses may also potentially be restricted by the limits of measurable glucose levels imposed by *CGMS*, due to the unknown degree of glycaemic change outside the specified measuring range so the "adjusted *CONGA*" was also formulated but the negligible difference between the two results shows no need for an additional calculation.

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### **4.3 Longitudinal study of the *CGMS* algorithm in a cohort of children with diabetes**

The second aim of the study was to describe the outcome of continuous glucose monitoring using the algorithm in a cohort of children with diabetes over a three day period. The *CGMS* monitor was worn on four consecutive occasions as part of the three monthly visit to assess the impact on glycaemic outcomes over a twelve month period. The continuous glucose monitoring analysis for the 3 day period for the study cohort at each individual timepoint are reported here and the relationship of the glycaemic outcomes to metabolic control as measured by HbA1c are summarised at the end of this section. The outcomes of the cohort over the twelve months as a whole are considered in section 4.4.

#### **4.3.1 *CGMS* analysis at baseline [Time = 0]**

##### Population:

Fifty two children completed the first run of the monitor.. Two traces failed completely [one due to the child knocking the sensor out while trampolining, the second was unexplained] but both were repeated a week later and the subsequent traces included in the analysis. One

trace contained only 10.5 hours of usable [i.e. calibrated] data out of 66 hours of data collection so was omitted from the analysis.

#### Data collection:

The mean total time per trace for the remaining 51 traces was 73.1 hours [range 36.1 to 98.1] prior to cleaning of the data as per the methods. The overall mean valid CGMS trace time [after data cleaning] was 69.9 hours [range 33.2 to 97.0 hours].

#### Clinical data:

The overall group mean HbA1C was 8.1% [Range 6.4-11.4%]. The mean BMI Z-score for the group on this occasion was 1.0 [range -1.3 to 3.3]. The mean total daily insulin dose for the group was 0.9iu/kg/day [range 0.6 to 1.2]. The data for each individual is summarised by clinical and continuous glucose monitoring outcomes in Appendix 6.

#### Mean blood glucose:

The mean CGMS glucose was calculated for each individual. The overall group mean glucose was 11.1mmol/L [Range 6.9-17.7mmol/l].

#### Mean of daily differences:

Inter-day variation, calculated by the Mean of Daily Differences [MODD] had a group mean of 5.1 [range 2.3 - 17.4].

#### Percent time spent in the low CGMS glucose range:

The mean percent time for CGMS reading <4mmol/L totalled 8.4% of the duration of the trace [Range 0-36%]. Only 53.6% of total episodes in the CGMS range <4.0 were identified by patients through the events marker on the CGMS, with 68% of overnight episodes occurring asymptotically.

#### Percent time spent in the normal CGMS glucose range:

Mean percentage time spent in the 4-12 mmol/l range was 51.2% of the total time [Range 6-88%]



Percent time spent in the high CGMS glucose range:

Mean percentage time spent in the >12 mmol/l glucose range the mean value was 40.4% [Range 10 to 93%]. The mean minutes per hour spent in each glycaemic range is stratified for time of day [morning, evening and night] in Table 4.3.

**Table 4-3: Mean minutes per hour in each range, by time of day, CGMS run 1.**

	Mean minutes/hr (min, max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)
Time	Total	8am-4pm	4pm-12	12-8am
CGMS range (<4mmol/L)	4.8 (0, 23.1)	3.0 (0, 18.8)	2.7 (0, 12.9)	8.4 (0, 37.8)
CGMS range (4-12mmol/L)	30.5 (5.4, 45.4)	25.9 (5.0, 37.0)	31.1 (3.8, 55.5)	33.8 (9.1, 43.9)
CGMS range (>12mmol/L)	24.7 (1.7, 53.9)	31.1 (5.6, 54.6)	26.2 (1.0, 56.1)	17.8 (0, 56.4)

Continuous overall net glycaemic action:

Intra-day variation was calculated by continuous overall net glycaemic action (CONGA). The overall group mean for the CONGA was calculated for 1 hour (3.1 [range 2.1-4.3]), 2 hour (4.7 [range 3.1-6.5]) and 4 hour (6.1 [range 4.1-8.7]) intervals.

#### 4.3.2 CGMS analysis at three months [Time=1]

##### Population:

Forty five children continued on to wear the monitor on a second occasion three months later. Seven dropped out. Reasons given for the dropouts are summarised in the table below.

**Table 4-4: Reasons for withdrawing from the study before the second occasion**

Study no.	Reason given for removal
2	Child refused to wear monitor again
13	Child refused to wear monitor again
16	Unrelated family conflict, withdrew child from study.
17	Child refused to wear monitor again
27	Child refused to wear monitor again
40	Parents withdrew child from study
50	Failed to attend outpatients on several occasions within the 3months

##### Data Collection:

Two traces were unsatisfactory with multiple interruptions in the data. During the wearing of the monitor the monitor had alarmed continuously despite recalibration. Both families declined the opportunity to repeat the trace and after data cleaning less than 24 hours of data remained so these traces were omitted from analysis. Both families had used the same monitor and after further investigation the cable was found to be faulty. Patient #44 failed to calibrate with blood glucose readings throughout the entire trace so the data was deemed invalid. Therefore the analysis was carried out on 42 children only. The mean total time per trace for the remaining 42 traces was 80.7 hours [range 62.9 to 98.3 hours] prior to data cleaning. The overall mean valid CGMS trace time [after data cleaning] was 74.6 hours [range 38.2 to 96.8 hours].

##### Clinical data:

The overall mean HbA1c was 8.6% [Range 6.7-10.8]. The mean BMI Z-score for the group on this occasion was 1.2 [range -0.9 to 3.3]. The mean insulin dose for the group was 0.9iu/kg/hr [range 0.6 to 1.3]. Details of the individual clinical and continuous glucose monitoring outcomes are summarised in Appendix 6.

#### Mean blood glucose:

Mean CGMS glucose was calculated for each individual CGMS trace with a group mean of 10.7mmol/L [Range 7.5 to 15.1mmol/l].

#### Mean of daily differences:

Inter-day variation, calculated by the Mean of Daily Differences [MODD] showed a group mean of 4.5 [Range 2.3 to 10.3].

#### Percent time spent in the low CGMS glucose range:

The mean percent time for CGMS reading <4mmol/L totalled 7.4% of the duration of the trace [Range 0-29.7%].

#### Percent time spent in the normal CGMS glucose range:

Mean percentage time spent in the CGMS range 4-12mmol/l was 53.8% of the total time [Range 29.1-89.1%].

#### Percent time spent in the high CGMS glucose range:

Mean percentage time spent in the CGMS glucose range >12mmol/l was 38.9% of the total time (Range 10.9-70.9%). The mean minutes per hour spent in each glycaemic range is stratified for time of day [morning, evening and night] in table 4.5.



**Table 4-5: Mean minutes per hour in glycaemic ranges, by time of day for CGMS run 2.**

	Mean minutes/hr (min, max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)
Time	Total	8am-4pm	4pm-12	12-8am
CGMS range (<4mmol/L)	4.6 (0, 24.5)	2.0 (0, 7.5)	3.0 (0, 16.3)	8.9 (0, 50.0)
CGMS range (4-12mmol/l)	31.5 (10.6, 50.2)	27.7 (5.6, 37.6)	29.4 (8.4, 53.4)	37.3 (51.1, 60.0)
CGMS range (>12mmol/l)	23.9 (4.3, 43.5)	30.3 (11.3, 49.2)	27.6 (1.2, 40.9)	13.9 (0, 40.2)

Continuous overall net glycaemic action:

The overall group mean for the CONGA was calculated for 1 hour (2.9 [range 1.9-4.2]), 2 hour ( 4.3 [ range 2.9 to 6.3]) and 4 hour ( 5.6 [range 3.3 to 8.5]) intervals.

**4.3.3 CGMS analysis at six months [Time = 2]**

Population:

Forty five children attended for the third run of the monitor.

**Table 4-6: Reasons for withdrawing from the study before the third occasion**

Study no.	Reason given for removal
7	Family decided not to proceed with study
22	Unable to attend due to commitments
34	Unable to attend due to commitments
44	Child failed to calibrate monitor again

#### Data Collection:

Patient 44 had now completed three unusable CGMS traces so was excluded from the remainder of the study. Patient 9 had a malfunction of the trace between day 1 and day 3 and was excluded from analysis on this occasion as less than 24 hours of continuous trace existed after data cleaning. This left 40 children available for analysis on this occasion. The mean total time per trace was 76.2 hours [range 50.5 to 99.8 hours] prior to data cleaning. The mean valid trace time [after data cleaning] was 73.8 hours [range 50.3 to 95.6 hours].

#### Clinical data:

The mean HbA1C was 8.8% [Range 7.2-10.3]. The mean BMI Z-score for the cohort on this occasion was 1.1 [Range -1.0 to 3.2]. The mean insulin dose per child on this occasion was 0.9iu/kg/hr [range 0.6 to 1.4]. Data for individual clinical and continuous glucose monitoring outcomes is summarised in Appendix 6.

#### Mean blood glucose:

Mean blood glucose was calculated for each individual continuous trace with a group mean of 11.6mmol/L [range 7.8 to 14.9mmol/l].

#### Mean of daily differences:

Inter-day variation, calculated by the Mean of Daily Differences [MODD] showed a group mean of 5.1 [range 2.3 to 8.1].

#### Percent time spent in the low CGMS glucose range:

The mean percent time for CGMS reading <4mmol/L totalled 4.7% of the duration of the trace [Range 0-17.4%].

Percent time spent in the normal CGMS glucose range:

Mean percentage time spent in the normal range was 50.5% of the total time [Range 23.3-91.3%],

Percent time spent in the high CGMS glucose range:

Mean percentage time spent in the high glucose range 44.8% [Range 7.0-76.7%]. The mean minutes per hour spent in each glycaemic range (low, normal and high) is stratified for time of day (morning, evening and night) in table 4.7.

**Table 4-7: Mean minutes per hour in glycaemic ranges, by time of day for CGMS run 3.**

	Mean minutes/hr (min, max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)
Time	Total	8am-4pm	4pm-12	12-8am
CGMS range (<4mmol/l)	2.8 (0, 16.1)	2.4 (0, 15.3)	1.7 (0, 7.9)	4.2 (0, 24.5)
CGMS range (4-12mmol/L)	30.1 ( 5.4, 50.2)	25.7 (10.6, 42.5)	28.6 (3.8, 50.7)	35.4 (7.5, 55.0)
CGMS range (>12mmol/l)	27.1 (2.9, 48.3)	31.9 (6.0, 49.2)	29.7 (4.4, 51.0)	20.4 (0.4, 42.3)



#### Continuous overall net glycaemic action:

Intra-day variation was calculated by continuous overall net glycaemic action analysis (CONGA). The overall group mean was calculated for 1 hour (2.8 [range 1.6-4.1]) 2 hour (4.3 [range 2.4 to 6.4]) and 4 hour (5.7 [range 3.1 to 8.2]) intervals.

#### **4.3.4 CGMS analysis at 9 months [Time = 3]**

##### Population:

Forty four children completed the final run of the monitor. Patient 7 decided to rejoin the study and all the other participants completed valid traces.

##### Data Collection:

The mean total time per trace was 76.1 hours (range 28.1-95.9) prior to data cleaning. The mean valid trace time (after data cleaning) was 73.2 hours (range 25.3-94.4 hours).

##### Clinical data:

The mean HbA1c was 8.5 [Range 6.4-10.1]. The mean insulin dose for this cohort on this occasion was 0.9iu/kg/min [Range 0.6 to 1.3]. The mean BMI Z-score was 1.1 [range -1.1 to 3.1]. Data for individual clinical and continuous glucose monitoring outcomes is summarised in Appendix 6.

##### Mean blood glucose:

Mean CGMS glucose was calculated for each individual trace with a group mean of 11.5mmol/L [Range 6.4-16.6mol/l].

##### Mean of daily differences:

Inter-day variation, calculated by the Mean of Daily Differences [MODD] had a group mean of 4.6 [range 1.3 to 7.7].

##### Percent time spent in the low CGMS glucose range:

The mean percent time for CGMS reading <4mmol/L totalled 7.9% of the duration of the trace [Range 0-28.5%].

Percent time spent in the normal CGMS glucose range:

Mean percentage time spent in the normal range was 47.2% of the total time [Range 2.6-82.1%].

Percent time spent in the high CGMS glucose range:

Mean percentage time spent in the high glucose range was 44.9% [Range 8.4-87.8%]. The mean minutes per hour spent in each glycaemic range [low, normal and high] is stratified for time of day [morning, evening and night] in table 4.8.

**Table 4-8: Mean minutes per hour in glycaemic ranges, by time of day for CGMS run 4.**

	Mean minutes/hr (min, max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)	Mean minutes/hr (min,max)
Time	Total	8am-4pm	4pm-12am	12am-8am
CGMS range (<4mmol/l)	4.7 (0, 23.1)	2.2 (0, 11.7)	3.0 (0, 16.3)	8.8 (0, 40.5)
CGMS range (4-12mmol/l)	28.6 (2.2, 41.3)	24.8 (4.0, 38.0)	27.6 (2.5, 40.0)	33.1 (0, 44.2)
CGMS range (>12mmol/l)	27.1 (6.3, 50.0)	33.0 (20.0, 45.9)	29.7 (1.0, 47.5)	19.1 (0, 42.7)

Continuous overall net glycaemic action:

Intra-day variation was calculated by continuous overall net glycaemic action analysis (CONGA). The overall group mean for CONGA was calculated at 1 hour (2.9 [range 1.8 to 4.0]), 2 hour (4.3 [range 2.4 to 6.4]) and 4 hour (5.6 [range 2.8 to 8.9]) intervals.

#### 4.3.5 Comparison of CGMS outcomes with metabolic control measured by HbA1c.

At each timepoint in the longitudinal study of the CGMS algorithm, the CGMS data was examined to ascertain whether any correlation existed between metabolic control reflected by HbA1c and values obtained from the CGMS algorithm. Bivariate regression analyses with HbA1c as the independent variable and each of the continuous glucose measures as the dependent variable are summarised in Table 4.9 for all four runs.

##### Baseline [Time =0]:

There were significant positive linear relationships between MBG and percent time spent in high CGMS range, and HbA1c, although in both cases the correlation coefficient was quite low. For the other continuous glucose measures, slope of the regression line was not significantly different from zero. Repeated regression analysis adjusted for age and sex showed similar relationships between the measures and HbA1c.

##### Three months [Time = 1]:

A positive association did exist between the percent time spent in the high CGMS range [ $>12\text{mmol/L}$ ] and HbA1c [ $p=0.01$ ] and between mean blood glucose and HbA1c [ $p=0.02$ ] on this occasion. Again, there were significant positive linear relationships between MBG and percent time spent in high CGMS range, and HbA1c, although in both cases the correlation coefficient remained low. For the other continuous glucose measures, the slope of the regression line was not significantly different from zero.

##### Six months [Time = 2]:

On this occasion, there were no significant positive linear relationships identified for mean glucose [ $p=0.27$ ] or time spent in the high glucose range [ $p=0.41$ ] and HbA1c, although in both cases the correlation coefficient was quite low. For the other continuous glucose measures, slope of the regression line was again not significantly different from zero.



### Nine months [Time = 3]:

On this occasion, the significant positive linear relationships were identified once more between mean glucose and time spent in the low [ $p=0.006$ ], normal [ $p=0.01$ ] and high [ $p<0.001$ ] glucose ranges and HbA1c, with higher correlation coefficients than reported for the other runs. For the other continuous glucose measures, slope of the regression line was again not significantly different from zero.

Table 4.9 summarises the relationship between all the glycaemic outcomes and HbA1c at each of the four individual timepoints. As summarised above positive correlation was mostly seen between both mean blood glucose and percentage time spent in the high glycaemic range and HbA1c. Negative correlations were seen between percentage time spent in the low or normal glycaemic ranges and HbA1c. No consistent relationship was demonstrated between either measure of glycaemic variation [CONGA or MODD] and HbA1c. The ongoing relationship between HbA1c and glycaemic outcomes over the twelve months is summarised in Section 4.4.3. and Table 4.10.

Table 4-9: Relationship between HbA1c and outcomes of continuous glucose monitoring

Continuous glucose monitor outcome	CGMS monitor run	Regression coefficient	95% Confidence Interval	P value	Correlation coefficient
Mean blood glucose (MBG)	0	0.8	(0.2,1.4)	0.01	0.35
	1	0.8	(0.1, 1.4)	0.02	0.36
	2	0.4	(-0.3,1.1)	0.27	0.18
	3	1.5	(0.8, 2.2)	0.00	0.56
Mean of daily differences (MODD)	0	-0.2	(-0.9,0.5)	0.59	0.08
	1	-0.5	(-0.6, 0.5)	0.86	-0.03
	2	-0.1	(-0.8, 0.5)	0.72	-0.06
	3	0.02	(-0.6, 0.6)	0.93	0.01
Percent time spent in low CGMS range	0	-2.1	(-4.8,0.5)	0.12	-0.22
	1	-2.4	(-5.7, 0.9)	0.15	-0.23
	2	0.3	(-2.0, 2.6)	0.78	0.05
	3	-4.3	(-7.4, -1.3)	0.006	-0.42
Percent time spent in normal CGMS range	0	-4.3	(-8.8,0.2)	0.07	-0.24
	1	-4.9	(-10.0, 0.2)	0.06	-0.3
	2	-3.2	(-10.2, 3.9)	0.37	-0.2
	3	-7.8	(-13.7, -1.7)	0.01	-0.38
Percent time spent in high CGMS range	0	6.4	(1.5,11.3)	0.01	0.34
	1	7.3	(2.1, 12.6)	0.008	0.41
	2	2.8	(-4.0, 9.7)	0.41	0.13
	3	12.1	(5.7, 18.5)	<0.001	0.52
Continuous overall net glucose action measured at 1hr intervals (CONGA1)	0	0.1	(0.0,0.3)	0.07	0.26
	1	0.1	(-0.2, 0.3)	0.51	0.11
	2	0.1	(-0.1, 0.4)	0.31	0.16
	3	-0.1	(-0.4, 0.1)	0.33	-0.15
Continuous overall net glucose action measured at 2hr intervals (CONGA2)	0	0.3	(0.0,0.5)	0.05	0.28
	1	0.1	(-0.3, 0.4)	0.71	0.06
	2	0.2	(-0.2, 0.6)	0.26	0.18
	3	-0.2	(-0.6, 0.3)	0.41	-0.13
Continuous overall net glucose action measured at 4hr intervals (CONGA4)	0	0.3	(0.0,0.7)	0.06	0.27
	1	-0.01	(-0.5,0.5)	0.95	0.01
	2	0.4	(-0.2, 0.9)	0.22	0.2
	3	-0.1	(-0.7, 0.6)	0.85	-0.03

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## 4.4 Long term impact of continuous glucose monitoring

The third aim was to compare continuous glucose monitoring between baseline and twelve months to ascertain whether repeated CGMS resulted in improved glycaemic outcomes.

### 4.4.1 Demographic data

Thirty eight children were included in this aspect of the study having completed all four runs of the study. The study group was comparable to the non-participating eligible clinic population when matched for age (8.2years vs. 7.7years,  $p=0.16$ ) and HbA1c (8.1% vs. 8.1%,  $p=0.55$ ).

The summary data from all four runs is summarised in Appendix 6.

Longitudinal plots were constructed for each glycaemic outcomes of the CGMS trace over time and are summarized in Figures 4.2 - 4.12. The results over the twelve month period are displayed in this series of spaghetti plots which graphically show the trend for each variable. The slope for each variable was then calculated from the raw data and paired student t-tests were used to calculate whether differences between variables from the start to the end of the project were significant.

### 4.4.2 Change in independent variables over the twelve month period of the study

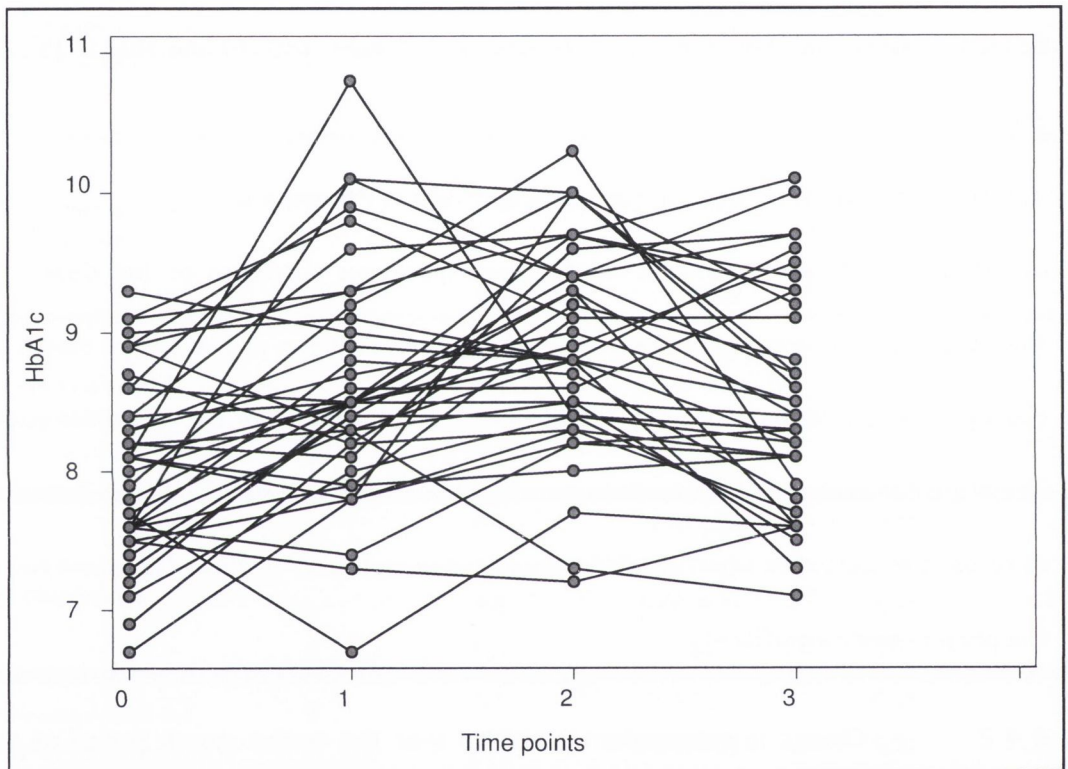
#### HbA1c:

A significant rise in group mean HbA1c over the 12 month period (figure 4.3) was noted within the study group (8.1 vs. 8.5,  $p=0.01$ ). To ascertain whether this change in HbA1c was unique to the study group or reflective of changes in the population, a comparison was made to the non-participant eligible population. This showed a non-significant but similar rise in HbA1c in the eligible population not included in the study over the duration of the project (8.1 vs. 8.4,  $p=0.13$ ). On closer examination there was a significant change noted in the mean HbA1c



between each visit. From time 0-1 HbA1c increased from 8.1-8.6% ( $p=0.0002$ ), from time 1-2 there was a further increase from 8.6-8.9% ( $p=0.03$ ) while in the last three months there was an improvement in HbA1c with a decrease from 8.9-8.5% ( $p=0.002$ ).

Figure 4-3: Change in HbA1c (%) of cohort at quarterly intervals.

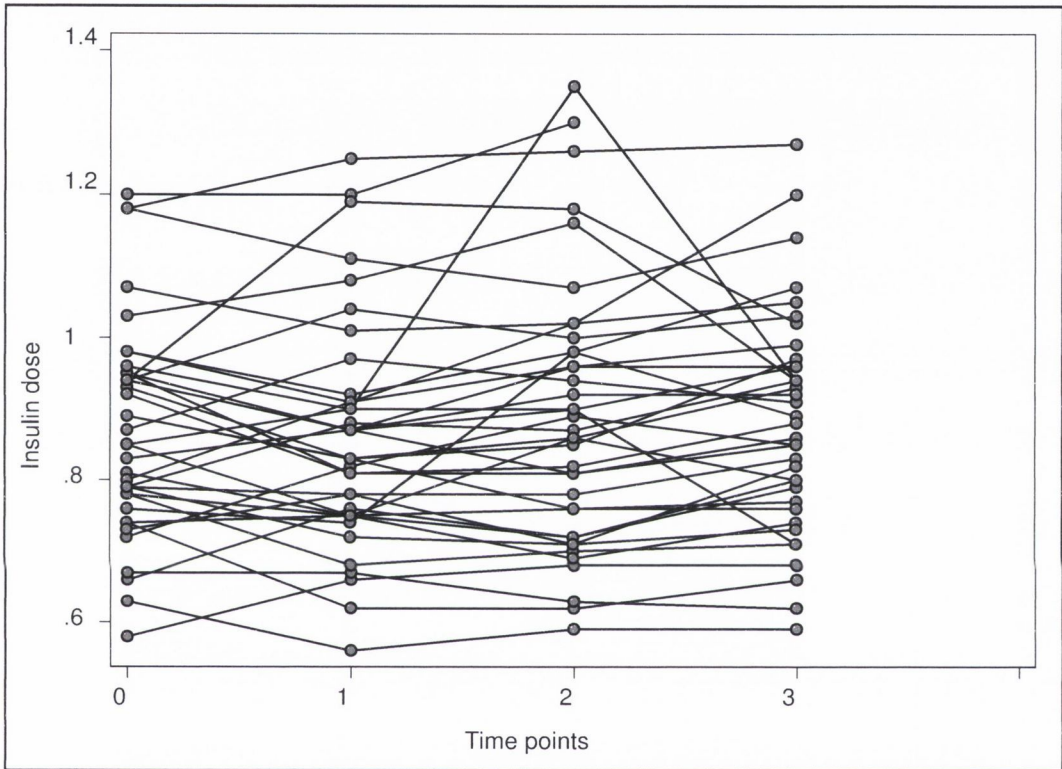


Over the four timepoints from baseline to twelve months the HbA1c shows a steady increase. This suggests that the findings from the CGMS traces did impact on metabolic control. Further analysis of the relationship between HbA1c and glycaemic outcomes is carried out in section 4.3.

Insulin dosage:

The insulin dosage for the group was recorded at each clinic visit over the twelve month period (figure 4.13). The mean insulin doses at 0, 3, 6 and 9 months were 0.87, 0.86, 0.89 and 0.89 u/kg/day respectively. There was no significant change in mean insulin dosage requirements over the twelve months ( $p=0.09$ ).

**Figure 4-4: Change in insulin dose (iu/kg/min) of cohort at quarterly intervals.**

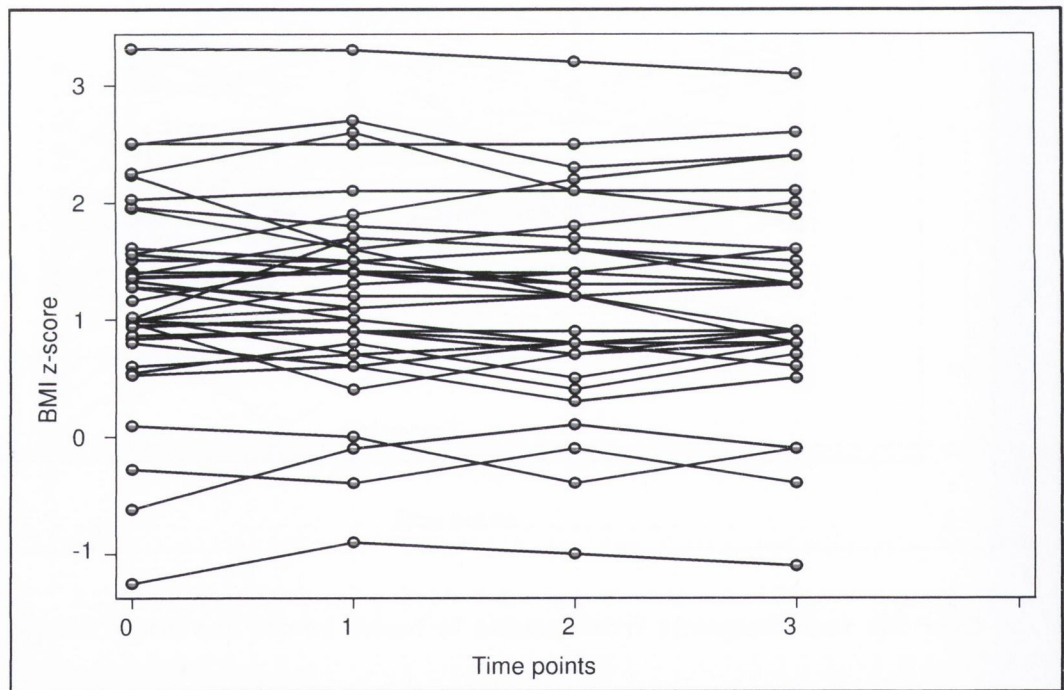


Over the four timepoints from baseline to twelve months the insulin dosage shows a steady state with minimal changes over the prescribed time. This indicates that the findings from the CGMS traces did not significantly impact on total insulin dose. This will be examined in more detail in section 4.5.

**BMI Z-score:**

BMI z-score was calculated based on height and weight measurements taken at each clinic visit (figure 4.5). The mean BMI z scores at 0, 3, 6 and 9 months were 1.1, 1.2, 1.2 and 1.1 respectively. There was no significant difference over the twelve month period ( $p=0.88$ ).

**Figure 4-5: Change in BMI z-score of cohort at quarterly intervals.**



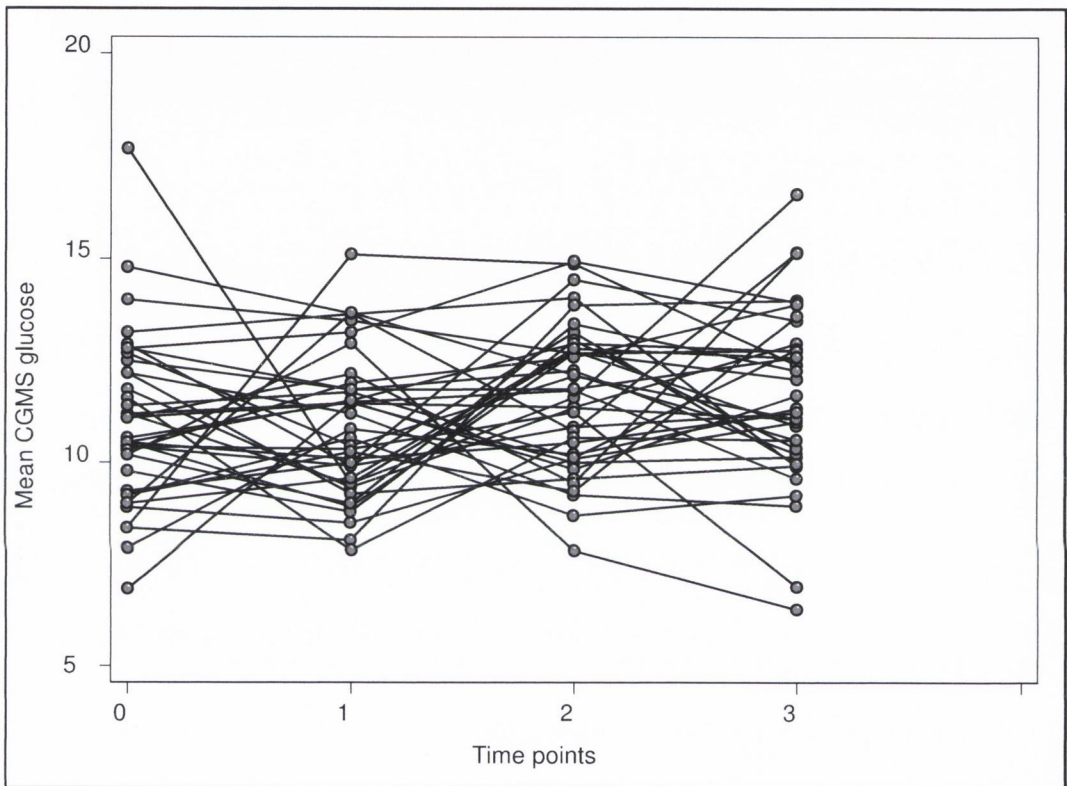


#### 4.4.3 Change in glycaemic variables over the twelve months

##### Mean CGMS glucose:

The mean CGMS glucose values at 0, 3, 6 and 9 months were 11.0, 10.9, 11.6 and 11.6 mmol/L respectively. There was no significant difference for mean blood glucose ( $p=0.08$ ) between the start and end of the study (figure 4.6).

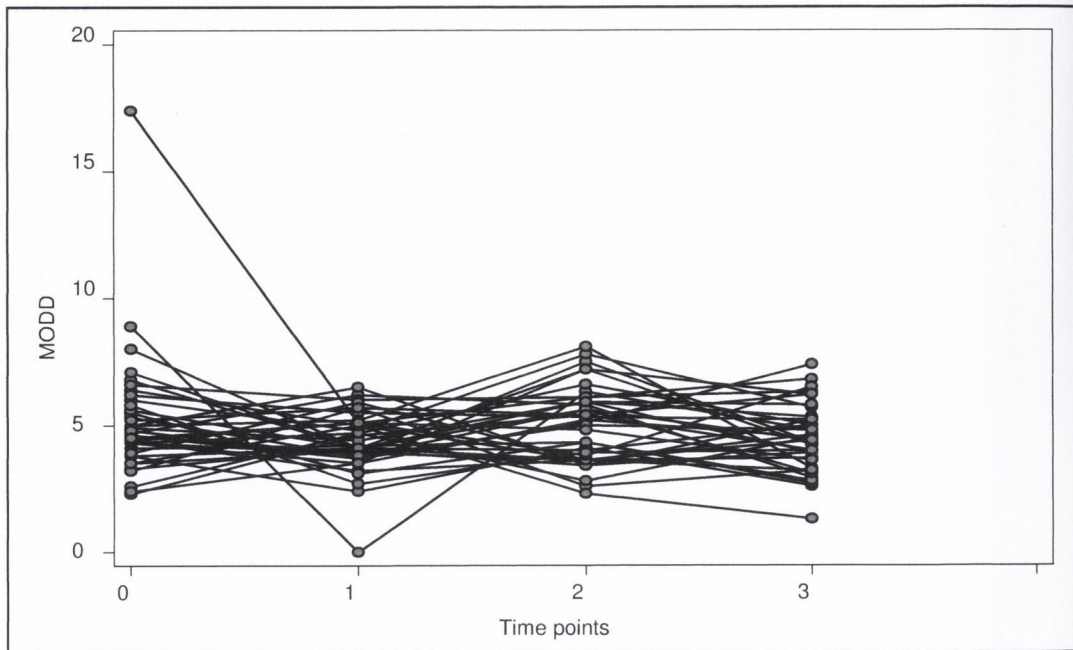
**Figure 4-6:** Change in mean blood glucose [mmol/L] of cohort at quarterly intervals.



##### Mean of daily differences:

The mean of daily differences at 0, 3, 6 and 9 months was 5.2, 4.5, 5.0 and 4.4 respectively. There was no significant difference between the start and end of the study ( $p=0.79$ ) for mean of daily differences (figure 4.7). The outlier at time 0 had erratic control over the three days of the study that was verified by at least four blood sugars on each day.

**Figure 4-7:** Change in mean of daily differences of cohort at quarterly intervals.



Percent time in low CGMS range

The mean percent time spent in the CGMS range <4.0mmol/L at 0, 3, 6 and 9 months were 9.9, 7.1, 4.9 and 7.8% respectively. There was no significant difference between the start and end of the study ( $p=0.46$ ) regarding percent time in the low CGMS range (figure 4.8).

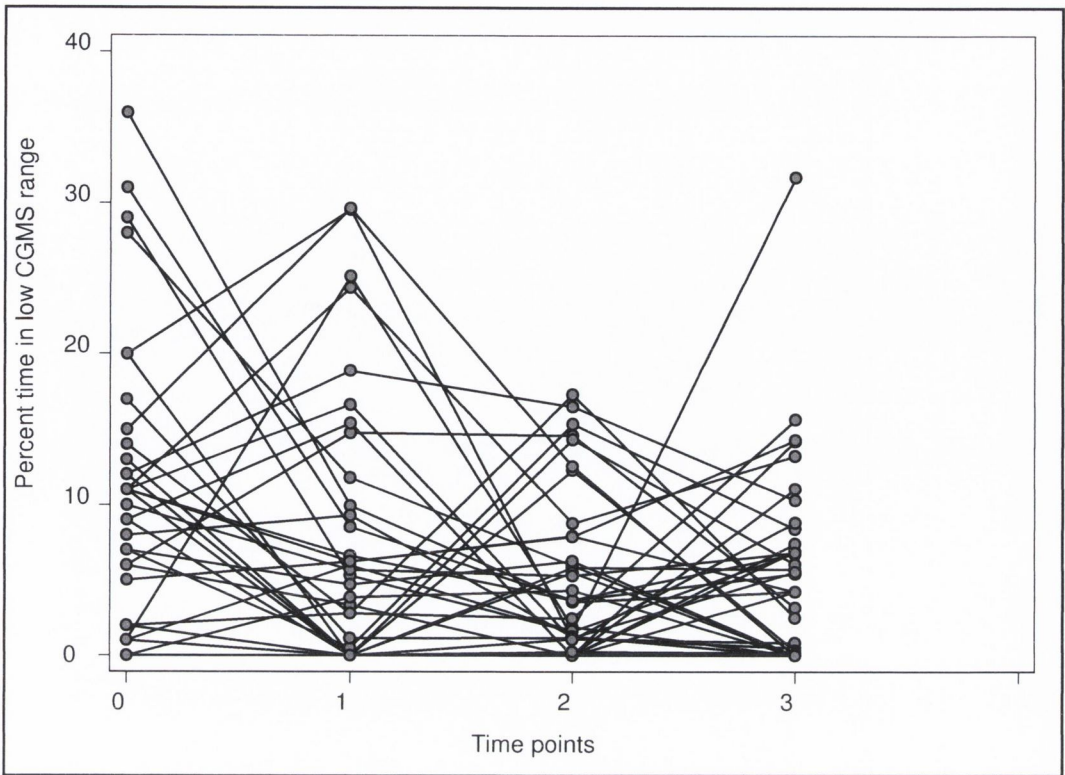
Percent time in normal CGMS range

The mean percent time spent in the CGMS range 4.0 - 12mmol/L at 0, 3, 6 and 9 months were 50.1, 53.4, 50.6 and 46.6% respectively. There was no significant difference between the start and end of the study ( $p=0.13$ ) for percent time spent in the normal range (figure 4.9).

Percent time in high CGMS range

The mean percent time spent in the CGMS range >12mmol/L at 0, 3, 6 and 9 months were 40.0, 39.5, 44.7 and 46.3% respectively. There was a significant increase ( $p=0.05$ ) in percent time spent in the high glycaemic range over the twelve months of the study (figure 4.10).

**Figure 4-8:** Change in time spent in the low CGMS range in the cohort over quarterly intervals.



**Figure 4-9:** Change in time spent in the normal CGMS range over quarterly intervals.

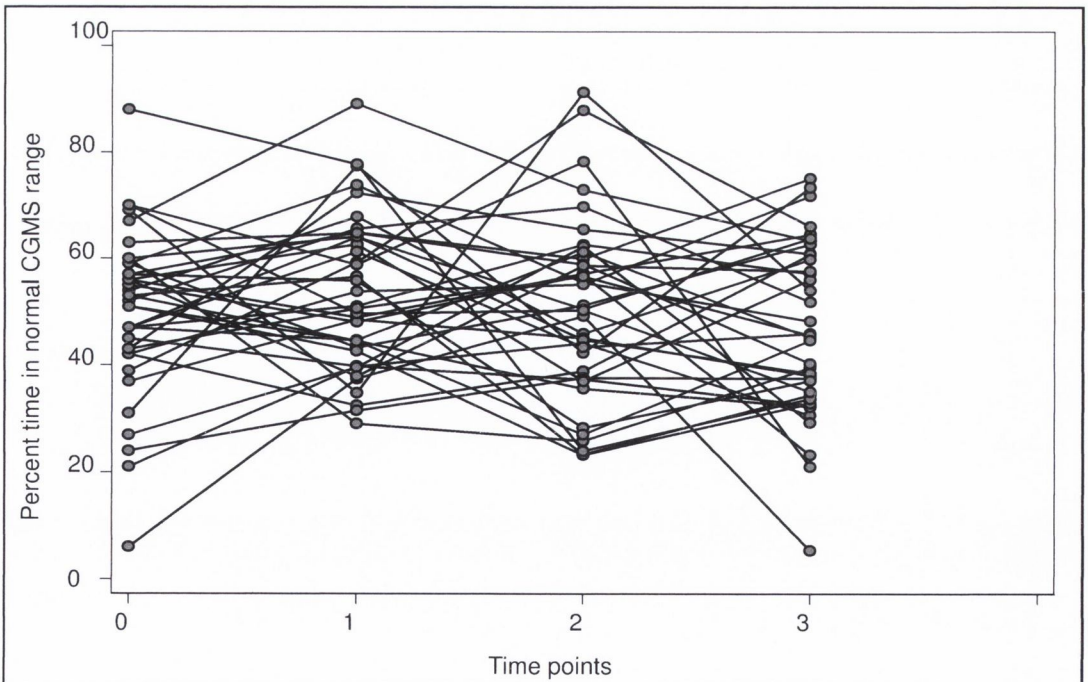
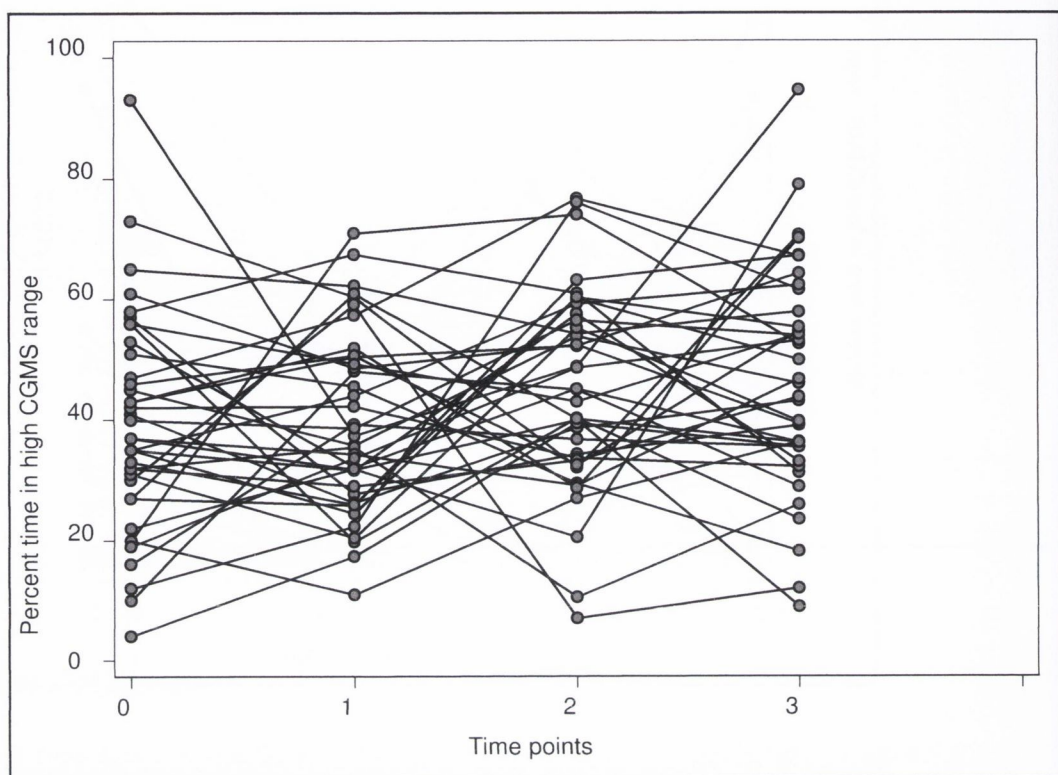




Figure 4-10: Change in time spent in the high CGMS range in the cohort over quarterly intervals.



### CONGA1

The mean for CONGA1 at 0, 3, 6 and 9 months was 3.3, 2.9, 2.9 and 2.9 respectively. There was a consistent and significant decrease in CONGA1 ( $p=0.03$ ) for the group over the twelve months of the study suggesting a reduction in glycaemic variation over this time period (figure 4.11).

### CONGA2

The mean for CONGA2 at 0, 3, 6 and 9 months was 4.7, 4.3, 4.3 and 4.3 respectively. There was a significant decrease in CONGA2 ( $p=0.05$ ) for the group over the twelve months of the study suggesting a reduction in glycaemic variation over this time period (figure 4.12).

Figure 4-11: Change in CONGA1 in the cohort over quarterly intervals.

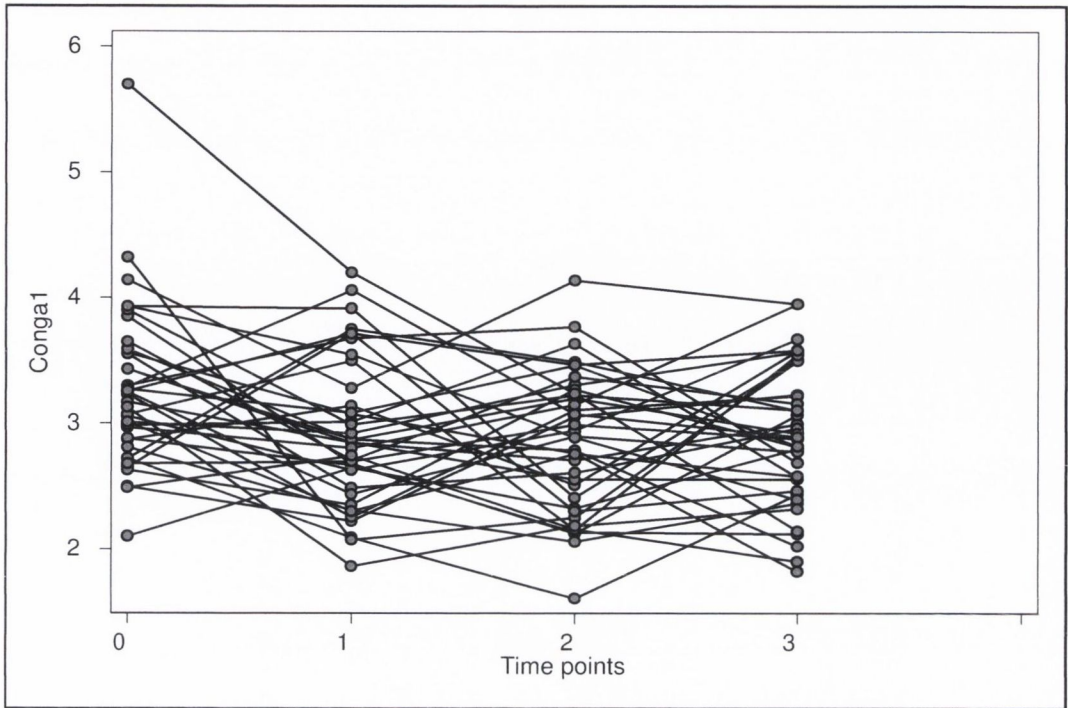
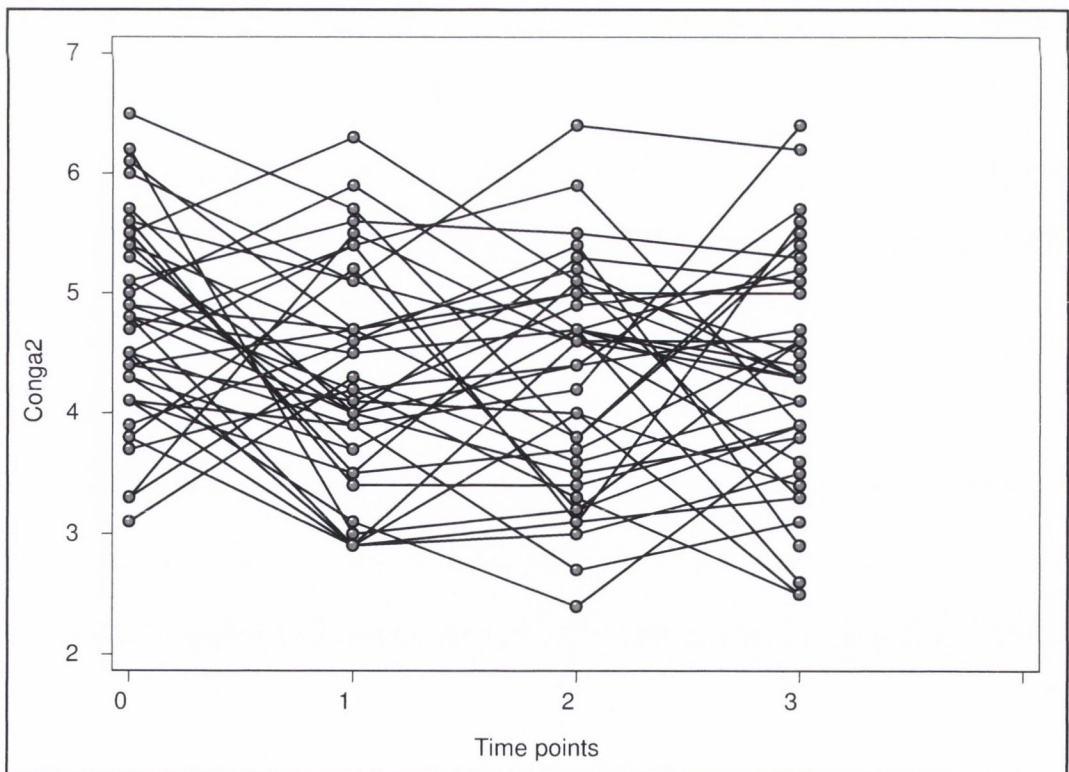


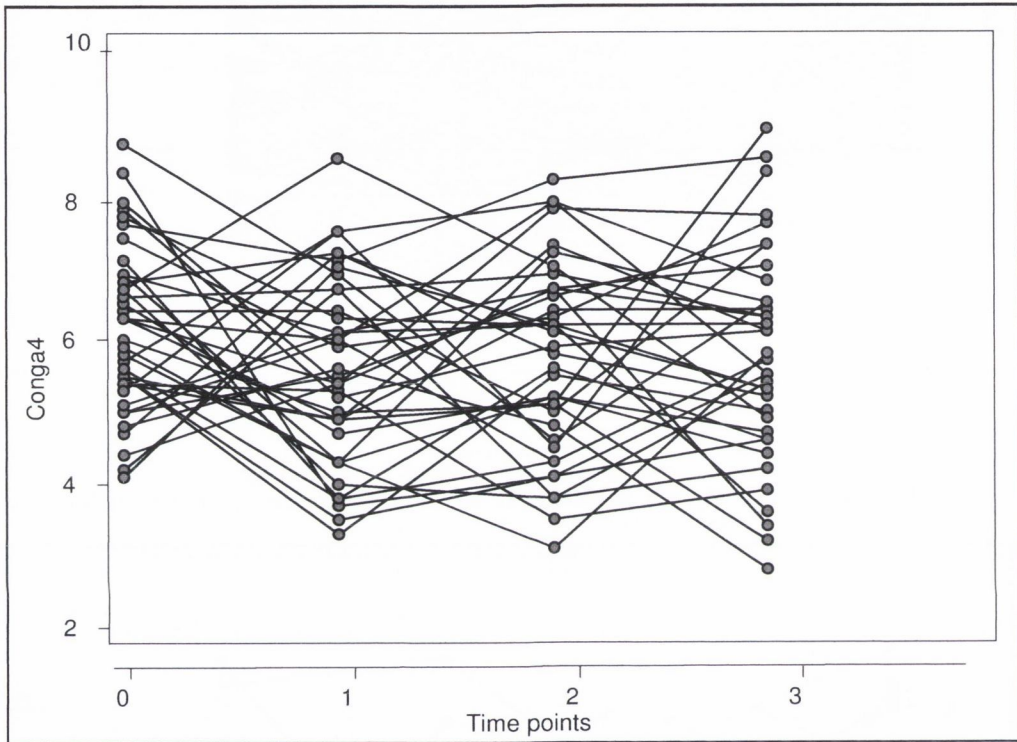
Figure 4-12: Change in CONGA2 in the cohort over quarterly intervals.



CONGA4

The mean for CONGA4 at 0, 3, 6 and 9 months was 6.1, 5.6, 5.7 and 5.7 respectively. There was a significant decrease in CONGA4 ( $p=0.03$ ) for the group over the twelve months of the study suggesting a reduction in glycaemic variation over this time period (figure 4.13).

Figure 4-13: Change in CONGA4 in the cohort over quarterly intervals.



Using the four point scales for each of the clinical and continuous glucose monitoring outcomes, linear regression over time was carried out to see if an ongoing relationship could be identified over the twelve month period. The results of this analysis are summarised in table 4.10. The data for each of the clinical and continuous glucose monitoring outcomes over the twelve month period for the eligible children are summarised in Appendix 6.



**Table 4-10: Summary of overall linear regression analysis between HbA1c vs. and CGMS outcomes.**

Continuous glucose monitor outcome	Regression coefficient	95% Confidence Interval	P value	Correlation coefficient
Mean blood glucose (MBG)	20.9	(-56.4, 98.3)	0.59	0.09
Mean of daily differences (MODD)	0.05	(-0.8, 0.9)	0.91	0.02
Percent time spent in low CGMS range	-6.5	(-10.5, -2.6)	0.002	-0.49
Percent time spent in normal CGMS range	-9.6	(-16.9, -2.3)	0.01	-0.41
Percent time spent in high CGMS range	16.9	(9.9, 23.9)	0.000	0.63
Continuous overall net glucose action measured at 1hr intervals (CONGA1)	-0.18	(-0.5, 0.1)	0.25	-0.19
Continuous overall net glucose action measured at 2hr intervals (CONGA2)	-0.1	(-0.5, 0.3)	0.59	-0.09
Continuous overall net glucose action measured at 4hr intervals (CONGA4)	-0.1	(-0.6, 0.5)	0.87	-0.03

No association was seen on comparison of the insulin dose or bmi z-score over the study period with the continuous glucose outcomes.

#### Glycaemic variables and HbA1c:

Mean blood glucose: A positive correlation was seen between the change in mean blood glucose and final HbA1c (0.355, 95% C.I. 0.07 to 0.59).

Time spent in the low CGMS range: A negative trend was reflected in the percent time in the low CGMS range and final HbA1c (-0.232, 95% C.I. -0.5 to 0.07).

Time spent in the high CGMS range: A positive correlation between change in percent time in the high CGMS range and final HbA1c (0.349, 95% C.I. 0.06 to 0.59) was also evident.

No changes in HbA1c were noted for the percentage of normal CGMS readings, intra (CONGA) or inter (MODD) day variation.

#### Hypoglycaemia and CGMS:

The mean percent time spent in the low CGMS range decreased from 8.8% on the first run to 7.9% in the final run with a corresponding reduction in percent time spent in the low CGMS range overnight (12midnight to 8am) from 5.4% on the first run to 5% on the final run.

Twenty six children demonstrated a reduction in percentage low CGMS readings over the 12 month period compared to the eighteen children whose percentage time in the low CGMS range increased with a corresponding difference in mean HbA1c (8.7 vs. 8.2,  $p=0.05$ ).

#### Metabolic control and CGMS:

A sub analysis was carried out comparing HbA1c in those whose glycaemic outcomes had improved against those who had deteriorated. A significant difference in HbA1c was noted in the seventeen children whose mean blood glucose improved over the 12 month period compared to the twenty seven children whose mean blood glucose deteriorated (Mean HbA1c 8.2 vs. 8.7,  $p=0.05$ ).

#### Glycaemic variables and BMI z score:

No correlation was found between the percent time spent in the low or high CGMS ranges and BMI z-score (0.03, 95% C.I. -0.268 to 0.325).

To ascertain whether a higher frequency of hypoglycaemia was associated with an increasing BMI z-score, the group were subdivided into those whose BMI z-score increased over the year against those whose BMI z-score decreased. Each group was compared using their change in percent time in the low CGMS range but no difference was found (p=0.7).

A negative correlation was however noted between the change in CONGA over the twelve month period and final BMI z-score (-0.337, 95% C.I. -0.58 to -0.04) indicating that as the glycaemic variation increased the BMI z-score fell.

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## **4.5 The relationship between Insulin regimen and CGMS**

The fourth aim of the study was to ascertain whether the insulin regimen used by children within the study cohort impacted on their glycaemic outcomes measured by CGMS. Valid data was obtained and pooled from 165 traces carried out over the time frame of the study from 49 of the 52 participants. Three children were excluded as two of the children switched to multiple dose injections during the course of the study while the third child [patient 44] failed to obtain a valid CGMS trace after three attempts as previously mentioned. Mean time per CGMS trace was 76.3±15.0 hours prior to data validation and 72.3±16.4 hours after data cleaning.

### **4.5.1 Demographic data**

Mean age of participants was 8.2 years [range 4.3-10.3]. Mean duration of diabetes was 3.5 years (range 2.1-7.2). Mean HbA1c was 8.1% [range 6.4- 11.4].



#### 4.5.2 Insulin regimens

Insulin was administered in twice daily combinations of intermediate and short acting insulins. The total daily insulin dose of the study group ranged from 0.6 to 1.3 unit/kg/day. Within the total daily dose the mean intermediate acting insulin was 73% [range 44-91%] while the mean short acting insulin was 27% [range 9-41%]. The mean morning to evening insulin dosage was 69% [range 50-90%] to 31% [range 10-50%] of the total insulin dose. Morning insulin consisted of a mean of 74% [range 60-93%] intermediate acting insulin combined with a mean of 26% [range 7-40%] short acting insulin. A similar pattern was seen in evening injections with a mean of 73% intermediate acting [range 0-100%] insulin given in combination with a mean of 27% short acting insulin [range 0-50%]. Short acting insulin refers to regular insulin which was given in 73% of patients in the morning and 32% of patients in the evening or insulin analogues which were given to 27% of patients in the morning and 68% of patients in the evening. No long acting insulin analogues were in use in this cohort at the time of this study. Insulin dosages are further summarised in Table 4.11.

**Table 4-11: Summary of insulin regimens [n=49]**

Variable	Mean $\pm$ sd	Range
Total insulin dose (units)	29.1 $\pm$ 9.4	12.5-60
Insulin dose to weight (units/kg/day)	0.88 $\pm$ 0.16	0.56-1.3
Total intermediate acting insulin (units)	21.3 $\pm$ 7.0	9-40
Total short acting insulin (units)	7.8 $\pm$ 3.4	2-20
Total morning insulin dose (units)	20.1 $\pm$ 7.0	9-40
Total evening insulin dose (units)	9.0 $\pm$ 3.6	2-20

### 4.5.3 Metabolic and Glycaemic outcomes

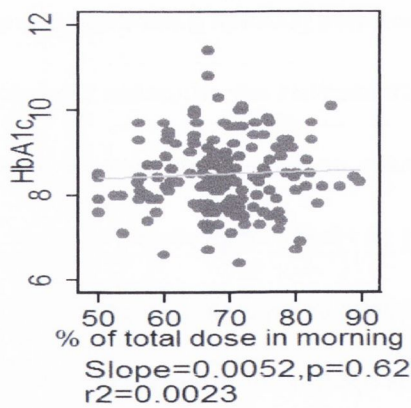
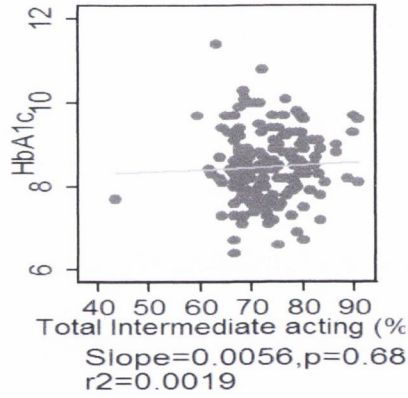
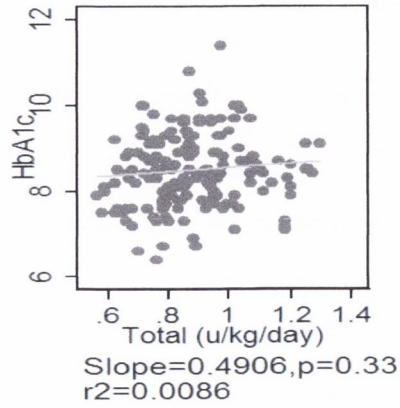
Metabolic control was summarised using mean HbA1c. The glycaemic outcomes were summarised according to the novel CGMS algorithm [section 4.2] in Table 4.12.

**Table 4-12: Summary of glycaemic outcomes [n=49]**

Variable	Mean $\pm$ sd	Range
HbA1c	8.5+0.9	6.4-11.4
Mean blood glucose (mmol/L)	11.3 $\pm$ 2.0	6.4-17.7
Mean of daily differences	4.8 $\pm$ 1.7	1.3-17.4
Percent time spent in low (<4mmol/L) CGMS range (%)	7.1 $\pm$ 7.9	0.0-36.0
Percent time spent in normal (4-12mmol/L) CGMS range (%)	50.5 $\pm$ 15.3	5.4-91.3
Percent time spent in high (>12mmol/L) CGMS range (%)	42.4 $\pm$ 16.7	7.0-94.6
CONGA 1hour	3.0 $\pm$ 0.6	1.6-5.7
CONGA 2hour	4.4 $\pm$ 0.9	2.4-6.5
CONGA 4hour	5.8 $\pm$ 1.3	2.8-8.7

The CGMS data was sub-analysed for comparison to either total daily insulin dose, total intermediate acting: short acting ratio insulin and total morning: evening dose. Metabolic [HbA1c] and glycaemic outcomes [mean CGMS glucose, percent time in various glycaemic ranges, inter-day variation [MODD] and intra-day variation [CONGA]] were correlated against insulin status. Linear regression analysis did not demonstrate that any adjustment in total insulin dose, balance between intermediate and short acting insulin, or distribution of dose between morning and evening dose resulted in a corresponding effect on HbA1c or glycaemic variation. Figures 4.14 - 4.20 illustrate the relationship between the metabolic or glycaemic outcomes and aspects of the insulin regimen namely total daily dose of insulin, proportion of intermediate acting insulin and the percentage of insulin given in the morning. This aspect of the study has been published during the course of this study [Appendix 7].

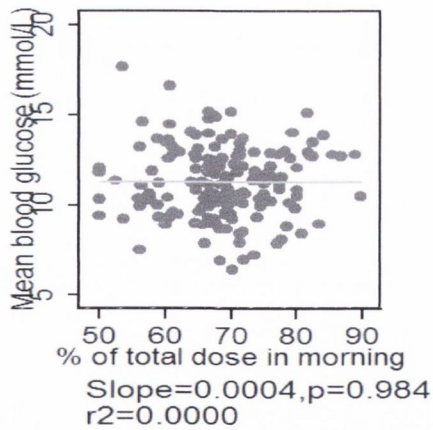
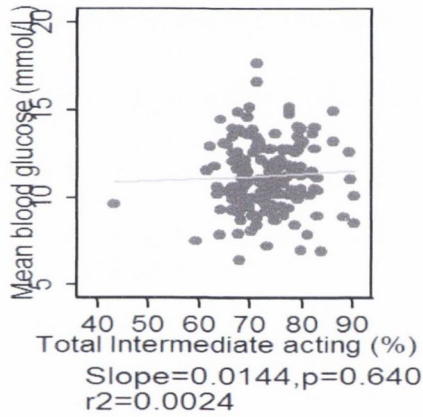
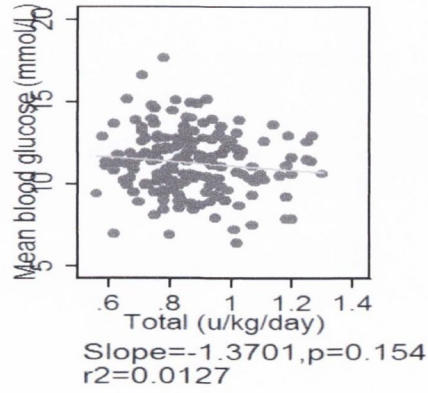
Figure 4-14: Linear regression analysis comparing HbA1c with total insulin dosage, total intermediate acting insulin and percentage of total insulin given in morning dose.



No relationship was shown between any aspect of the insulin regimen and metabolic control [HbA1c].

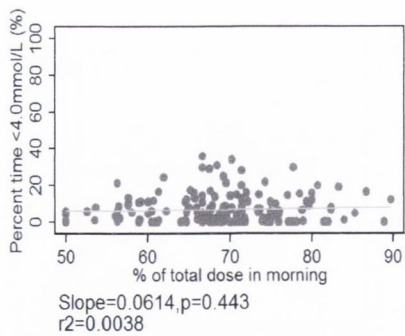
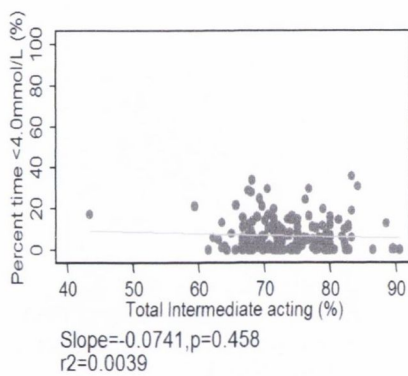
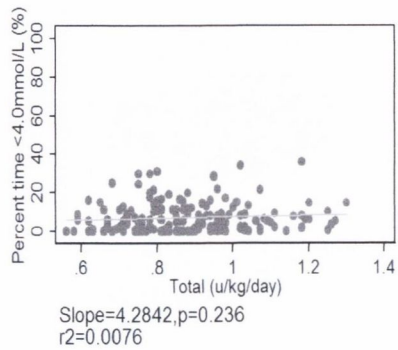


Figure 4-15: Linear regression analysis comparing mean blood glucose with total insulin dosage, total intermediate acting insulin and percentage of total insulin given in morning dose.



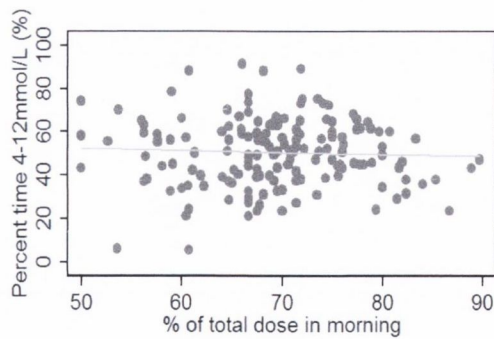
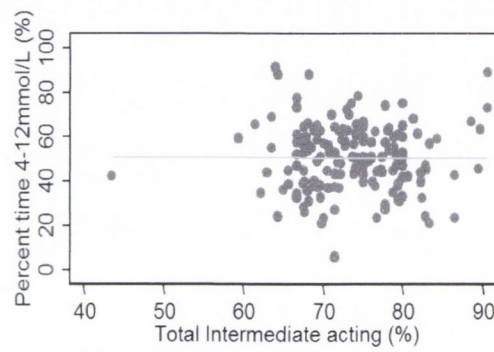
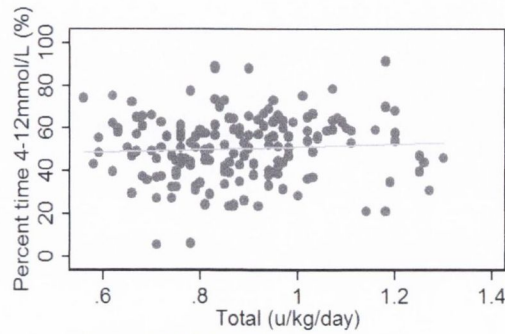
No relationship was shown between aspects of the insulin regimen and mean blood glucose as evidenced by the flat slope, low regression coefficient and p values that are non significant.

Figure 4-16: Linear regression analysis comparing percent time <4.0mmol/L with total insulin dosage, total intermediate-acting insulin and percentage of total insulin given in morning dose.



No relationship was shown between any aspect of the insulin regimen and percent time in CGMS range <4.0mmol/L showing no direct link between insulin proportions and hypoglycaemia on a CGMS trace.

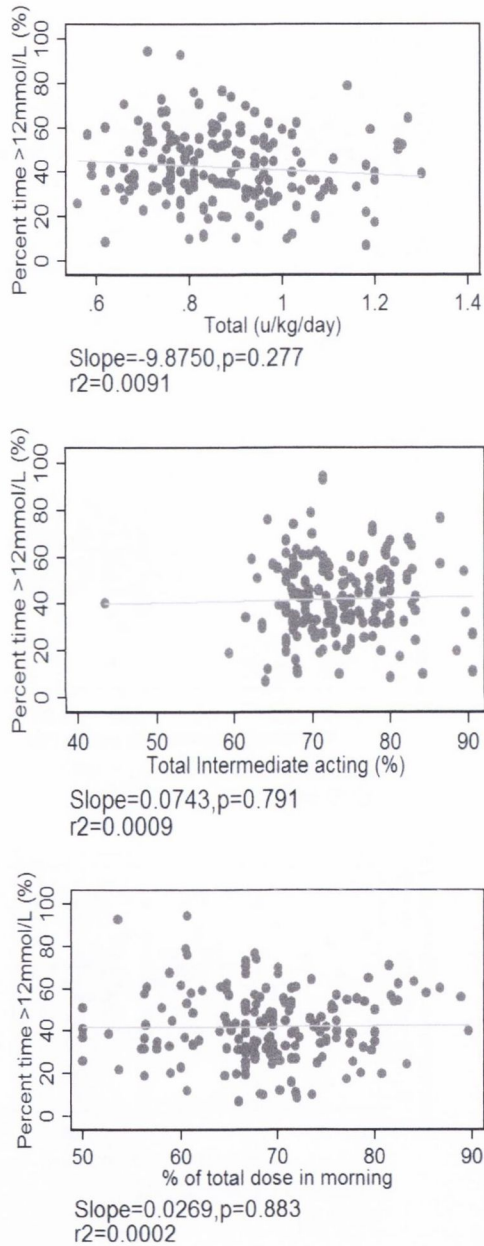
Figure 4-17: Linear regression analysis comparing percent time 4.0 - 12.0 mmol/L with total insulin dosage, total intermediate acting insulin and percentage of total insulin given in morning dose.



No relationship was shown between any aspect of the insulin regimen and percent time in CGMS range 4.0 - 12mmol/L showing no direct link between insulin proportions and normoglycaemia on a CGMS trace.



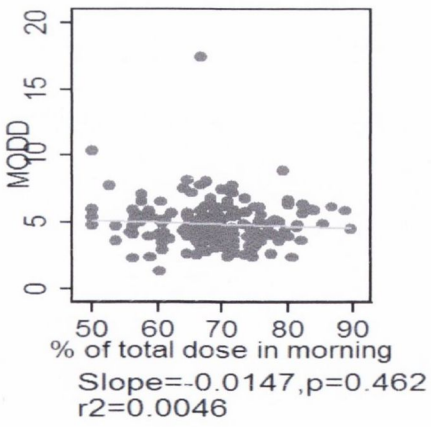
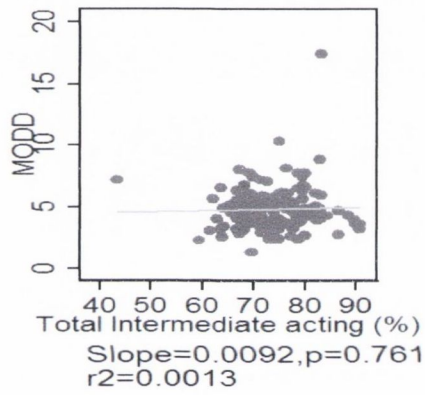
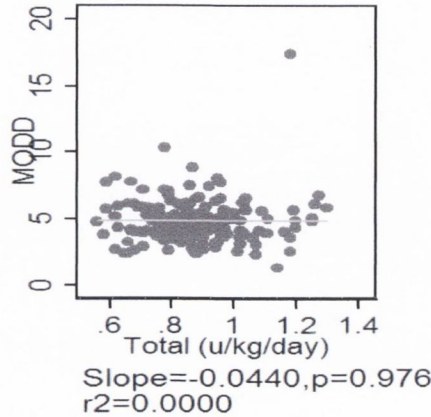
Figure 4-18: Linear regression analysis comparing percent time >12.0 mmol/L with total insulin dosage, total intermediate acting insulin and percentage of total insulin given in morning dose.



No relationship was shown between the insulin regimen and percent time in CGMS range >12mmol/L indicating no link between insulin proportions and hyperglycaemia on a CGMS trace.

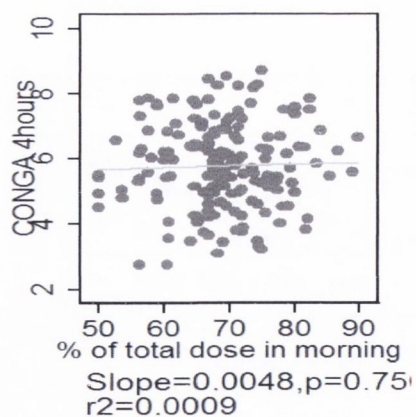
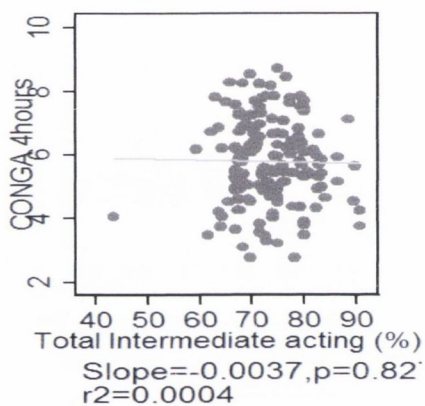
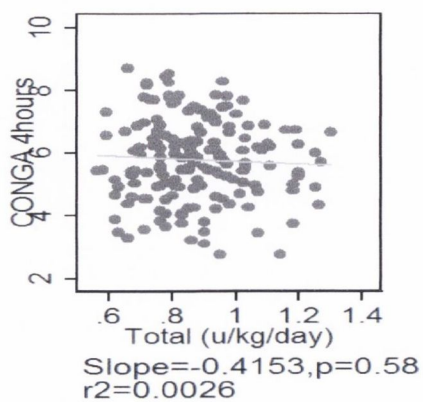
Figure 4-19: Linear regression analysis comparing mean of daily differences [MODD] with

total insulin dosage, total intermediate acting insulin and percentage of total insulin given in morning dose.



No relationship was shown between any aspect of the insulin regimen and interday glycaemic variation calculated by the mean of daily differences [MODD].

Figure 4-20: Linear regression analysis comparing CONGA at 4 hours with total insulin dosage, total intermediate acting insulin and percentage of total insulin given in morning dose.



No relationship was shown between any aspect of the insulin regimen and any interval of intraday glycaemic variation including CONGA1, CONGA2 and CONGA4 [illustrated in figure 4.20].



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## 4.6 Behavioural assessment and outcomes measured by CGMS

The fifth aim of the study was to ascertain whether types of behaviour reflect glycaemic outcomes measured by CGMS analysis. Parents were asked to complete the Behavioural Assessment system for children [BASC], a standardized, validated, parent-report instrument reflecting the child's typical behaviour over the previous six months. All patients completed assessment according to the protocol with the behavioural questionnaire applied at time points 1 [three months] and 3 [six months]. Data from both time-points were pooled providing 84 glycaemic and behavioural paired data sets.

### 4.6.1 Demographic data

Forty-two children aged 5-10 years (27 female, 15 male) completed this component of the study, and were representative of the total clinic population for age (mean 8.0 years vs. 7.6 years,  $p=0.25$ ) and HbA1c (8.2 vs. 8.1,  $p=0.60$ ). Forty patients received insulin in a twice daily mixing regime, two patients received insulin in a 3-4 injection regime. The mean number of valid hours per CGMS trace was 73.9 hours. Glycaemic and behavioural data were normally distributed.

**Table 4-13: Comparison of demographic data between the two time points of the study at 3 months [time=1] and 9 months [time =3].**

	Time = 1 [sd]	Time =3 [sd]
Number of patients	42	42
Age [years]	8.3 [1.4]	8.9 [1.4]
Males: females	15:27	15:27
Total daily insulin dose [u/kg/day]	0.87 [0.2]	0.9 [0.2]
HbA1C [%]	8.6 [0.8]	8.5 [0.8]

#### 4.6.2 Behavioural data

Childhood behaviour was characterised into externalizing or internalizing behaviour. Externalizing behaviour comprises hyperactivity, aggression and conduct scores, whereas internalizing behaviour comprises anxiety, depression and somatization scores. High scores indicate greater psychopathology. Maximum score is 100.

Externalising behaviour scores: The mean externalising behaviour *T*-score was  $48.3 \pm 10.3$ . There was a high correlation between intra-individual externalising behaviour scores at the time points studied [ $r=0.88, p<0.001$ ] see table 4.13 and figure 4.10.

Internalising behaviour scores:

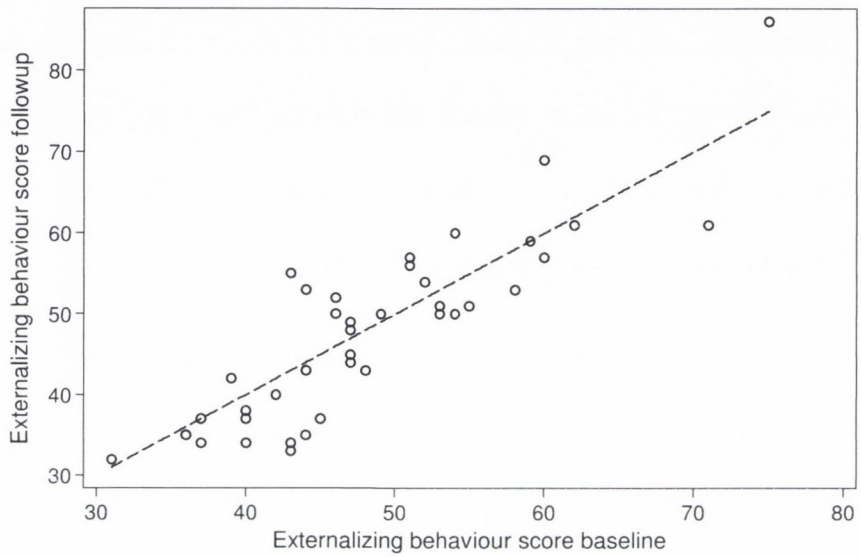
The mean internalising behaviour *T*-score was  $53.5 \pm 13.1$ . There was a high correlation between intra-individual internalising behaviour scores across the two time points studied [ $r=0.81, p<0.001$ ] see table 4.14 and figure 4.21.

**Table 4-14: Comparison of behavioural data between the two time points of the study at 3 months [time=1] and 9 months [time =3].**

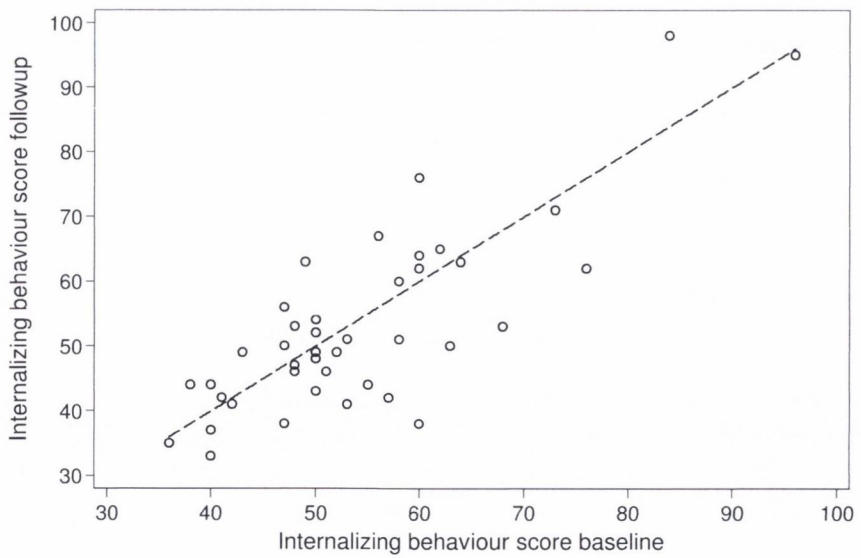
Behavioural variables	Time = 1 [sd]	Time = 3 [sd]
Number of patients	42	42
Externalising behaviour score	48.6 [9.3]	48.1 [11.3]
Internalising behaviour score	54.2 [12.2]	52.9 [14.0]

Figure 4-21: Intra-individual association of behavioural measures between baseline [time =1] and follow-up [time=3].

a) Externalising behaviour scores



b) Internalising behaviour scores





There was a high correlation between intra individual behavioural scores for both externalizing behaviour [figure 4.21a] and internalizing behaviour [figure 4.21b] at both timepoints studied so the data was pooled to produce overall mean externalizing and internalizing behaviour scores.

#### 4.6.3 Glycaemic data

##### Mean blood glucose:

The overall MBG value was  $11.2 \pm 2$  mmol/l [mean +SD]. The intra-individual correlation of MBG values between the 2 time-points studied was not significant [ $r=0.04$ ,  $p=0.83$ ]. In individual patients, MBG was moderately correlated with intercurrent HbA1c ( $r = 0.43$ , [95% CI 0.24 to 0.59],  $p<0.001$ ).

##### Mean of daily differences:

The overall mean of daily differences was  $4.5 \pm 1.4$  with poor correlation of the intra-individual measures at the 2 time points.

##### Percent of time in the low CGMS glucose range:

The overall mean percentage of time spent in the low [ $<4$  mmol/l], CGMS range was  $7.1 \pm 8.2\%$  with poor correlation of intra-individual measures at the two points [ $r=0.14$ ,  $p=0.36$ ].

##### Percent time spent in the normal CGMS glucose range:

The overall mean percentage of time spent in the normal [4-12 mmol/l] CGMS range was  $50.4 \pm 15.1\%$  again with poor correlation of intra-individual measures at the two points [ $r=0.33$ ,  $p=0.03$ ].

Percent time spent in the high CGMS glucose range:

The overall mean percentage of time spent in the high [ $>12$  mmol/l] CGMS range was  $42.4 \pm 16.7\%$  with further poor correlation of the intra-individual measures at the 2 time points [ $r=0.10$ ,  $p=0.53$ ].

Continuous overall net glycaemic action:

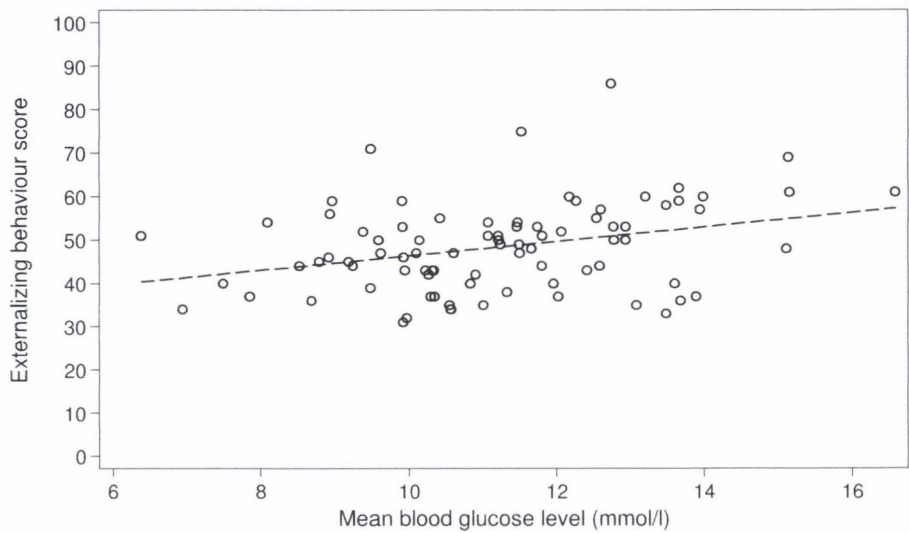
Mean CONGA1, CONGA2, and CONGA4 values were  $2.9 \pm 0.6$ ,  $4.3 \pm 1.0$  and  $5.6 \pm 1.4$  respectively. Again there was poor correlation of the intra-individual measures at the 2 time points (CONGA1,  $r=0.16$ ,  $p=0.3$ , CONGA2,  $r=0.22$ ,  $p=0.17$ , CONGA4,  $r=0.28$ ,  $p=0.07$ ).

**Table 4-15: Comparison of glycaemic outcomes between the two time points of the study at 3 months [time=1] and 9 months [time =3].**

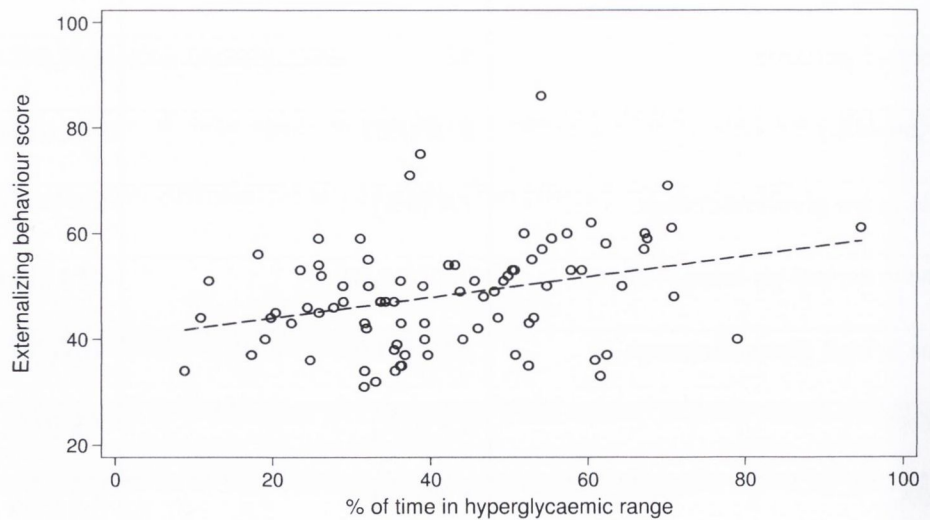
Glycaemic measures	Time = 0 months [sd]	Time = 6 months [sd]
Number of patients	42	42
MBG (mmol/L)	10.8 [1.8]	11.6 [2.1]
% time in low glycaemic range	7.4 [8.6]	6.9 [7.9]
% time in normal glycaemic range	53.7 [14.0]	47.1 [15.6]
% time in high glycaemic range	38.9 [14.9]	46.0 [17.8]
CONGA1	2.9 [0.6]	2.9 [0.6]
CONGA2	4.3 [0.9]	4.2 [1.0]
CONGA4	5.6 [1.2]	5.7 [1.5]

Figure 4-22: Association between externalising behaviour score and glycaemic outcomes including mean blood glucose [figure 4.22a], percent time in high glycaemic range [figure 4.22b] and percent time in normal glycaemic range [figure 4.22c].

a): Externalising behaviour and MBG

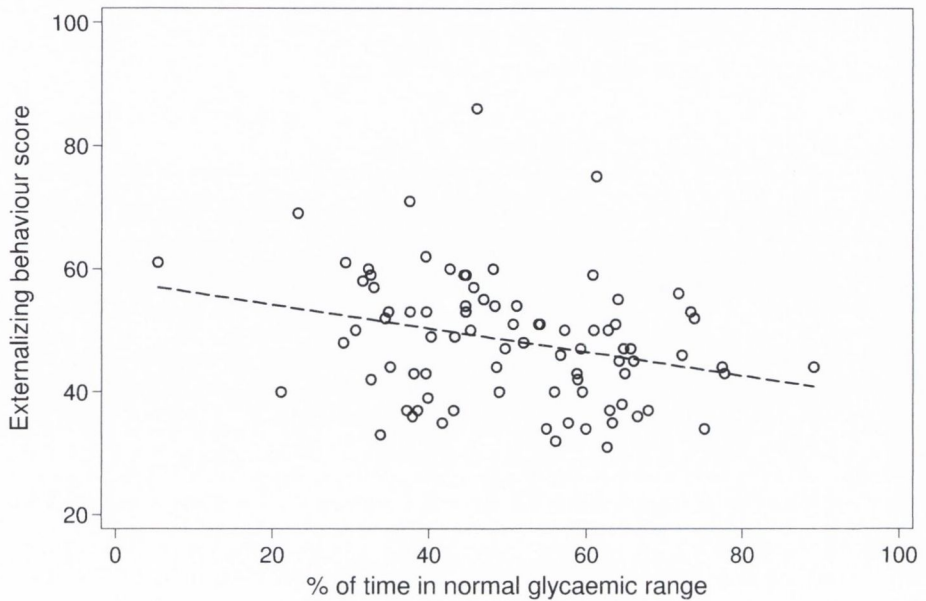


b): Externalising behaviour and percent time in high glycaemic range





**c): Externalising behaviour and percent time in normal glycaemic range**



**4.6.4 Association between glycaemic measures and behaviour scores**

Mean blood glucose:

Mean blood glucose was significantly associated with the externalizing behaviour score [regression co-efficient =1.7 (95% C.I. 0.6-2.8) adjusted  $r^2 = 0.088$ ] This indicates that on average, for every 1mmol/L rise in mean blood glucose, there was a concomitant rise of 1.7 in the externalizing behaviour score, and variation in the mean blood glucose explained 8.8% of the variance in the group mean externalizing behaviour score [figure 4.22a]. There was no significant association between mean blood glucose and the mean internalizing behaviour score.

Mean of daily differences:

There were no significant associations between Mean of daily differences and mean externalizing or internalizing behaviour scores.

Percentage of time in the low glycaemic range:

There were no significant associations between percentage of time in the low glycaemic range and mean externalizing or internalizing behaviour scores.

Percentage of time in the normal glycaemic range:

Percentage of time in the normal glycaemic range was significantly associated with the mean externalizing behaviour score [ $r = -0.2$  (95% CI -0.3 to -0.5) adjusted  $r^2 = 0.068$ ]. This data indicated that for every 5% increase in time in the normal glycaemic range, there was a decrease in externalizing behaviour score of 1.0 [figure 4.22c]. Variation in percentage of time in the normal glycaemic range explained 6.8% of the variance in the overall mean externalizing behaviour score. Significant associations were not found between internalizing behaviour scores and the percentage of time spent in the normal glycaemic range.

Percentage of time in the high glycaemic range:

Percentage of time in the high glycaemic range was also significantly associated with mean externalizing behaviour score [ $r = 0.2$  (95% C.I. 0.07-0.3) adjusted  $r^2 = 0.089$ ]. This data indicated that for every 5% increase in time in the high glycaemic range there was an increase in the externalizing behaviour score of 1.0 [figure 4.22b]. Variation in percentage of time spent in the high glycaemic range explained 8.9% of the variance in the overall mean externalizing behaviour score. A significant association was not found between internalizing behaviour scores and the percentage time spent in the high glycaemic range.

Continuous overall net glycaemic action:

There were no significant associations with CONGA and externalizing or internalizing behaviour scores.

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## 5 Discussion

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### 5.1 Study design: population and methods

The purpose of this study was to ascertain if information gathered from continuous glucose monitoring was associated with other outcomes of diabetes control using a prepubertal cohort of children. The planning of the project took place in late 2002 - early 2003 at a time when only minimal utilisation of continuous glucose monitoring was available. A longitudinal, observational, prospective study looking at outcomes over a twelve month period was designed to allow adequate time to examine whether any associations existed between continuous glucose monitoring and behavioural variables, insulin dosage and metabolic outcomes. Calculation of sample size presented an obstacle to this study. Prior to this study, no studies had examined associations between short term glycaemic outcomes calculated using continuous glucose monitoring, and behaviour.

#### 5.1.1 Calculation of sample size

As no comparisons existed and a novel method of glycaemic variation was utilised, an adequate sample size was difficult to establish. A review of the available descriptive studies of continuous glucose monitor use showed a maximum of forty seven patients studied in any one cohort. These numbers were adequate to show a relationship between these short term glycaemic outcomes and the current gold standard HbA1c (153; 195; 196) therefore it was considered that a similar number would be adequate to demonstrate the differences within this study. The methodology outlined in the study (particularly the glucose limits) correspond with more recent guidelines set by the International Society of Paediatric and Adolescent Diabetes (233). If such information had been available at the time of planning it may have enabled us to model a target reduction in



glycaemic variation and estimate sample size. However, the decision reached to proceed with the number of participants recruited was made based on the literature available at the commencement of the study. Retrospectively, it can be theorised that larger study numbers may have enabled subtle yet significant differences in glycaemic variation to be identified.

### **5.1.2 Recruitment of longitudinal study cohort**

Ninety-nine children (of the appropriate age and duration of type 1 diabetes) were attending the diabetes outpatient clinic of the Royal Children's hospital at the time of recruitment and were eligible for participation in this study. Prepubertal children were chosen to avoid complicating CGMS analysis with pubertal insulin resistance (including the dawn phenomenon) commonly seen in adolescents. All children included in the study met the Tanner Stage 1 criteria (ie no pubertal development). Minimum duration of diabetes was set at two years to avoid the inclusion of children in the honeymoon period whose glycaemic control may be affected by residual endogenous insulin. All eligible families were approached. Participation in repeated subcutaneous monitoring does however require a highly motivated cohort due to the invasiveness of the procedure. The insertion of a subcutaneous monitor was the deterrent for the majority of the 47% of non participants who declined entry into the study. Particularly the author encountered difficulty encouraging young boys to enter the study who were surprisingly less impressed by the technology than their female counterparts. There is scant evidence within the literature suggesting that gender difference impacts on glycaemic variation prior to puberty. Recently, Hochhauser et al demonstrated no gender difference in the HbA1c values of a prepubertal cohort more than twelve months from diagnosis in a small single centre retrospective study (234). While this author would not use the results of a single study to infer that no gender influence exists on the metabolic control of prepubertal children with diabetes, I am satisfied that there is

no conclusive proof to the contrary. Despite the difference in gender participation this author was satisfied that the study group remained representative of the eligible clinic population of children with diabetes as they were a prepubertal cohort with no difference from non participants with regard to age, HbA1c and duration of diabetes (see table 4.1).

Within the participating group, there was an 85% completion rate. This was in spite of technical difficulties encountered using the monitor in a young cohort (mostly associated with length of the cable which could get caught or pulled easily) and in some cases, requiring repetition of the trace. Fifty two children agreed to partake in the study, a participation rate of fifty three percent of the total study population available. Fifteen percent of participating children withdrew from the study between the first and second monitor runs. The invasive nature of the continuous glucose monitor was the main reason for the moderate participation and fall-out rates as many parents felt that their children endured enough invasive procedures in the course of a day with diabetes due to their insulin injections and blood glucose testing.

### **5.1.3 Representative nature of study cohort to diabetes clinic population**

The study group remained consistently representative of the eligible clinic population as defined by age and HbA1c. Between the first and second continuous monitor applications, the drop in patient numbers resulted in a significant difference between the study group and eligible population regarding duration of diabetes. With regard to experience with use of the continuous glucose monitor, verbal feedback from families suggested that they found it difficult to remember to record hypoglycaemic episodes and add event markers into the continuous glucose monitor. Anecdotally, most families did feel that using the continuous glucose monitor did provide added insight into their child's glycaemic control.

#### **5.1.4 Study limitations**

Continuous monitoring is not without limitation (187; 235). The monitor can be cumbersome and restrict vigorous exercise. As the monitor is not waterproof, children are restricted from swimming and other water activities during the three-day recording. The subcutaneous sensors occasionally fail and this may not be detected until the end of the trace period. CGMS glucose recordings lag real-time blood glucose by up to 5-10 minutes (200) and have maximum and minimum cut-off values of 22.2 and 2.2 mmol/l respectively. The CGMS used was not a real-time blood glucose measuring device and is not designed to detect isolated aberrations of blood glucose. It is a tool designed for pattern recognition or for the detection of particular problems in glucose profiles. The strengths of CGMS are its ability to reflect continuous ambulant glycaemic control in a manner that allows for other dimensions of glycaemia such as variation to be quantified. Although, the company have now produced the Guardian RT (a real time continuous glucose monitor) and there are competing brands on the market, these products were not available for consideration at the time of the study.

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## **5.2 A novel algorithm for continuous glucose monitoring analysis**

The first specific aim of this study was to devise an algorithm which would reflect the many aspects of continuous glucose monitoring in diabetes, including time spent in the high and low glucose ranges and describing the variation in glycaemia seen in a trace over three days; to develop a novel approach which would describe such intra-day glycaemic variation.



### **5.2.1 Use of software provided by continuous glucose monitoring system**

Some information can be provided through the use of the solutions software provided by Minimed for analysis of the data obtained from the CGMS. This will provide the average blood glucose reading for the trace and the area under or above the curve at various glucose levels. Although the upgraded version 3.0 became available during the study period we analysed all traces on version 1.7A to maintain consistency throughout the project. Differences between the two analytical programs are based on the algorithm used to analyse the data and the graphics used to illustrate the results. Neither program provides the ability to verify, clean or exclude data that is considered inaccurate. As the software was not utilised to analyse the data, there was no disadvantage in continuing with the older version. Refabrication and upgrading of the CGMS sensors occurred prior to the study onset so did not impact on this project.

The software calculations available are carried out regardless of the quality of the data. Interruptions of the glucose curves, unexpected alarms and nonfunctioning wires all decrease the quality of the trace and can result in inaccurate summary scores (187). The advantage of the novel algorithm is that data is only analysed after interruptions and dysfunctions are excluded.

### **5.2.2 Development of a new measurement for glycaemic variation**

A retrospective analysis of the methods utilised for both continuous glucose monitoring and intermittent glucose monitoring as discussed in the background for this thesis demonstrated the need for a method to adequately describe glycaemic variation in this setting (157; 159). The role of glycaemic variation on diabetes control has been previously postulated (80; 156). Until now, there has been no single measure which adequately summarises the variability of data collected by continuous monitoring in uncontrolled conditions. Many methods were trialled including variations of area under the curve, slopes of the curve and adaptations of the MAGE formula

(144; 156). However, none of these methods could be adapted for use in continuous glucose monitoring. In addition, they did not demonstrate a clear differential between subjects with type 1 diabetes and those without.

The Mean Amplitude of Glycaemic Excursions (MAGE) devised in 1970 (145) was devised for the assessment of the variability of intermittent blood glucose measures in controlled post-prandial conditions. Whilst MAGE has been used as a tool to assess CGMS data in two previous studies (158; 161), the use of MAGE to analyse CGMS data is inappropriate. This is due to reservations regarding the inability of MAGE to reliably reflect all the peaks and nadirs in truly continuous glycaemic data and the requirement of an arbitrary decision by the data analyst as to what constitutes a peak or a nadir. No mathematical system could be devised that reliably, objectively and consistently identified glycaemic peaks and nadirs required for this calculation to be used with CGMS. These shortcomings of MAGE led to the conception of the new continuous measure of glycaemic variability (CONGA).

### 5.2.3 Elements of the algorithm

For every continuous glucose monitoring trace the following information is accrued.

- Mean blood glucose
- Mean of daily differences
- Percent time spent in the low CGMS glucose range
- Percent time spent in the normal CGMS glucose range
- Percent time spent in the high CGMS glucose range
- Continuous overall net glycaemic action

The second part of the first aim of this study was to apply the algorithm to demonstrate the differences in continuous monitoring between people with and without diabetes. The premise of

this approach to continuous glucose analysis is that there is no one measure that covers all aspects of glycaemic control, and hence a multifaceted approach is warranted. Each aspect of the algorithm demonstrated clearly the difference between the two groups of subjects [diabetes vs. controls] and these differences are delineated for each glycaemic variable below.

#### Mean blood glucose of subjects with diabetes vs. controls:

Mean blood glucose is an easily reproducible calculation which has been shown to be associated with concurrent HbA1c levels (153-155). As expected when the mean blood glucose was calculated for the diabetes and control groups the range for mean blood glucose was much narrower in the cohort without diabetes (4.8-5.8mmol/L) compared to the subjects with diabetes (6.0-16.2mmol/L).

#### Mean of daily differences (MODD) of subjects with diabetes vs. controls:

The mean of daily differences relates the days in the trace to each other evaluating whether the data obtained is reproducible on a day to day basis. A MODD score of <1.0 is indicative of a low degree of variation between days. Low variation from day to day in a child with a large percentage of time in the high glucose range suggests that an alteration in insulin dosage may help achieve a more stable profile. Large day to day differences suggests that diet and/or exercise have a bigger role to play in achieving normalised glucose values.

In the trial application of the CGMS algorithm the group mean MODD value for the group with diabetes (4.3 [range 2.9-8.1]) was much higher than the group mean MODD value of the control group without diabetes (0.8 [range 0.5- 1.2]). There was considerable variability in the group with diabetes suggesting varied levels of routine and control on a day to day basis. The MODD value of <1.0 indicating a high level of correlation between all three days of the trace and was not



attained by any of the subjects with diabetes. This is not a frequently used derivation and the last published paper incorporating the use of MODD was in 1986 (236). However, it is a useful tool as one of the aims of glycaemic control should be consistency in glucose levels on a day to day basis. By assessment of the degree of consistency in a CGMS trace, the degree to which observed daily patterns are ongoing and representative can be assessed. The introduction of continuous subcutaneous insulin infusion where the basal bolus technique can be altered minutely should result in very low MODD values if used effectively.

Percent time spent in the low, normal or high CGMS glycaemic ranges by subjects with diabetes vs. controls:

Percent time in the three defined glucose ranges [low, normal and high] is also easily calculable. The ranges chosen reflected the age of the study population and were representative of the clinical practice of the author [CMcD] and supervisor [FC] involved in the study. The normal glucose range extends from 4 to 12mmol/L although this is reduced as the child gets older. Bearing in mind that the ADA criteria for diagnosis of diabetes includes a random blood glucose above 11.1mmol/L (237), this cut-off was considered appropriate for study purposes, so high glucose readings in the CGMS range were related to a glucose measurement greater than 12mmol/L. Prepubertal children are advised to treat hypoglycaemia for any blood sugar level <4mmol/L. The percentage time analyses proposed in this study have the advantage that CGMS data is summarised only in terms of time, an easily reproducible and comparable measure between subjects. Also, the percent of time spent within various glycaemic ranges is a useful clinical concept that can be used to adjust therapy. Notwithstanding the clinical utility of percentage time, it is still a limited measure of continuous CGMS data. The figures obtained are sum totals and not indicative of whether the time spent at or below a particular glucose value is within one

prolonged episode or consists of many shorter episodes. Subanalysis of percentage time during various periods [night, school time etc] may be more helpful in specific clinical contexts. The subanalysis of the time spent in each glycaemic range for specified periods of the day is expressed in the results of this thesis as mean minutes per hour rather than percent time to allow easy comparison between results from different monitor runs independent of the length of the trace.

During the trial application of the algorithm the control group spent 79.5-100% time in the normal range [as defined by the limits for this study and not by normal definitions of hypoglycaemia (238)] and no time in the high glucose range as would be expected in a group without diabetes, whereas the subjects with diabetes spent a varied range of time in the normal and high glucose ranges reflecting the spread of HbA1c values within this group. In this limited study, patients and controls experienced similar percentage of times of CGMS readings below 4mmol/L [6% vs. 8%, respectively]. There are potentially two explanations for this. First, the normal range for blood glucose levels extends down to 2.6mmol/L (238). Thus CGMS readings between 3 and 4 mmol/L can be viewed as normal for the control subjects. In this study a cutoff value of 4mmol/L was chosen because in patients with diabetes this is the level at which patients with diabetes frequently experience symptoms of hypoglycaemia and the level at which paediatric patients are counseled to maintain before bedtime according to ISPAD international guidelines (233). The second reason pertains to the accuracy of interstitial tissue CGMS readings compared with blood glucose at low glucose levels. In the control traces, the lowest recorded glucose level was 2.7mmol/L. Out of a total of 6,488 5-min control readings the glucose level was recorded between 2.7 and 3.0mmol/L in only 35 occasions (0.5% of readings). The issue of sensor accuracy has been discussed in the literature with discrepancies occasionally seen between interstitial tissue and blood glucose levels in detecting low glucose values (87; 160; 179). It is for these

reasons that this thesis does not advocate the use of the CGMS to assess the absolute degree of glycaemic excursions (i.e. percentage time in and out of various glycaemic ranges). In addition, CGMS values are not referred to as hyper-, normo- or hypoglycaemia; but as high, normal or low CGMS glycaemic ranges in order to reinforce the point that the CGMS is not measuring blood glucose levels *per se*.

#### Continuous overall net glycaemic action (CONGA):

This novel measurement illustrates intra-day (or within day) variation. The CONGA formula developed specifically for this study is based on the premise that normoglycaemia inherently allows for little glycaemic variation, whereas glycaemic control in diabetes will result in greater variation in blood glucose levels. A score of  $<1.5$  appears to reflect the degree of glycaemic variability in a person without diabetes mellitus. High CONGA values will therefore reflect increased glycaemic excursions consistent with less stable control, and low CONGA values will reflect stable glycaemic control.

CONGA is defined as the standard deviation of the differences and measures the overall intra-day variation of glucose recordings. The CONGA does not require arbitrary glucose cut-offs, logarithmic transformation, chosen peaks and nadirs or defined meal or exercise times. The CONGA calculation is an expression of the variation shown during normal, ambulatory activity which is more useful in the assessment of a child with diabetes. The choice of the time difference,  $n$ , will depend on the clinical question being addressed. Three time periods were selected as most pertinent for the age group to be studied namely CONGA1, CONGA2, and CONGA4, the time periods 1 h, 2 h, and 4 h, corresponding approximately to time between different activities in school [1 hour], time between snacks [2 hours], and time between meals [4 hours].



CONGA analyses may potentially be restricted by the limits of measurable glucose levels imposed by CGMS, due to the unknown degree of glycaemic change outside the specified measuring range. In order to test this hypothesis all CGMS values less than 2.2 mmol/l or greater than 22.2 mmol/l were excluded and re-calculated as "adjusted CONGA". Only minimal differences were identified. Adjusting CONGA values may be considered a form of selection bias and as the glucose reading must approach the cut-off to exceed it, a wide standard deviation already occurs. Therefore I do not recommend the adjustment of CONGA to account for the artificial limitations of the CGMS.

When assessing the CONGA values between people with and without diabetes, this data demonstrates the dramatic difference in glycaemic variation between people with diabetes and healthy controls. None of the subjects with diabetes had a CONGA score less than 1.7 indicating a much higher degree of glycaemic variation compared to the healthy controls. Whilst both distributions are centred around zero, a much wider range of differences is evident in the subjects with diabetes. The differences between the two groups increased as the time interval increased. This makes sense as the healthy controls are controlled internally in a narrow glycaemic range whilst in diabetes such tight control is lost.

The lower the number, the less variation and more stable glycaemic control evident within the trace of a person with diabetes mellitus. This finding suggests that those children with the greatest degree of intra-day glycaemic variation are more likely to have the most amounts of low glucose values and this has direct implications for clinical management. Strategies designed to avoid or prevent hypoglycaemia in pre-pubertal diabetic children should be designed to minimise intra-day glycaemic variation focussing upon balance of insulin doses and regularity of lifestyle habits. The relevance of this derivation to the other outcomes of diabetes will be discussed in subsequent sections of this discussion. The derivation of the CONGA formula completed the

formulation of a reproducible algorithm that summarises the findings of continuous glucose monitoring that are relevant to diabetes control (figure 5-1). The presence of such an algorithm enabled the exploration of the effects of various aspects of glycaemic control including percentage hyperglycaemia and glycaemic variation on the behavioural health of the child with type 1 diabetes mellitus. This algorithm has subsequently showed that social consumption of alcohol in adolescents with Type 1 diabetes is associated with increased glucose lability (Appendix 9).

As previously mentioned the planning of this dissertation took place in 2002. Since then there has been a steady increase in the focus of diabetologists on glycaemic variation. Further methods to calculate glycaemic variation using continuous glucose monitoring have been developed since this study commenced e.g. GRADE (165) [Glycaemic Risk Assessment Diabetic Equation] which suggests that the degree of risk associated with glycaemic variability should be considered in conjunction with HbA1c. Monnier et al recently illustrated how glycaemic variability can also lead to increased oxidative stress (120) which has been implicated in the pathogenesis of diabetes complications (119). Glycaemic variability could therefore be a very important independent risk factor of diabetes complications and requires further investigation with a reproducible measure like CONGA to ascertain its significance in long term studies of diabetes control.

Measurement	Abbreviation	Indication	Advantage	Disadvantage
Mean blood glucose	MBG	Measure of daily blood glucose average	Good indicator of concurrent HbA1c	Fails to identify periods of high or low glucose readings in patients who have periods of both resulting in an average reading in the normal range.
Mean of daily differences	MODD	Measure of inter day glycaemic variation	Identifies areas where lifestyle rather than insulin dose may require modification.	Cut off points suggested with this measure are arbitrary so the level of acceptable day to day variation in the management of diabetes is unclear.
Percentage time spent in the low/normal/high CGMS glucose range	% low/normal/high	Measures time spent in hypoglycaemia /normoglycaemia /hyperglycaemia.	Normal glucose limits can be reduced as children grow older to enable tighter control.	Period of time is a sum total and not indicative of whether the time spent in this range occurred in one block or over shorter intervals.
Continuous overall net glycaemic action	CONGA	Measure of intra day glycaemic variation	Good glycaemic control in diabetes is reflected by small degrees of variation in blood glucose levels	May potentially be restricted by the limits of measurable glucose levels imposed by CGMS, due to the unknown degree of glycaemic change outside the specified measuring range.

**Table 5-1: Components of algorithm recommended for incorporation into clinical analysis of continuous glucose monitoring systems in children with type 1 diabetes.**



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### **5.3 Longitudinal study of the CGMS algorithm in a cohort of children with diabetes**

The next aim of the project was to apply the approach to a defined cohort of children with diabetes and to describe the outcomes of continuous glucose monitoring in this cohort over a three day period with emphasis on whether outcomes of continuous glucose monitoring outcomes reflect metabolic control measured by HbA1c. The following subsections discuss the progress of each of the glycaemic variables over the twelve months of the study as demonstrated by continuous glucose monitoring.

#### **5.3.1 The relationship between Mean blood glucose (MBG) and HbA1c using continuous glucose monitoring.**

The pre-pubertal cohort were found to have HbA1c levels typical of most international and Australian tertiary diabetes care centres (67; 239; 240) with the mean HbA1c ranging from 8.1 - 8.8% over the twelve months of the study. The Mean blood glucose for the cohort ranged from 10.7-11.6mmol/L at the same four timepoints. The mean blood glucose correlated with HbA1c on three of the four monitor runs. On the third run when there was no correlation [0.18 p=0.27], the study number had dropped to forty patients and this decrease in numbers may have contributed to the decreased correlation between these variables. The repeat finding on the other three occasions does substantiate previous documentation by other groups of the relationship between mean blood glucose calculated by short term continuous glucose monitoring [using an average of the glucose readings over the three days] and the HbA1c which remains the gold standard indicating level of metabolic control (154; 155). This association infers that a low mean blood glucose in a three day trace corresponds to an acceptable HbA1c over a corresponding

three month period. While mean CGMS glucose values cannot account for glycaemic variation they can potentially be utilized as surrogate markers for HbA1c within trace analysis. The advantage of continuous glucose monitoring is to expand on the information provided by mean blood glucose and ensure that large periods of low and high glucose readings do not contribute to an acceptable HbA1c measurement due to the documented deleterious effects of repeated low and high blood sugars. In particular, to document and address the presence of low glucose readings (particularly nocturnal) that have been identified in other cohorts by continuous glucose monitoring (155; 198).

### **5.3.2 Mean of daily differences (MODD)**

The inter-day variation on each occasion ranged from a mean of 4.5 to 5.1. As mentioned previously, a MODD value <1.0 indicates a similar pattern of trace on each of the days involved. This high level of variation on a day to day basis in comparison to normal individuals suggests the difficulty encountered in maintaining good glycaemic control while pursuing a normal lifestyle particularly that of a schoolchild. On personal experience, CGMS traces with high MODD values are indicative of irregular habits and require detailed contemporaneous lifestyle information prior to interpretation.

### **5.3.3 Percentage time spent in the low, normal and high CGMS glycaemic ranges**

At time = 0, the analysis from the continuous glucose monitoring showed that children experience an average of 1.9 hours per day (mean = 4.8 minutes per hour) of glucose levels below 4mmol/L. This compares with a previous report of an average of 2.6 hours per subject per day (for glucose levels <3.5mmol/L) in a cohort of 28 children under 12 years of age (155). On each occasion, the analysis was shown to the family and changes were made to limit the amount of time spent in the

low CGMS range. This advice included change in insulin dosage, timing of injections, treatment of low blood sugars to avoid prolonged hypoglycaemia and dietary intervention. The second and fourth monitor runs showed a similar time spent in the low CGMS range (means = 4.6 and 4.3 minutes per hour respectively). The third monitor run fell to a mean of 2.8 minutes per hour. This may be related again to the smaller group on this occasion or that those who failed to participate were having higher rates of low blood sugar values. When the percent time spent in the low CGMS range was reviewed for the fourth run, an outlier was identified (see appendix 6). Patient 33 spent 33% of the total trace time in the low glucose range compared to less than five percent on each of the first three occasions. A review of the CGMS graph reveals that the majority of this time was at night and was unrecognised. The sensor readings correlated with the capillary blood glucose measurements and the entire trace was validated according to the set criteria without any data cleaning required. If the percent time spent in the low CGMS range is recalculated without patient 33, a fall from 7.9% to 6.2% is seen. This is a fall from 4.3 to 3.4 mean minutes per hour per patient for the cohort. An alternative explanation for this occurrence could be the time of year as the third run (time =2) occurred between August and November which is the the low season for sport and some of the coldest months of the year in Australia when children spend more time indoors and less exercise. It could be postulated that less exercise related hypoglycaemia occurred during this period. However, the impact of exercise was not examined as part of this study so this finding cannot be evidence based but would be an interesting point to follow up in future studies.

Previous reports (155; 198) have also illustrated a significant percentage of undetected nocturnal hypoglycaemia. An average of 1.1 hours (mean = 8.4 minutes per hour) was spent in the CGMS range <4mmol/L between midnight and 8am on the first monitor run. This returned to a mean of 1.2 hours ( mean = 8.8 minutes per hour) on the fourth monitor run having reached a nadir of 0.6



hours (mean = 4.2 minutes per hour) on the third occasion. Again, the exclusion of patient 33 on the fourth run would have seen the period of time spent in the low CGMS range nocturnally drop from means of 8.8 to 7.9 minutes per hour. These prolonged periods of time reflective of hypoglycaemia are cause for concern particularly as most episodes appear to be unrecognised by either child or care-giver. "Hypoglycaemia unawareness", especially during sleep, is a well recognised phenomenon (241) and is in part caused by recurrent hypoglycaemia (217; 242). Thus intermittent finger-prick testing has many limitations in detecting the true frequency of this complication and ambulant continuous glucose appears to be the detection method of choice. The reason why a significantly increased rate of hypoglycaemia occurs in sleep compared to wakefulness remains unclear although, blunted counterregulatory hormone responses to hypoglycaemia have been previously reported in children during sleep (142; 243).

Whether interstitial glucose readings are a marker of clinically significant nocturnal hypoglycaemia in venous blood also remains debated. Experimental studies have shown that at least slow alterations in circulating glucose are mirrored by the interstitial glucose levels (244; 245). Studies of the Minimed continuous glucose monitoring system estimate that the delay in glucose equilibration in the interstitial fluid is most likely less than ten minutes with an error margin of less than 6% (87; 179). In addition, sleep studies have detected disturbances in sleep architecture in children with documented periods of spontaneous nocturnal hypoglycaemia during continuous glucose monitoring. If interstitial fluid was not reflective of physiological hypoglycaemia then changes in central nervous functions such as sleep should not be observed.

The time spent in the normal glucose range (4-12mmol/L) showed very little difference over the twelve months. Despite a wide range of blood sugars the mean time spent in the normal CGMS glycaemic range was approximately fifty percent of the time. Those who improved their percentage time in the low glucose range rebounded into the high glucose range. This may be a

result of increased parental anxiety due to the identification of nocturnal low glucose readings with subsequent changes in insulin management. One of the priorities for clinicians managing children with diabetes is to address the challenge of nocturnal hypoglycaemia while maintaining a HbA1c in the target range. This will involve a multidisciplinary approach including nutrition, psychological and clinical aspects of care. Review of the mean minutes per hour stratified for time of day shows that children tend to be hyperglycaemic in daytime and hypoglycaemic at night. This could be related to either poor adherence to diet or a delay in presentation of exercise induced hypoglycaemia. Both areas would benefit from further investigation in younger people with diabetes.

#### **5.3.4 Continuous overall net glycaemic action (CONGA)**

The CONGA calculation is an expression of the variation shown during normal ambulatory activity which is more useful in the assessment of a child with diabetes. CONGA does not require arbitrary glucose cut-offs, logarithmic transformation, chosen peaks and nadirs or defined meal or exercise times. The CONGA calculation is based on the premise that normoglycaemia inherently allows for little glycaemic variation, whereas glycaemic control in diabetes will result in greater variation in blood glucose levels. High CONGA values will therefore reflect increased glycaemic excursions consistent with less stable control, and low CONGA values will reflect stable glycaemic control. The intra-day glycaemic variation is calculated as CONGA increases while the time period lengthens from 1-4 hours. The largest increases are seen in those patients with large glycaemic swings over the period of the trace. In contrast, healthy controls show little change in CONGA values over time due to the limited degree of glycaemic variation. CONGA values remained within consistent ranges over the four monitor runs when calculated for 1 hour (2.8-3.1) 2 hour (4.3-4.7) and 4 hour (5.6 -6.1) intervals. Neither MODD or CONGA correlated with HbA1c

on any of the four occasions. There was however, no clinical reason why these metrics should correlate with HbA1c as clinical experience can attest to patients having the same HbA1c but markedly varying degrees of clinical variation. Conversely, there can be two patients with the same degree of intra-day or inter-day glycaemic variation who have very different mean glucose levels or HbA1c values.

During the study a consistent decrease was shown in CONGA1 values for all patients over the twelve months ( $p=0.03$ , figure 4-11). This result reflects the trend downward of the mean of each individual patient's four readings and was not affected by the obvious outlier evident on the graph. Such an improvement could be due in part to improved parent and patient management of diabetes secondary to the knowledge provided by the continuous glucose monitor.

As CONGA is a new measure of glycaemic variation there is no comparison in the literature with which to discuss the outcomes. Over the four runs of continuous glucose monitoring, CONGA has been demonstrated to be a valid, reliable and reproducible measure. Glycaemic variation has been postulated to be an important factor in those with diabetes who consistently report HbA1c levels within the target range but still develop complications of diabetes at an early age. This suggests that measures of glycaemic variation may show their greatest utility in longitudinal studies where children and adults with diabetes reporting similar targeted HbA1c levels but with variable glycaemia, demonstrate improvement in hypo- and hyperglycaemia with a subsequent reduction in complications associated with low CONGA levels.

### **5.3.5 Insulin requirements**

Insulin requirements were tracked over the duration of the study. Insulin dosage [units/kg/day] did not differ over the duration of the study indicating that insulin resistance did not impact on the cohort.



### 5.3.6 Body mass index

Body mass index was normalised for age and gender using the z-score based on available reference populations and showed only a slight increment over the twelve months with means between 1.0 and 1.2 on each occasion and minimal deviation in range. This is most likely due to the selection of prepubertal children for the study and may have been more significant in an older pubertal cohort.

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## 5.4 Long term impact of continuous glucose monitoring

The advent of continuous glucose monitoring has provided a clearer picture of ambulatory glycaemic control. The impact of repeated continuous glucose monitoring over the twelve months was assessed by calculating the slope for each glycaemic variable from the raw data and utilising paired student t-tests to assess whether differences between glycaemic or metabolic variables from the start to the end of the project were significant. The continued participation of thirty eight children through the study gave ample opportunity to monitor the effects of clinic visits and continuous glucose monitoring over a twelve month period. The results are discussed individually over the subsequent sections.

The longer-term results reported in this study support the value of repeated CGMS in a clinical setting but not with the expectation of improved metabolic control. Parallel to this study, most of the data obtained from continuous glucose monitoring was achieved using cross-sectional studies or short term longitudinal studies. Outcomes from these studies indicated that improvements in HbA1c were only transient and that repeated use of continuous glucose monitoring did not result in sustained improvements in metabolic control (187; 246).

#### 5.4.1 HbA1c

HbA1c significantly increased over the time frame of the study [ $p=0.05$ ]. This was mirrored by a similar but non significant increase in the non participating clinic population. This finding was in contrast to other studies published at this time by Salardi, Chase and Kaufman (154; 195; 196). These studies demonstrated an improvement in HbA1c despite the identification of nocturnal hypoglycaemia. However, the HbA1c returned to pre study levels once continuous glucose monitoring was discontinued. Ludvigson and Hanas in their controlled cross over study demonstrated that improvements in HbA1c only occurred in the open arm of the study when patients were briefed and followed up after continuous glucose monitoring. As this was not an interventional study, continuous glucose monitoring traces were shown and explained to the families after each visit but no additional phone calls or clinic visits were instigated until the next three month clinic review and this may have played a factor in the difference in results. Yates et al in 2006 followed thirty children with a HbA1c  $<10\%$  on either CSII or glargine with repeated CGMS on a three weekly basis versus self monitoring of blood glucose. Again HbA1c improved at baseline but this change was no longer evident by 12 weeks. Their conclusion was that CGMS was no more useful than intermittent monitoring and frequent review in improving diabetes control with near physiological insulin regimens (246).

It is possible to conclude that an observer effect plays a significant part in initial improvement in HbA1c. It is also important to remember that the aim of this aspect of the study was not to look at HbA1c in isolation but at glycaemic variation and such attempts to reduce variation (not examined in the other studies) may result in higher baseline glycaemia. Although HbA1c did increase, glycaemic variation was decreased. This long term effect of this could be important in the pathogenesis of complications in the child or young adult with diabetes.

#### **5.4.2 Mean blood glucose**

Repeated continuous glucose monitoring had no effect on mean blood glucose levels over a twelve month period. Mean CGMS glucose values are calculated as the arithmetic mean of the glucose values within a given period and their fundamental presence in the algorithm is as a short term marker for metabolic control within the CGMS trace. The mean blood glucose consistently positively correlated with HbA1c as did the percent time spent in the high glycaemic range confirming that the CGMS trace is representative of long term metabolic control. The mean blood glucose did not change overall but the decrease in fluctuations would impact on glycaemic variation without altering the arithmetic mean.

#### **5.4.3 Mean of daily differences (MODD)**

As with the individual timepoints over the twelve month period, mean of daily differences did not correlate with HbA1c over the twelve month period. Again, there is no clinical reason why inter day glycaemic variation should correlate with HbA1c which can reflect a mixture of days of erratic glycaemic control with a similar value to a person with consistent glycaemic control. The values for Mean of daily differences remained stable over the twelve month period [5.2, 4.5, 5.0 and 4.4] with no significant difference achieved over the twelve months of the study [ $p=0.79$ ]. These scores are indicative of a large glycaemic difference between days. Reduction in the mean of daily differences may be difficult to achieve in prepubertal children with half of the traces taken on weekdays and the other half on weekends where they tend to have an entirely different routine.



#### 5.4.4 Percentage time spent in the low, normal and high CGMS ranges

Repeated use of continuous glucose monitoring over a 12 month period led to a reduction in the percent time spent in the low CGMS range with an improvement in the overall percent time spent in the low glycaemic range seen in 26/44 (59%) of children in the study. Unlike previous short term studies this improvement was not associated with an improvement in HbA1c (187; 195). As in previous studies, the majority of hypoglycaemia seen in this cohort was nocturnal and unrecognised (155; 198). Episodes of unrecognised hypoglycaemia should be considered clinically when low HbA1c values are recorded especially in children. Documented effects of acute hypoglycaemia on the developing brain include poor cognition, altered mood and conscious state (138; 247) whereas prolonged, recurrent or severe hypoglycaemia appears to cause long-term neuropsychological deficits (207; 208; 210; 211).

Overall, the change in HbA1c of the group as a whole negatively correlated with the change in time spent in the low CGMS glucose range (-0.49, 95% C.I. -0.7 to -0.2) and with an decrease in the time spent in the normal CGMS glucose range (-0.4, 95% C.I. -0.64 to -0.1). This was mirrored by a rise in the time spent in the high CGMS glucose range which positively correlated with HbA1c (0.63 95% C.I.0.39 to 0.79). This may explain the rise in HbA1c reported in some CGMS studies that by reducing the frequency of hypoglycaemia, the glycaemic balance shifts towards hyperglycaemia. This could be explained by overcompensation by the parents on the recognition of hypoglycaemia through reduction of insulin or increase of nutrition resulting in a higher percentage of values in the high CGMS range. The overall increase in HbA1c contrasts with the outcome reported by Ludvigsson and Hanas in their cross-over study where the HbA1c was shown to fall in an intensive three month period (187). In that study, the CGMS was applied

on a six week basis and repeated use of the monitor on such a frequent basis would not be feasible over a twelve month period.

#### **5.4.5 Continuous overall net glycaemic action (CONGA)**

All three measures of glycaemic variation namely CONGA1, 2 and 4 improved over the longitudinal study to a significant level [CONGA1 decreased from 3.3 to 2.9  $p=0.03$ , CONGA2 decreased from 4.7 to 4.3,  $p=0.05$  and CONGA4 decreased from 6.1-5.7,  $p=0.03$ ]. As with the mean of daily differences there was no correlation with HbA1c but no correlation was expected.

One of the reasons for developing a method to describe glycaemic variation was to ascertain if repeated CGMS monitoring could improve glycaemic variation as well as improve HbA1c. As previously discussed there was a net deterioration in HbA1c over the twelve months and it was postulated that attempts to reduce variation (not examined in the other studies) may result in higher baseline glycaemia. This study has proven that fluctuations can be improved with a reduction in overall glycaemic variation without a corresponding improvement in overall metabolic control. With the rising importance of glycaemic variation in the pathogenesis of diabetes and the ongoing concerns that an aggregate measure like HbA1c will not predict all subjects with diabetes predisposed to complications, the need for another measure of glycaemic control is highlighted.

These findings underline the complexity of diabetes management where improvements in percent time in the low glucose range and reductions in the level of glycaemic variation may not be sufficient to improve metabolic control. This is a multidimensional problem where balanced nutrition, active lifestyle and adequate insulin dosage must be achieved in tandem with good glycaemic control to allow the child with diabetes the best opportunity for good long-term health.

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## 5.5 The relationship between Insulin regimen and CGMS.

The fourth aim of this study was to ascertain if proportions of free mixing insulins impact on glycaemia.

Data presented in this study showed that in sequentially assessed children with type 1 diabetes aged 4.3 to 10.3 years, there was no association between glycaemic outcomes and insulin proportions in a twice daily insulin regimen using isophane insulin. Within a range of insulin dose of 0.6 to 1.3 units/kg/day, metabolic and glycaemic outcomes (including HbA1c, mean CGMS glucose, percent time in various glycaemic ranges and intra-day and inter-day glycaemic variability) were not associated with the proportions of insulin delivery- specifically the amount of insulin given in the morning or night, or the amount of insulin given as either isophane, intermediate-acting or regular/analogue short-acting insulin.

These results were surprising and appear counter-intuitive. The notion that in a twice-daily regimen a balance of insulin doses between morning and night and intermediate-acting and short-acting is important in determining glycaemic stability has been current for some time and to some extent underpins the philosophy behind pre-mixed insulins (26; 248). Attempts to achieve a stable pharmacokinetic insulin profile in a twice daily regimen by manipulations in the proportions of short and intermediate-acting insulins is a clinical goal referred to in many diabetes texts (70-72). It would appear from this study that when using isophane insulins this goal is illusory. Whether these findings are also found when longer-acting analogue insulins are substituted for isophane insulins is yet to be determined.

There is cumulating evidence that "intensive therapy" in paediatrics is associated with better metabolic outcomes (42; 249-251). In the DCCT "intensive therapy" incorporated an intensive insulin regimen (more than 2 injections per day), frequent measuring of blood glucose, increased



contact with the diabetes care team and the setting of metabolic goals (75). In a comparative analysis of varying insulin regimens in an international Hvidoere group study of 2269 adolescents, twice-daily free-mixed insulin regimens were associated with marginally better metabolic outcomes than thrice daily, basal: bolus and insulin pump regimens (79). These findings are similar to a previous study of the Hvidoere group that showed a greater mean HbA1C level after patients were changed from twice daily to 4 times daily insulin regimens (77). In further analyses of the 2007 Hvidoere study, the major determinants of metabolic outcome were family dynamics (252) and glycaemic goal-setting by diabetes care teams (79). Self management competence has been found to be a key predictor as to which children and adolescents will benefit from intensive therapy (253). Socioeconomic status (254), mental health of parents and child (255; 256) diabetes-related family conflict (257-260), agreement within families as to who is responsible for diabetes care (261) and frequency of blood glucose monitoring (262-264) are also critical determinants of metabolic outcome. Thus the relative importance of the insulin regimen itself, within the context of "intensive therapy" appears to be of lesser importance and it appears likely that twice-daily, free-mixing insulin regimes will continue to be utilized in many centers for some time.

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## 5.6 Behavioural assessment and outcomes measured by CGMS

The fifth aim of this study was to ascertain whether types of behaviour are associated with glycaemic outcomes using continuous glucose monitoring. In this study of a primary school-aged cohort of children with type 1 diabetes higher MBG values, increased percentage time in the high glycaemic range and decreased percentage time in the normal glycaemic range were associated with higher externalising behaviour scores. Consistency is demonstrated in association between

the three glycaemic measures and externalising behaviour, with higher MBG values and percentage time in high glycaemic ranges having a negative impact on behaviour and higher percentage time in the normal glycaemic range having a positive impact. Overall, MBG and percent time in high and normal glycaemic ranges explained between 7-9% of the variance in externalising behaviours. Multiple independent and interacting factors are likely to influence behaviour, hence identifying a single factor that explains this amount of the variance in behavioral outcome is clinically meaningful (265). Scale scores correlate highly with The Child Behaviour Checklist (266) which has been validated for use in Australian populations (267). BASC is a parent rating scale used to assess the behaviour of children. The questionnaire was applied on the second visit to establish whether any connection existed between behaviour and presence of subclinical hypoglycaemia. The questionnaire was then repeated on the fourth visit to establish whether any change in glycaemic control was reflected in an improvement in behavioural summary scores.

Although, this study examined short-term glycaemic control only using a CGMS trace, previous studies have illustrated that mean blood glucose levels from the trace correlate strongly with HbA1c (153; 154). A positive correlation between MBG and HbA1c was again illustrated in this section of the study indicating that the short term CGMS trace is representative of long term metabolic control. This is important as it demonstrates the consistency with previously reported associations between high HbA1C levels and high externalising behaviour scores (228; 268).

Externalising behaviour identified on the BASC includes hyperactivity, aggression and conduct disorders. These behaviours are similar to those identified anecdotally by parents as being associated with higher blood glucose levels. Little evidence is available in the literature to clarify whether a child with externalizing behaviour problems has poorer control because they tend to be non-adherent with the diabetes regimen or whether poor metabolic control results in externalizing behaviour. The absence of a significant change in externalizing behaviour t-score

between the two time-points with a deterioration in mean blood glucose suggests that the externalizing behaviour problems may be an important maintaining factor in chronic hyperglycaemia. This study offered the opportunity to observe the associations between behaviour and blood glucose control in a naturalistic experiment as the scores from the BASC questionnaire were not calculated until the end of the study period and no behaviour modifications were put in place for the duration of the study.

In this study we were unable to determine causality in the association between the behaviour variables and glycaemia. However the tight intra-individual correlation between externalising behaviour scores at both time points and lack of correlation between intra-individual MBG at both time points suggests that externalising behaviour problems may be an important maintaining factor in determining hyperglycaemia. This notion is supported by other studies showing that externalising behaviour scores at the time of diagnosis are important determinants of poor metabolic outcomes up to 10-15 years later (228; 268).

There is evidence in previous literature of an association between internalizing symptoms and better regimen adherence/metabolic control (269-272), suggesting that neurotic symptoms may either contribute to, or result from obsessive pre-occupation with the demands of strict adherence to treatment regimens. Despite internalising behaviour scores showing the same intra-individual consistency as externalising behaviour scores across the 2 time points, there was no demonstration of any significant association between these and glycaemic measures. It is not clear why this association was not apparent in the current study but it is possible that internalising symptoms emerge over a longer period, making it difficult to identify associations in the six-month time frame used in this study. Interestingly, there was no correlation found between any of the behavioural variables and percent time spent in the low glucose range or glycaemic variation. Diaries linked to the CGMS traces showed high levels of "hypoglycaemic



unawareness" with poor identification of long periods of hypoglycaemia especially at night. It is possible that when children lack a sympathetic counter-regulatory response to hypoglycaemia and are "unaware" of events, their experience of hypoglycaemia does not arouse anxiety and has no lasting impact on either internalising or externalising behaviour. These hypotheses remain to be tested.

This is the first report of intercurrent changes in behaviour and continuous glycaemia in an ambulant, non-controlled setting. These findings provide some support for parental observation of an association between externalising behaviour problems and intercurrent hyperglycaemia. As such, these findings are consistent with the previously recognised association between behaviour problems and longer-term metabolic control as reflected in HbA1c levels. The current findings are also consistent with the developmental psychopathology literature that shows moderate to high stability in externalizing behaviours, particularly in the absence of treatment (273). Whilst causality between externalising behaviour and hyperglycaemia remains an open question, these findings underscore the importance of understanding the mechanisms of this association and how it might impact upon ultimate diabetes outcomes. Externalising behaviour problems are easily identifiable and effective treatments are available, particularly if implemented early (274). An effective and timely intervention with young children presenting with externalizing behaviour problems may have dual benefit in reducing morbidity in both mental and physical health outcomes.

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## 6 Conclusions

Continuous glucose monitoring provides a useful tool for assessment of glycaemic control specifically glycaemic variation, an aspect of glycaemia which remains undetected by self monitoring of blood glucose. Continuous glucose monitoring offers the opportunity to study more concisely the effects of glycaemia on children with diabetes. The measurement and analysis of continuous glucose readings increases our understanding of glycaemic control but good analytical tools are required to assess continuous glucose monitoring in this age group. This study provides such a tool for the assessment of glycaemic variation namely, continuous onset net glycaemic action [CONGA]. A larger sample size may have shown clearer differences with regard to glycaemic variation. In particular, relationships were stronger when there was more than forty children in the cohort. Future studies should aim for a higher number of participants.

The algorithm developed in this study allows for corroboration of glucose values with HbA1C, an analysis of intra-day glycaemic variation, description of amount of time spent within various glucose ranges, and finally an analysis of inter-day glycaemic variation. The CONGA value within this algorithm is a novel measure that is suitable for use in ambulant continuous glucose monitoring systems and should be an integral component in the assessment of CGMS data. Whether or not the CONGA value proves to be associated with other glycaemic outcomes e.g hypoglycaemia rates or risk of diabetic ketoacidosis, remains an area of ongoing enquiry. Use of this measure should be examined further in children on continuous subcutaneous insulin infusions and those trialling integrated infusion/monitoring systems.

Continuous glucose monitoring has been successfully used to describe a cohort of representative pre-pubertal children with type 1 diabetes over a twelve month period. Use of the CGMS has confirmed that high mean CGMS glucose levels are associated with higher HbA1c levels.

Glycaemic variation adds another dimension to analysis. Specifically, children with the greatest degree of intra-day glycaemic variation are more likely to have the highest amount of low glucose values. Glycaemic variation should be viewed as an independent outcome measure and improvement in glycaemic variation may lead to less hypoglycaemia and associated clinical sequelae and should be the subject of prospective studies in children with diabetes.

Prior to this study, most of the data obtained from continuous glucose monitoring was achieved using cross-sectional studies or short term longitudinal studies. These long-term results suggest the value of utilizing CGMS in a clinical setting but not with the expectation of improved metabolic control. These findings underline the complexity of diabetes management where improvements in percent time in the low glucose range and reductions in the level of glycaemic variation may not be sufficient to improve metabolic control.

This study has shown that the details of insulin proportions in twice-daily regimens are not associated with any short or medium term glycaemic outcomes. These findings are consistent with the notion that insulin regimens themselves do not appear to be the major determinants of metabolic outcome but insulin therapies need to be tailored to the needs of individual patients in conjunction with diet, exercise and comprehensive education. The maintenance of fixed-proportions of insulins appears to be misguided.

The importance of continuous glucose monitoring in clinical situations is demonstrated by the relationship between short term measures of glycaemic control with clinically significant externalizing behaviour. Further studies are required to establish whether behavioural symptoms are reduced with improved metabolic control [i.e. avoidance of both hyper and hypoglycaemia with resultant reduction in glycaemic variation].

This research establishes the framework for analysis of continuous glucose monitoring in both research and clinical settings. Further work should extrapolate the role of glycaemic variation in



type 1 diabetes using this innovative technology. The role of glycaemic variation in brain development could be explored. Such studies may provide further insight into aspects of glycaemic control that remain unexplained.

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## 8 Appendices

1. Ethics agreement dated November 2002
2. Demographics record form for each child
3. Sample trace from the Minimed® continuous glucose monitoring system
4. A novel approach to continuous glucose analysis utilising glycaemic variation.
5. Behavioural Assessment System for Children questionnaire
6. Raw data from all four time points of CGMS analysis
7. Proportions of mixed insulins have little impact upon glycaemia in primary school-aged children.
8. Hyperglycaemia and Externalizing Behaviour in children with type 1 diabetes.
9. Social consumption of alcohol in adolescents with Type 1 diabetes is associated with increased glucose lability, but not hypoglycaemia.



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## Appendix 1: Ethics agreement dated November 2002

- Ethics committee approval form
- Parent information statement for research protocol
- Parent consent form for research protocol
- Parent information statement regarding use of *CGMS*
- Consent form for use of *CGMS*

**ROYAL CHILDREN'S HOSPITAL**  
**ETHICS IN HUMAN RESEARCH COMMITTEE**

Dr Ciara McDonnell  
Endocrinology and Diabetes.  
RCH

19 November, 2002

Dear Dr McDonnell,

Please find attached the Approval for the project **EHRC 22070A** entitled "New approaches to assessing the impact of hypoglycaemia on children. PIS dated 1 Nov 2002. Protocol dated 18 Oct 2002. Consent form dated 18 Nov 2002.," for which you are listed as the contact person.

Please note that this project was recommended for Chairman's approval (expedited review). All projects approved by the Chairman require ratification at the next full Ethics in Human Research Committee meeting, at which time there is the possibility that your project may be reconsidered by the Committee, and further questions raised.

Please also note the conditions on which this approval is granted.

Thank you

Judy Davey  
Coordinator, Human Ethics  
Ethics in Human Research Committee

## TITLE

New approaches to assessing the impact of hypoglycaemia on the developing brain.

## INVESTIGATORS

Ciara McDonnell, Fergus Cameron, George Werther.

## CO-OPERATING DIVISIONS / SUPPORT SERVICES

See part B - previously submitted

## BACKGROUND INFORMATION

The impact of low blood glucose on the brain has arguably become the major factor influencing clinical management of Type 1 diabetes. The Diabetes Control and Complications Trial (DCCT) has shown conclusively that intensive insulin treatment is the most effective strategy for preventing the microvascular complications of type 1 diabetes, but is also associated with a three-fold increase in the incidence of severe hypoglycaemia<sup>1</sup>. The brain is critically dependent upon blood glucose for cerebral energy needs and inadequate supplies (hypoglycaemia) trigger a continuum of events ranging from confusion through seizures, coma and ultimately death unless appropriate action is taken. Neuropsychological deficits were evident in children with a history of severe hypoglycaemia involving seizure/coma in our own studies<sup>2,3,4</sup> and in reports from other centres<sup>5,6</sup>. Adults appear more resilient. These findings suggest that children are more sensitive to hypoglycaemia than adults, possibly because of the higher cerebral energy needs associated with growth and neural 'pruning' during brain development<sup>7</sup>. Individual differences in susceptibility to hypoglycaemia-related brain damage have been noted and are as yet unexplained, while the age/stage of neuromaturation at which greater resilience to hypoglycaemia is achieved has not been established. A related issue is the 'hypoglycaemic dose' required to irreversibly impair brain function. Previous studies have shown that children with early-onset diabetes exhibit deficits in information processing and executive skills<sup>4</sup> that cannot be explained by a known history of hypoglycaemic seizures or coma. Unrecognised episodes of hypoglycaemia short of seizures or coma, particularly nocturnal events, are common in very young children<sup>8</sup> and provide a plausible, but as yet unproven explanation for the 'early onset' phenomenon. Current management of type 1 diabetes emphasizes the importance of avoiding hyperglycemia (abnormally high blood glucose levels) in order to minimize the risk of diabetic complications in later life. However, tight metabolic control increases the risk of hypoglycemia (abnormally low blood sugar) which may be harmful to the developing brain. To date, the incidence of subclinical hypoglycaemia (low blood sugar without symptoms) remains undefined and risk factors for developing a major hypoglycaemic event (defined as a seizure or altered conscious state) have relied on retrospective studies and clinical observations.

## POTENTIAL RISKS AND BENEFITS

The only risk identified in conjunction with the continuous glucose monitoring system (CGMS) is that of infection at the insertion site. Insertion of the probe will be carried out by a trained nurse educator /doctor under sterile conditions. If infection does occur (redness or tenderness at the site of probe insertion), the CGMS will be removed and the child treated with antibiotics if necessary. Patients will continue to carry out routine blood sugar measurements while the monitor is being worn and the results will be correlated. Immediate adjustment of insulin doses will be taken if any child is identified with subclinical or nocturnal hypoglycaemia.

This study will precisely define the incidence of hypoglycaemia in a paediatric population with type 1 diabetes mellitus. The information gained from this study will facilitate the development of clinical protocols aimed at balancing the respective risks of both high and low blood sugar levels, while protecting the integrity of the CNS during development. Study findings will lead to the development of treatment strategies designed to prevent hypoglycaemia.

## PROJECT SUMMARY AND TRIAL OBJECTIVES

This study follows a group of prepubertal children (<10 years old) with established diabetes (>2 years since diagnosis) over twelve months to monitor and identify the incidence of symptomatic and asymptomatic hypoglycaemia. Previously, this information has only been available through parental observation which can be unreliable as not all hypoglycaemia causes symptoms. New technology facilitates continuous glucose monitoring (using a probe placed just under the skin for a 72hour period) which will be used at three monthly intervals to detect both symptomatic and asymptomatic



hypoglycaemia. This information will be correlated with patient glucometer readings and with routine information collected at outpatients (height, weight and HbA1c measurements).

#### **AIMS:**

- To assess the frequency, incidence and somatic consequences of major hypoglycaemic events and hypoglycaemia in a prepubertal population of children with type 1 diabetes mellitus.

#### **Hypotheses:**

- Major hypoglycaemic events and hypoglycaemia are frequent occurrences in a well-controlled population of children with type 1 diabetes mellitus.
- Symptomatic and asymptomatic hypoglycaemia occur independently of age and background HbA1c
- Recurrent hypoglycaemia may be associated with a consequent increase in body weight due to maternal overfeeding.

#### **STUDY DESIGN**

Children aged <10years old and diagnosed with diabetes >2years prior to study commencement who attend the routine outpatient diabetes clinic at the Royal Children's hospital will be asked to participate in the study. Each diabetic will have HbA1c and continuous glucose monitoring carried out at routine outpatient clinic attendance at 0,3,6,9 and 12 months.

#### **STUDY POPULATION**

Any child <10years old (n=100), currently attending Royal Children's hospital diabetes outpatient clinic with a history of diabetes > 2years duration. Physicians in the diabetic clinic are aware of the study and have given their permission for their patients to be approached.

<b>TIMELINE:</b>	<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>
HbA1c	+	+	+	+	+
Continuous glucose monitor	+	+	+	+	+
Auxology	+	+	+	+	+

#### **ENTRY CRITERIA**

Children aged <10years of age who have been diagnosed with insulin dependent diabetes at least 2 years prior to participation in the study.

#### **EXCLUSION CRITERIA**

Any child <10 years old with duration of diabetes <2 years.

Informed consent is to be obtained from the parents for the study. Confidentiality will be maintained for the data obtained from the results of the study.

#### **JUSTIFICATION OF SAMPLE SIZE**

N=100 patients. There are currently 115 patients on our database who fit these criteria. This is a highly motivated patient group and previous studies have recorded >90% participation rates.

#### **TREATMENT OF SUBJECTS**

Children aged less than 10years old and diagnosed  $\geq 2$  years ago, prior to study will be invited on routine attendance to clinic to participate in this project. Baseline glycaemic control will be assessed by calculation of HbA1c and 72 hour continuous glucose monitoring and repeated at 3,6,9 and 12 months.

#### **MARKERS OF GLYCAEMIC CONTROL**

**HbA1c** - is a standard laboratory test used to calculate glycaemic control over a three month period by measurement of % glycosated haemoglobin subunit A. All diabetic children have a HbA1c measurement carried out by fingerprick in outpatients on a three monthly basis. No extra measurements will be required in this study. Assay is carried out by Bayer DC2000 immunoassay.

**Continuous 72 hour glucose monitoring** - (Minimed continuous glucose sensor system) records glucose over a continuous period of 72 hours maximum using a sensor approximately the size of a 24 gauge needle inserted just beneath the skin typically in the abdominal area. A custom cable links the sensor to a monitor similar in size to the cardiac holter monitor. Patients in the diabetic clinic routinely use this monitor. Glucose readings are based on interstitial fluid glucose in the subcutaneous tissue, which have been shown to be comparable to blood glucose values. Patients will continue to test blood

sugar via finger prick during continuous glucose measurements. This method provides the current best estimate of hypoglycaemia including nocturnal hypoglycaemia.

#### **ADDITIONAL INFORMATION**

Height, weight, insulin dose, frequency of injections, recent glucometer readings, and history of recurrent hypoglycaemia are all routine elements of patient outpatient attendance. This information will also be used to identify any predisposing factors to severe or recurrent hypoglycaemia.

#### **ASSESSMENT OF SAFETY**

All tests are in current use for patients in our clinics. No change in patient treatment will be required to participate in this study. Throughout the study patients will continue to monitor their diabetes care by regular finger prick testing and insulin injections.

#### **DATA COLLECTION AND ANALYSIS**

All patients will be assessed by the same clinician throughout the studies. The research fellow will carry out data collection and analysis. Continuous data will be compared using chi-squared analysis and categorical data using the student t-test.

#### **TIME ALLOCATION**

The research is to be conducted over eighteen months, with patients being recruited into the study as near as possible to commencement. It is anticipated that total patient recruitment will take up to 6 months with assessment being completed in the subsequent twelve months.

#### **DATA HANDLING AND RECORD KEEPING.**

Data will be recorded in the hospital unit record, subject to hospital regulation as to privacy. The privacy of participants will be protected by patient data being identified by UR number and initials.

Personal information will be kept separately from data for purposes of collection of information and analysis.

Data will be pooled for analysis.

#### **ETHICAL ISSUES**

It is unlikely that adverse events will occur during this project.

Informed consent: The study will be fully explained to eligible children and their parents and signed consent obtained before any procedures are performed. Families will be told that they may withdraw from the study at any time, even after agreeing to participate. The children will be too young to give informed consent on their own behalf.

The CGMS still requires Individual Patient Usage (IPU) Approval by the Therapeutic Goods Administration. We have been using CGMS under this proviso for some time in routine clinical care in our unit. As part of this process we have had to obtain approval by the RCH Ethics committee after assessment of the CGMS by the Biomedical Engineering Department (Ethics Committee Endorsement dated 27/8/02). The CGMS information statement will be attached to the study information statement (see attachment A). We will continue to obtain IPU Approval for the study participants.

#### **FUNDING**

There is no outside sponsor for the trial.

There is no reimbursement or payment to participants.

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## ETHICS IN HUMAN RESEARCH COMMITTEE APPROVAL

**EHRC REF. No:** 22070 B

**PROJECT TITLE:** New approaches to assessing the impact of hypoglycaemia on children

**Date of Parent/Participant Information Statements and Consent Form:** P/GIS & Consent form v 2 dated 7 April 2003.

**Date of Study Protocol:** Protocol dated 7 April 2003.

**INVESTIGATOR(S):** C McDonnell, F Cameron, G Werther

**DATE OF MODIFICATION APPROVAL:** 22 April 2003

**DURATION:** 43 months

**SIGNED:** .....  
COMMITTEE REPRESENTATIVE

### APPROVED SUBJECT TO THE FOLLOWING CONDITIONS:

#### ALL PROJECTS

1. Any proposed change in protocol and the reasons for that change, together with an indication of ethical implications (if any), must be submitted to the Ethics in Human Research Committee for approval.
2. The Principal Investigator must notify the Secretary of the Ethics in Human Research Committee of:
  - Actual starting date of project.
  - Any adverse effects of the study on participants and steps taken to deal with them.
  - Any unforeseen events.
3. A progress report must be submitted annually and at the conclusion of the project, with special emphasis on ethical matters.

#### DRUG TRIALS

4. The investigators must maintain all records relating to the study for a period of 23 years.
5. The investigator(s) must report to the Sponsor and the Ethics in Human Research Committee within 24 hours of becoming aware of any serious adverse event experienced by any subject during the trial.
6. The investigators must ensure that all externally sponsored Clinical Drug Studies have insurance coverage that is current for the entirety of the study.

**TITLE**

**New approaches to assessing the impact of hypoglycaemia on the developing brain.**

**INVESTIGATORS**

Ciara McDonnell, Fergus Cameron, George Werther.

**CO-OPERATING DIVISIONS / SUPPORT SERVICES**

See part B - previously submitted

**BACKGROUND INFORMATION**

The impact of low blood glucose on the brain has arguably become the major factor influencing clinical management of Type 1 diabetes. The Diabetes Control and Complications Trial (DCCT) has shown conclusively that intensive insulin treatment is the most effective strategy for preventing the microvascular complications of type 1 diabetes, but is also associated with a three-fold increase in the incidence of severe hypoglycaemia<sup>1</sup>. The brain is critically dependent upon blood glucose for cerebral energy needs and inadequate supplies (hypoglycaemia) trigger a continuum of events ranging from confusion through seizures, coma and ultimately death unless appropriate action is taken. Neuropsychological deficits were evident in children with a history of severe hypoglycaemia involving seizure/coma in our own studies<sup>2,3,4</sup> and in reports from other centres<sup>5,6</sup>. Adults appear more resilient. These findings suggest that children are more sensitive to hypoglycaemia than adults, possibly because of the higher cerebral energy needs associated with growth and neural 'pruning' during brain development<sup>7</sup>. Individual differences in susceptibility to hypoglycaemia-related brain damage have been noted and are as yet unexplained, while the age/stage of neuromaturation at which greater resilience to hypoglycaemia is achieved has not been established. A related issue is the 'hypoglycaemic dose' required to irreversibly impair brain function. Previous studies have shown that children with early-onset diabetes exhibit deficits in information processing and executive skills<sup>4</sup> that cannot be explained by a known history of hypoglycaemic seizures or coma. Unrecognised episodes of hypoglycaemia short of seizures or coma, particularly nocturnal events, are common in very young children<sup>8</sup> and provide a plausible, but as yet unproven explanation for the 'early onset' phenomenon. Current management of type 1 diabetes emphasizes the importance of avoiding hyperglycemia (abnormally high blood glucose levels) in order to minimize the risk of diabetic complications in later life. However, tight metabolic control increases the risk of hypoglycemia (abnormally low blood sugar) which may be harmful to the developing brain. To date, the incidence of subclinical hypoglycaemia (low blood sugar without symptoms) remains undefined and risk factors for developing a major hypoglycaemic event (defined as a seizure or altered conscious state) have relied on retrospective studies and clinical observations.

**POTENTIAL RISKS AND BENEFITS**

The only risk identified in conjunction with the continuous glucose monitoring system (CGMS) is that of infection at the insertion site. Insertion of the probe will be carried out by a trained nurse educator /doctor under sterile conditions. If infection does occur (redness or tenderness at the site of probe insertion), the CGMS will be removed and the child treated with antibiotics if necessary. Patients will continue to carry out routine blood sugar measurements while the monitor is being worn and the results will be correlated. Immediate adjustment of insulin doses will be taken if any child is identified with subclinical or nocturnal hypoglycaemia.

This study will precisely define the incidence of hypoglycaemia in a paediatric population with type 1 diabetes mellitus. The information gained from this study will facilitate the development of clinical protocols aimed at balancing the respective risks of both high and low blood sugar levels, while protecting the integrity of the CNS during development. Study findings will lead to the development of treatment strategies designed to prevent hypoglycaemia.

**PROJECT SUMMARY AND TRIAL OBJECTIVES**

This study follows a group of prepubertal children (<10 years old) with established diabetes (>2 years since diagnosis) over twelve months to monitor and identify the incidence of symptomatic and asymptomatic hypoglycaemia. Previously, this information has only been available through parental observation which can be unreliable as not all hypoglycaemia causes symptoms. New technology facilitates continuous glucose monitoring (using a probe placed just under the skin for a 72hour period) which will be used at three monthly intervals to detect both symptomatic and asymptomatic



hypoglycaemia. This information will be correlated with patient glucometer readings and with routine information collected at outpatients (height, weight and HbA1c measurements). We will ask each family to complete questionnaires on behaviour and quality of life to ascertain the impact of hypoglycaemia.

#### **AIMS:**

- To assess the frequency, incidence and somatic consequences of major hypoglycaemic events and hypoglycaemia in a prepubertal population of children with type 1 diabetes mellitus.

#### Hypotheses:

- Major hypoglycaemic events and hypoglycaemia are frequent occurrences in a well-controlled population of children with type 1 diabetes mellitus.
- Symptomatic and asymptomatic hypoglycaemia occur independently of age and background HbA1c
- Recurrent hypoglycaemia may be associated with a consequent increase in body weight due to maternal overfeeding.

#### **STUDY DESIGN**

Children aged <10years old and diagnosed with diabetes >2years prior to study commencement who attend the routine outpatient diabetes clinic at the Royal Children's hospital will be asked to participate in the study. Each diabetic will have HbA1c and continuous glucose monitoring carried out at routine outpatient clinic attendance at 0,3,6,9 and 12 months.

#### **STUDY POPULATION**

Any child <10years old (n=100), currently attending Royal Children's hospital diabetes outpatient clinic with a history of diabetes > 2years duration. Physicians in the diabetic clinic are aware of the study and have given their permission for their patients to be approached.

<b>TIMELINE:</b>	<b>0</b>	<b>3</b>	<b>6</b>	<b>9</b>	<b>12</b>
HbA1c	+	+	+	+	+
Continuous glucose monitor	+	+	+	+	+
Auxology	+	+	+	+	+
BASQ	-	+	-	+	-
CHQ	-	+	-	-	-

#### **ENTRY CRITERIA**

Children aged <10years of age who have been diagnosed with insulin dependent diabetes at least 2 years prior to participation in the study.

#### **EXCLUSION CRITERIA**

Any child <10 years old with duration of diabetes <2 years.

Informed consent is to be obtained from the parents for the study. Confidentiality will be maintained for the data obtained from the results of the study.

#### **JUSTIFICATION OF SAMPLE SIZE**

N=100 patients. There are currently 115 patients on our database who fit these criteria. This is a highly motivated patient group and previous studies have recorded >90% participation rates.

#### **TREATMENT OF SUBJECTS**

Children aged less than 10years old and diagnosed  $\geq 2$  years ago, prior to study will be invited on routine attendance to clinic to participate in this project. Baseline glycaemic control will be assessed by calculation of HbA1c and 72 hour continuous glucose monitoring and repeated at 3,6,9 and 12 months.

#### **MARKERS OF GLYCAEMIC CONTROL**

**HbA1c** - is a standard laboratory test used to calculate glycaemic control over a three month period by measurement of % glycosated haemoglobin subunit A. All diabetic children have a HbA1c measurement carried out by fingerprick in outpatients on a three monthly basis. No extra measurements will be required in this study. Assay is carried out by Bayer DC2000 immunoassay.

**Continuous 72 hour glucose monitoring** - (Minimed continuous glucose sensor system) records glucose over a continuous period of 72 hours maximum using a sensor approximately the size of a 24



gauge needle inserted just beneath the skin typically in the abdominal area. A custom cable links the sensor to a monitor similar in size to the cardiac holter monitor. Patients in the diabetic clinic routinely use this monitor. Glucose readings are based on interstitial fluid glucose in the subcutaneous tissue, which have been shown to be comparable to blood glucose values. Patients will continue to test blood sugar via finger prick during continuous glucose measurements. This method provides the current best estimate of hypoglycaemia including nocturnal hypoglycaemia.

#### **ADDITIONAL INFORMATION**

Height, weight, insulin dose, frequency of injections, recent glucometer readings, and history of recurrent hypoglycaemia are all routine elements of patient outpatient attendance. This information will also be used to identify any predisposing factors to severe or recurrent hypoglycaemia. The BASQ and CHQ are standardised, validated, parent-report instruments that assess behavioural and functional health status in children. Both questionnaires have been adapted and validated for use with Australian children<sup>9</sup>.

#### **ASSESSMENT OF SAFETY**

All tests are in current use for patients in our clinics. No change in patient treatment will be required to participate in this study. Throughout the study patients will continue to monitor their diabetes care by regular finger prick testing and insulin injections.

#### **DATA COLLECTION AND ANALYSIS**

All patients will be assessed by the same clinician throughout the studies. The research fellow will carry out data collection and analysis. Continuous data will be compared using chi-squared analysis and categorical data using the student t-test.

#### **TIME ALLOCATION**

The research is to be conducted over eighteen months, with patients being recruited into the study as near as possible to commencement. It is anticipated that total patient recruitment will take up to 6 months with assessment being completed in the subsequent twelve months.

#### **DATA HANDLING AND RECORD KEEPING.**

Data will be recorded in the hospital unit record, subject to hospital regulation as to privacy.

The privacy of participants will be protected by patient data being identified by UR number and initials.

Personal information will be kept separately from data for purposes of collection of information and analysis.

Data will be pooled for analysis.

#### **ETHICAL ISSUES**

It is unlikely that adverse events will occur during this project.

**Informed consent:** The study will be fully explained to eligible children and their parents and signed consent obtained before any procedures are performed. Families will be told that they may withdraw from the study at any time, even after agreeing to participate. The children will be too young to give informed consent on their own behalf.

The CGMS still requires Individual Patient Usage (IPU) Approval by the Therapeutic Goods Administration. We have been using CGMS under this proviso for some time in routine clinical care in our unit. As part of this process we have had to obtain approval by the RCH Ethics committee after assessment of the CGMS by the Biomedical Engineering Department (Ethics Committee Endorsement dated 27/8/02). The CGMS information statement will be attached to the study information statement (see attachment A). We will continue to obtain IPU Approval for the study participants.

#### **FUNDING**

There is no outside sponsor for the trial.

There is no reimbursement or payment to participants.

#### **REFERENCES**

1. The Diabetes Control and Complications Trial Research Group: Effects of intensive diabetes therapy on neuropsychological function in adults in the diabetes control and complications trial. *Annals of Internal Medicine*, 124: 379-388, 1996

## PARENT / GUARDIAN INFORMATION STATEMENT

Project No 22070A

Version 2 Date 07.04.2003

### **Title of Project**

The impact of hypoglycaemia on children

### **Thank you for taking the time to read this Information Statement.**

This information statement is 4 pages long. Please make sure you have all the pages.

### **For people who speak languages other than English:**

**you would also like information about the research and the Consent Form in your language, please ask the person explaining this project to you.**

**Our child is invited to participate in a Research Project that is explained below.**

### **What is an Information Statement ?**

These pages contain information about a research project we are inviting you and your child to take part in. The purpose of this information is to explain to you clearly and openly all the steps and procedures of this project. The information is to help you to decide whether or not you and/or your child would like to take part in the research.

Please read this information carefully. You can ask us questions about anything in it. You may also wish to talk about the project with others eg. friends or a health care worker. When you understand what the project is about, you can sign the consent form attached if you agree for your child to take part. You will be given a copy of this information and the consent form to keep.

### **What is the Research Project about?**

Almost all children with diabetes experience episodes of hypoglycaemia (low blood sugar). If hypoglycaemia is not treated promptly, children may become confused or have a seizure. Young children seem to be more affected by hypoglycaemia than adults. This project will study how often hypoglycaemia occurs at different ages during childhood. The study will help doctors understand better how often mild, moderate and severe hypoglycaemia occurs and which age groups are most affected.

### **Who are the Researchers?**

Dr. Ciara McDonnell, Professor George Werther and Dr Fergus Cameron from the Royal Children's Hospital will conduct the study.

### **Why am I and my child being asked to be in this research project?**

You and your child have been invited to take part in this study because your child has diabetes and is at risk of

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having episodes of hypoglycaemia

### **What are my child's alternatives to participating in this project?**

Your child may choose to have the continuous glucose monitor attached on one occasion only or to continue to monitor his/her sugars using the glucometer.

### **What does my child need to do to be in this research project?**

If you agree for your child to take part in this study, then, every three months over the next year, we will ask your child to wear a continuous glucose monitor for 72 hours. This monitor measures glucose levels in the body through a probe which is inserted into the skin at the waistline. The probe is inserted using a needle which will immediately be removed and disposed of. Your child will attend clinic as normal on a three monthly basis and have height, weight and HbA1c level measured. We will ask you to complete two questionnaires regarding behaviour and quality of life during your clinic attendance. Each questionnaire takes approximately 20 minutes. We will time the monitor use to coincide with your usual clinic visit but we will require you to attend on two occasions in the week (once to connect the monitor and once to remove it). The monitor will measure your child's blood sugar level continuously for the 72 hour period. This will tell us if your child is having low blood sugars during the night. We will explain exactly how the monitor works and your child can have local anaesthetic cream applied to the region of skin on the stomach before the probe is inserted. If your child becomes upset at any point, we will not continue with the monitor. Each child is still asked to monitor their blood sugar levels using their glucometer while the monitor is in place.

### **Is there likely to be a benefit to my child?**

There may not be a direct benefit to your child, although better understanding of hypoglycaemia and how it affects children may be important in the care of your child in the future. The results of each monitor recording will be discussed with you and adjustments made to your child's insulin dosage if any hypoglycaemia is detected.

### **Is there likely to be a benefit to other children in the future?**

Yes, the results of this study will provide information that will help doctors care for other children with diabetes.

### **What are the possible risks and/or side effects for my child?**

Apart from the minor discomfort of the insertion of the electrode there is no expected discomfort associated with the device. We encourage normal daily activities, though contact sports should be avoided for the 72 hours that the meter is connected. No body fluids are removed from the body and no electrical currents are sent into the body by the CGMS.

Whenever any foreign material is inserted into the body there is a small risk of infection. This risk will be minimised by having a trained nurse educator/doctor performing the insertion under sterile conditions. If infection were to occur (the area around the electrode would become red and painful), the CGMS would be removed and your child would, if necessary, be treated with antibiotics. The electrode will be removed by hospital staff (or alternatively can be removed by you/your child) at any time if there is concern, although this will prevent continuation of glucose monitoring from that time.

### **What are the possible discomforts and/or inconveniences for me or my child?**

The only inconvenience noted is the necessity of two visits per week every three months to allow connection and disconnection of the monitor. Some children may find the insertion of the probe uncomfortable, which is why we will offer the use of local anaesthetic cream.

### **What will be done to make sure the information is confidential?**

Each child will be assigned a number when they enter the study. All analyses of the continuous glucose monitors will be identified through this number, rather than by individual names and results will be entered into the computer using numbers rather than names. There will be one list that matches children's names with their number and this will be stored in a separate file on the computer. Computer files will have passwords, known



only to people working on the study. We will not discuss your child's test results with anybody other than ourselves unless you ask us to do so in writing.

### **Will I be informed of the results when the research project is finished?**

A statement of the group results will be sent out to each family at the conclusion of the study. This group report will not contain the names of individual children

**You can decide whether or not you give permission for your child to take part in this research project.**

**You can decide whether or not you would like to withdraw your child from this research project at any time. No explanation is needed.**

You may like to discuss your participation in this research project with your family and with your doctor. You can ask for further information before deciding if your child will take part.

### **If you would like more information about the study or if you need to contact a study representative in an emergency, the person to contact is :**

Name: **Ciara McDonnell**

Contact telephone: Business Hours (03) 9345 5981

After Hours (0415) 778 470 mobile

### **What are my child's rights as a Participant?**

1. I am informed that except where stated above, no information regarding my child's medical history will be released. This is subject to legal requirements.
2. I am informed that the results of any tests involving my child will not be published so as to reveal my child's identity. This is subject to legal requirements.
3. The detail of the procedure proposed has also been explained to me. This includes how long it will take, how often the procedure will be performed and whether any discomfort will result.
4. It has also been explained that my child's involvement in the research may not be of any benefit to him or her. I understand that the purpose of this research project is to improve the quality of medical care in the future.
5. I have been asked if I would like to have a family member or a friend with me while the project is explained to me.
6. I understand that this project follows the guidelines of the National Statement on Ethical Conduct in Research Involving Humans (1999).
7. I understand that this research project has been approved by the Royal Children's Hospital Ethics in Human Research Committee on behalf of Women's and Children's Health Board.
8. I have received a copy of this document.

If you have any concerns about the study, and would like to speak to someone independent of the study, please contact Consumer Liaison, Clinical Support Services Team at the Executive Office, RCH. Telephone 9345 5676 (Monday to Friday 9am-5pm).

**STANDARD INFORMED CONSENT  
FOR PARENT / GUARDIAN TO GIVE CONSENT  
FOR THEIR CHILD TO PARTICIPATE IN A RESEARCH PROJECT**  
(Attach to Parent Information Statement)

**Project No** 22070A

Version 2 Dated 07.04.2003

**Title of Project**

The impact of hypoglycaemia on children

**Principal Investigator(s)** Dr. Ciara McDonnell, Prof. George Werther, Dr. Fergus Cameron.

(Parent/Guardian name) \_\_\_\_\_

Parent / Guardian of (child's name) \_\_\_\_\_

I voluntarily consent to him / her taking part in the above titled Research Project, explained to me by

Mr / Ms / Dr / Professor

- I have received a Parent/Guardian Information Statement to keep and I believe I understand the purpose, extent and possible effects of my child's involvement
- I have been asked if I would like to have a family member or friend with me while the project was explained
- I have had an opportunity to ask questions and I am satisfied with the answers I have received
- I understand that the researcher has agreed not to reveal results of any information involving me/my child, subject to legal requirements
- If information about this project is published or presented in any public form, I understand that the researcher will not reveal my/my child's identity
- I understand that if I refuse to consent, or if I withdraw my child from the study at any time without explanation, this will not affect my child's access to the best available treatment options and care from Women's and Children's Health (The Royal Women's Hospital OR The Royal Children's Hospital).
- I understand I will receive a copy of this consent form.

**PARENT GUARDIAN SIGNATURE** \_\_\_\_\_ **Date** \_\_\_\_\_

**I have explained the study to the parent/guardian who has signed above, and believe that they understand the purpose, extent and possible effects of their involvement in this study.**

**RESEARCHER'S SIGNATURE** \_\_\_\_\_ **Date** \_\_\_\_\_

*Note:* All parties signing the Consent Form must date their own signature.



## PARENT / GUARDIAN INFORMATION STATEMENT

Project No 22070A

### **Title of Project**

The impact of hypoglycaemia on children

### **Thank you for taking the time to read this Information Statement.**

This information statement is 3 pages long. Please make sure you have all the pages.

### **Your child is invited to participate in a Research Project that is explained below.**

#### **What is the Research Project about?**

Almost all children with diabetes experience episodes of hypoglycaemia (low blood sugar). If hypoglycaemia is not treated promptly, children may become confused or have a seizure. Young children seem to be more affected by hypoglycaemia than adults. This project will study how often hypoglycaemia occurs at different ages during childhood. The study will help doctors understand better how often mild, moderate and severe hypoglycaemia occurs and which age groups are most affected.

#### **Who are the Researchers?**

Dr. Ciara McDonnell, Professor George Werther and Dr Fergus Cameron from the Royal Children's Hospital will conduct the study.

#### **Why am I and my child being asked to be in this research project?**

You and your child have been invited to take part in this study because your child has diabetes and is at risk of having episodes of hypoglycaemia

#### **What does my child need to do to be in this research project?**

If you agree for your child to take part in this study, then, every three months over the next year, we will ask your child to wear a continuous glucose monitor for 72 hours. This monitor measures glucose levels in the body through a probe which is inserted into the skin at the waistline. The probe is inserted using a needle which will immediately be removed and disposed of. Your child will attend clinic as normal on a three monthly basis and have height, weight and HbA1c level measured. We will time the monitor use to coincide with your usual clinic visit but we will require you to attend on two occasions in the week (once to connect the monitor and once to remove it). The monitor will measure your child's blood sugar level continuously for the 72 hour period. This will tell us if your child is having low blood sugars during the night. We will explain exactly how the monitor works and your child can have local anaesthetic cream applied to the region of skin on the stomach before the probe is inserted. If your child becomes upset at any point, we will not continue with the monitor. Each child is asked to monitor their blood sugar levels using their glucometer while the monitor is in place.

#### **Is there likely to be a benefit to my child?**

There may not be a direct benefit to your child, although better understanding of hypoglycaemia and how it affects children may be important in the care of your child in the future. The results of each monitor recording will be discussed with you and adjustments made to your child's insulin dosage if any hypoglycaemia is detected.



**Is there likely to be a benefit to other children in the future?**

Yes, the results of this study will provide information that will help doctors care for other children with diabetes.

**What are the possible risks and/or side effects for my child?**

Apart from the minor discomfort of the insertion of the electrode there is no expected discomfort associated with the device. We encourage normal daily activities, though contact sports should be avoided for the 72 hours that the meter is connected. No body fluids are removed from the body and no electrical currents are sent into the body by the CGMS.

Whenever any foreign material is inserted into the body there is a small risk of infection. This risk will be minimised by having a trained nurse educator/doctor performing the insertion under sterile conditions. If infection were to occur (the area around the electrode would become red and painful), the CGMS would be removed and your child would, if necessary, be treated with antibiotics. The electrode will be removed by hospital staff (or alternatively can be removed by you/your child) at any time if there is concern, although this will prevent continuation of glucose monitoring from that time.

**What are the possible discomforts and/or inconveniences for me or my child?**

The only inconvenience noted is the necessity of two visits per week every three months to allow connection and disconnection of the monitor. Some children may find the insertion of the probe uncomfortable, which is why we will offer the use of local anaesthetic cream.

**What will be done to make sure the information is confidential?**

Each child will be assigned a number when they enter the study. All analyses of the continuous glucose monitors will be identified through this number, rather than by individual names and results will be entered into the computer using numbers rather than names. There will be one list that matches children's names with their number and this will be stored in a separate file on the computer. Computer files will have passwords, known only to people working on the study. We will not discuss your child's test results with anybody other than ourselves unless you ask us to do so in writing.

**Will I be informed of the results when the research project is finished?**

A statement of the group results will be sent out to each family at the conclusion of the study. This group report will not contain the names of individual children.

**You can decide whether or not you give permission for your child to take part in this research project.****You can decide whether or not you would like to withdraw your child from this research project at any time. No explanation is needed.**

You may like to discuss your participation in this research project with your family and with your doctor. You can ask for further information before deciding if your child will take part.

**The name and telephone number of the person to contact for more information or in an emergency is:**

Dr. Ciara McDonnell at (03) 9345 5522 pager 5952

**For parents/guardians who speak languages other than English**

**If you would also like information about the research and the Consent Form in your language, please ask for it to be provided.**

## What are my child's rights as a Participant?

I am informed that except where stated above, no information regarding my child's medical history will be released. This is subject to legal requirements.

I am informed that the results of any tests involving my child will not be published so as to reveal my child's identity. This is subject to legal requirements.

The detail of the procedure proposed has also been explained to me. This includes how long it will take, how often the procedure will be performed and whether any discomfort will result.

It has also been explained that my child's involvement in the research may not be of any benefit to him or her. I understand that the purpose of this research project is to improve the quality of medical care in the future.

I have been asked if I would like to have a family member or a friend with me while the project is explained to me.

I understand that this project follows the guidelines of the National Statement on Ethical Conduct in Research Involving Humans (1999).

I understand that this research project has been approved by the Royal Children's Hospital Ethics in Human Research Committee on behalf of Women's and Children's Health Board.

I have received a copy of this document.

**If you have any questions about patient rights contact**

<p><b>The RCH Patient Representative RCH Hospital Support Unit Phone 9345 5676</b></p>
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**STANDARD INFORMED CONSENT  
FOR PARENT / GUARDIAN TO GIVE CONSENT  
FOR THEIR CHILD TO PARTICIPATE IN A RESEARCH PROJECT**

Project No 22070A

**Title of Project**

The impact of hypoglycaemia on children

**Principal Investigator(s)** Dr. Ciara McDonnell, Dr. Fergus Cameron, Prof. George Werther

**Brief outline of research project including benefits, possible risks, inconveniences and discomforts** (12 lines maximum)

We are inviting you and your child to take part in a study examining the impact of hypoglycaemia on children. If you agree to take part, we will arrange for your child to have continuous glucose monitoring every three months for the next year. Your child will attend clinic as normal on a three monthly basis and have height, weight and HbA1c level measured. During the clinic visit, we will attach the monitor by inserting a probe under the skin at the level of the waistline. During the monitoring your child will continue to test blood sugars using the glucometer. After three days your child will return to have the monitor removed. This test is completely safe.

(Parent/Guardian name) \_\_\_\_\_

Parent / Guardian of (child's name) \_\_\_\_\_

I voluntarily consent to him / her taking part in the above titled Research Project, explained to me by

Mr / Ms / Dr / Professor \_\_\_\_\_

**I have received a Parent/Guardian Information Statement to keep and I believe I understand the purpose, extent and possible effects of my child's involvement. I have been asked if I would like to have a family member or friend with me while the project was explained.**

**I understand that if I refuse to consent, or withdraw my child from the study at any time without explanation, this will not affect my child's access to the best available treatment and care from Women's and Children's Health. (The Royal Women's Hospital OR The Royal Children's Hospital). I understand I will receive a copy of this consent form.**

**PARENT GUARDIAN SIGNATURE** \_\_\_\_\_

Date \_\_\_\_\_

**I have explained the study to the participant who has signed above, and believe that they understand the purpose, extent and possible effects of their involvement in this study.**

**RESEARCHER'S SIGNATURE** \_\_\_\_\_

Date \_\_\_\_\_





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Victoria, Australia, 3052

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Facsimile (03) 9345 5789

**Parent information statement regarding your child  
and the Minimed continuous glucose monitoring system (CGMS)**

Your doctor has recommended that continuous glucose monitoring would be helpful in the clinical care of you/your child. Currently there is only one continuous glucose monitoring system (CGMS) available in Australia. This system is made by an American company called Minimed. Whilst the Minimed CGMS has the approval of the Food and Drug Administration (FDA) in America, it has not been submitted for approval by the Therapeutic Goods Administration (TGA) in Australia for commercial reasons. Because of this, the Minimed CGMS cannot be used at the Royal Children's Hospital without your consent. In addition, the Biomedical Engineering Department and The Human Ethics Committee at the Royal Children's Hospital have reviewed the use of the Minimed CGMS. Finally, your doctors must apply to the TGA for an authority to use this in individual patients.

**CGMS-what is involved?**

Your child will be required to attend a brief outpatient visit to have the CGMS connected by a Diabetes Nurse Educator/Doctor. The Nurse Educator/Doctor will insert a subcutaneous needle into your child's tummy wall or hip. The needle will be removed leaving a small (approximately twice the of an insulin needle) teflon electrode which sits under the skin for 72 hours. The electrode is attached to a wire that is attached to a small meter (the size of a small mobile phone). The electrode is covered by a transparent adhesive dressing. Your child will wear the meter in a small harness clipped to their belt continuously for 72 hours. Every 10 seconds the glucose levels in the tissue under the skin will be measured by the electrode, and then 5 minutes average measurements are recorded in the meter. The meter is soundless. The meter DOES NOT give real time measurements and is NOT connected to an alarm should the glucose levels go too high or low. It does NOT show readings while it is being worn. The meter can only be downloaded or read after the system is disconnected. Your child will still need to use their normal blood glucose monitors.

**How will CGMS help my child?**

CGMS will provide a very accurate picture of the glucose levels in your child over a 72 hour period. It will allow your doctors to treat your child in a way that should minimise major swings in glucose levels.

**What are the risks/potential side effects for my child?**

Apart from the minor discomfort of the insertion of the electrode there is no expected discomfort associated with the device. We encourage normal daily activities, though contact sports should be avoided for the 72 hours that the meter is connected. No body fluids are removed from the body and no electrical currents are sent into the body by the CGMS.

Whenever any foreign material is inserted into the body there is a small risk of infection. This risk will be minimised by having a trained nurse educator/doctor performing the insertion under sterile conditions. If infection were to occur (the area around the electrode would become red and painful), the CGMS would be removed and your child would, if necessary, be treated with antibiotics. Any concerns should be dealt with by contacting the Diabetes Nurse Educator (9am-5pm Monday to Friday) or the Diabetes Research Fellow (after regular working hours) by telephoning 9345 5522. The electrode will be removed by hospital staff (or alternatively can be removed by you/your child) at any time if there is concern, although this will prevent continuous glucose monitoring from that time.

Dr Fergus Cameron,  
Deputy Director,  
Department of Endocrinology and Diabetes,  
27/8/02.

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**Appendix 2: Demographics record form for each subject.**



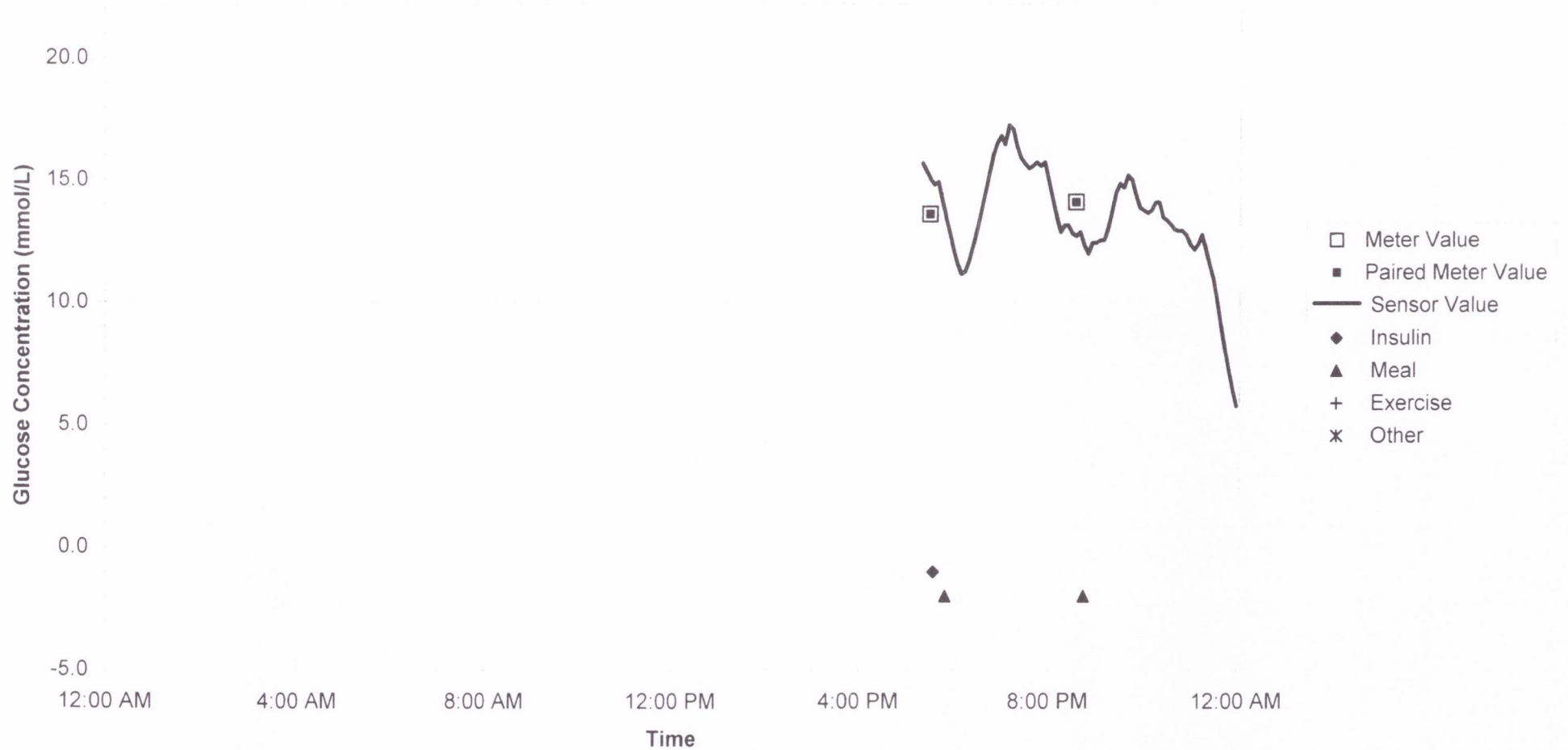


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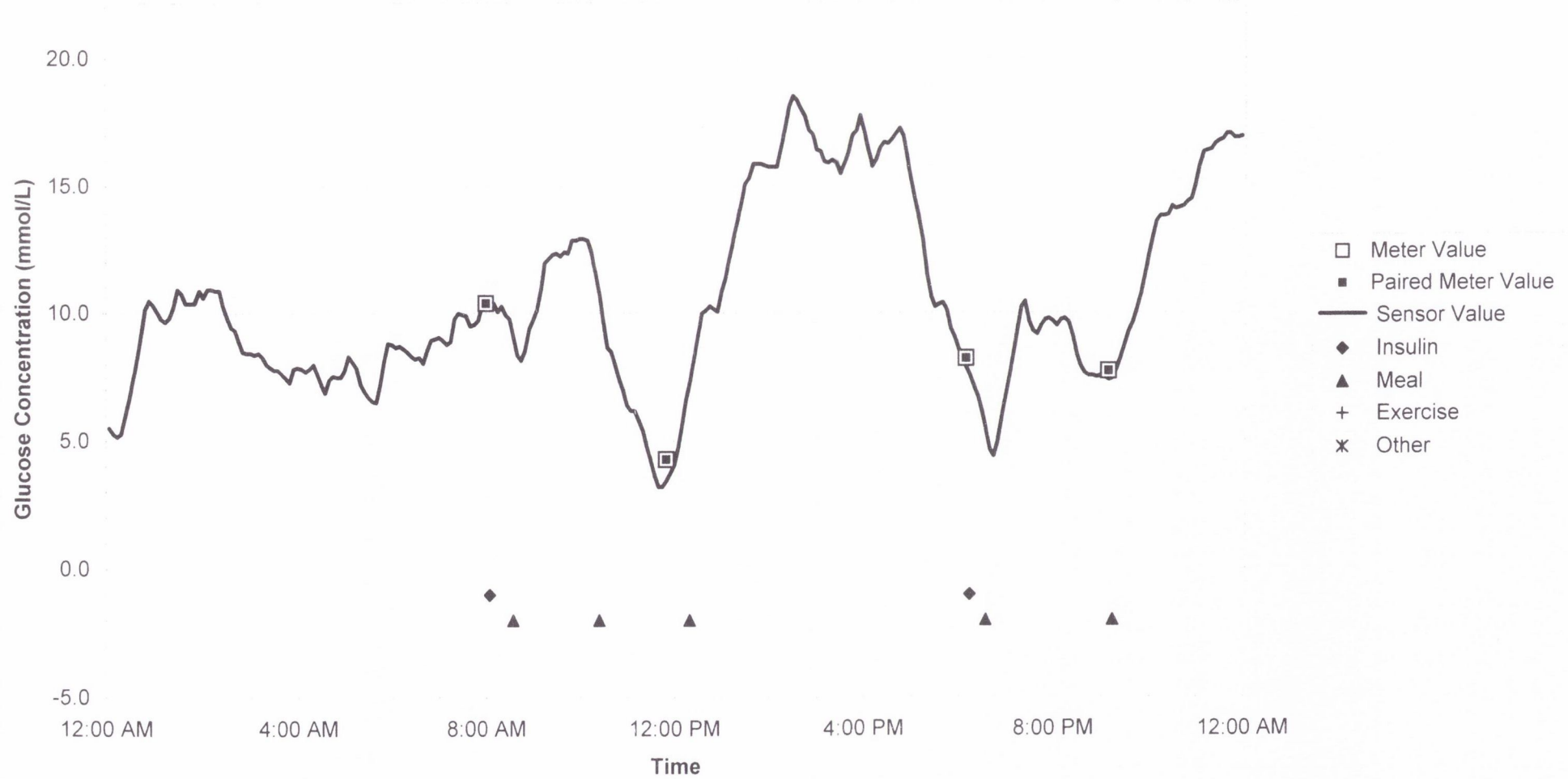
**Appendix 3: Sample trace from the Minimed® continuous  
glucose monitoring system.**

# Glucose Sensor Profile: 06-Jan-04 x

\*x: This day does not satisfy the criteria for optimal accuracy as indicated by the shaded entries in the summary table. Please use your clinical judgment in evaluating the graph.

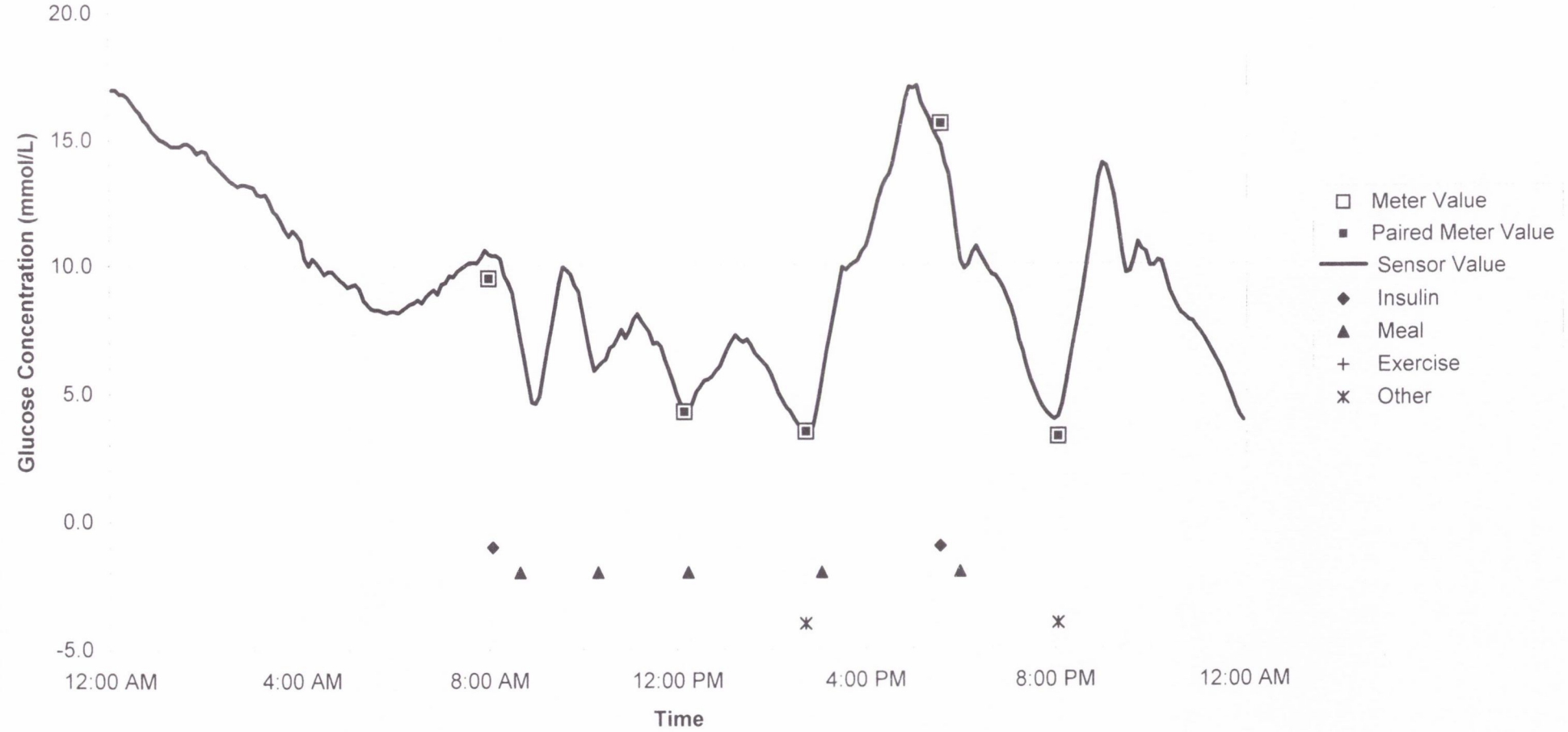


# Glucose Sensor Profile: 07-Jan-04



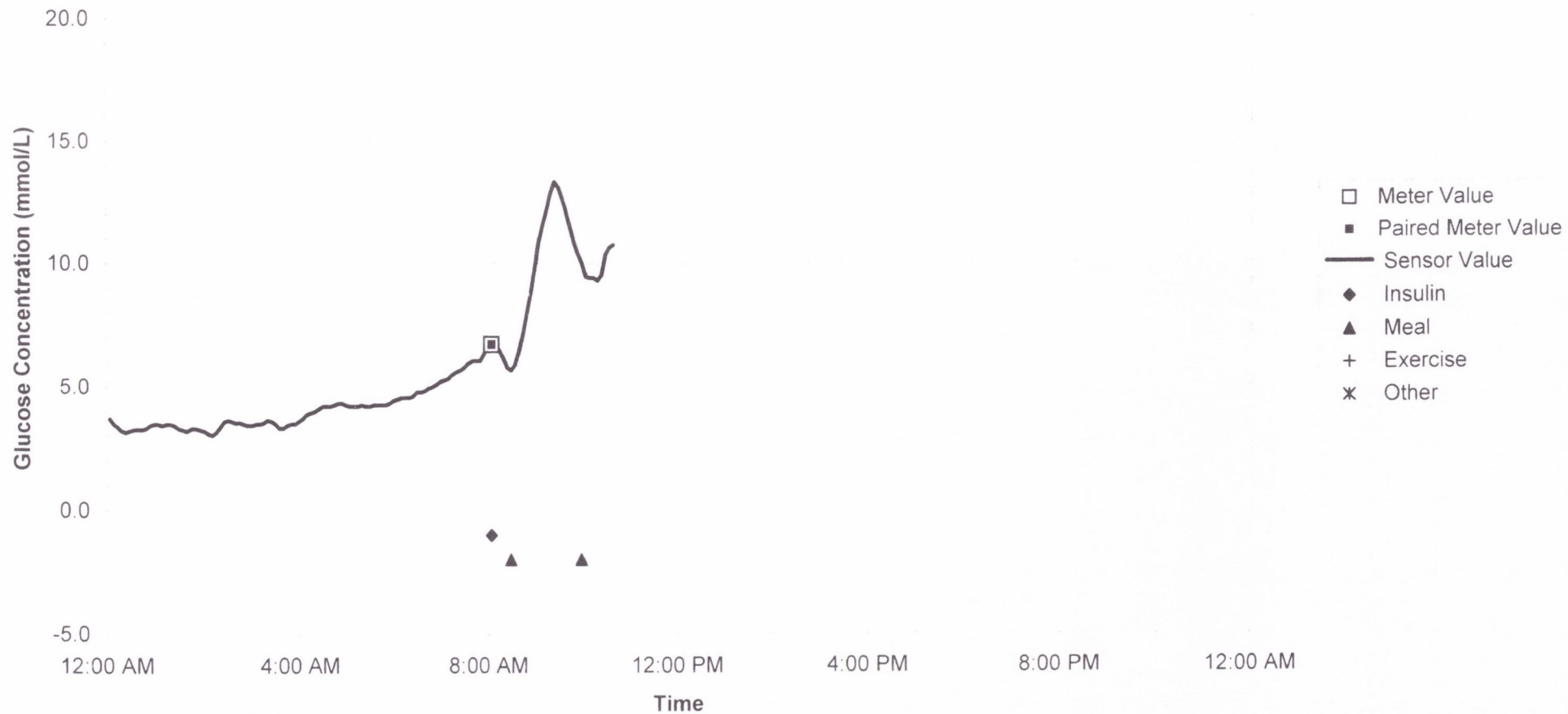


# Glucose Sensor Profile: 08-Jan-04



# Glucose Sensor Profile: 09-Jan-04 x

\*x: This day does not satisfy the criteria for optimal accuracy as indicated by the shaded entries in the summary table. Please use your clinical judgment in evaluating the graph.



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## **Appendix 4: A novel approach to continuous glucose**

**analysis utilising glycaemic variation.**



# A Novel Approach to Continuous Glucose Analysis Utilizing Glycemic Variation

C.M. MCDONNELL, M.B.B.S., M.R.C.P.I.,<sup>1,\*</sup> S.M. DONATH, M.A.,<sup>2</sup>  
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and F.J. CAMERON, M.D., F.R.A.C.P.<sup>1</sup>

## ABSTRACT

**Background:** Various methodologies have been proposed for analysis of continuous glucose measurements. These methods have mainly focused on the proportion of low or high glucose readings and have not attempted to analyze other dimensions of the data obtained. This study proposes an algorithm for analysis of continuous glucose data including a novel method of assessing glycemic variability.

**Methods:** Mean blood glucose and mean of daily differences (MODD) assessed the degree that the Continuous Glucose Monitoring System (CGMS<sup>®</sup>, Medtronic MiniMed, Northridge, CA) trace was representative of the 3-month glycemic pattern. Percentages of times in low, normal, and high glucose ranges were used to assess marked glycemic excursion. Continuous overall net glycemic action (CONGA), a novel method developed by the authors, assessed intra-day glycemic variability. These methods were applied to 10 CGMS traces chosen randomly from those completed by children with type 1 diabetes from the Royal Children's Hospital, Melbourne, Victoria, Australia and 10 traces recorded by healthy volunteer controls.

**Results:** The healthy controls had lower values for mean blood glucose, MODD, and CONGA. Patients with diabetes had higher percentages of time spent in high and low glucose ranges. There was no overlap between the CONGA values for patients with diabetes and for controls, and the difference between controls and patients with diabetes increased markedly as the CONGA time period increased.

**Conclusions:** We advocate an approach to the analysis of CGMS data based upon a hierarchy of relevant clinical questions alluding to the representative nature of the data, the amount of time spent in glycemic excursions, and the degree of glycemic variation. Integrated use of these algorithms distinguishes between various patterns of glycemic control in those with and without diabetes.

## INTRODUCTION

**T**HE AIM OF DIABETES THERAPY is to maintain blood glucose levels as close to normal as possible without compromising patient safety due to hypoglycemia.<sup>1</sup> Daily self-monitoring of

blood glucose levels provides a limited view of glycemic control, while glycosylated hemoglobin (HbA1c) has proved to be a reliable marker reflecting average glucose control over a 2–3-month period.<sup>2</sup> Several methodologies have been proposed for the analysis of the intermit-

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tent glucose measures produced by daily self-monitoring. Kovatchev et al.,<sup>3,4</sup> for instance, have developed various indices for summarizing non-continuous data downloaded from a patient's glucose meter, including low blood glucose index, high blood glucose index, blood glucose risk index, and blood glucose rate of change.

In the past 10 years, various technologies allowing ambulatory continuous glucose measurement have become available. Such continuous glucose monitoring has demonstrated the wide degree of glycemic variation occurring in children with type 1 diabetes, even those with excellent HbA1c levels.<sup>5-7</sup> Whilst the hardware aspect of continuous glucose sensing has attained a reasonable degree of reliability and reproducibility, methods to analyze continuous glucose data are yet to be fully developed; methods suitable for analyzing intermittent data do not fully utilize the wealth of information provided by continuous glucose monitoring.

The purpose of this paper was to develop a new approach to the interpretation of continuous glucose data, utilizing the data obtained from a continuous glucose monitoring system. The method proposed is to answer a series of relevant hierarchical clinical questions:

1. How representative are the continuous glucose monitoring system data of average glycemic control within a 3-month period?
2. What is the amount of time with marked glycemic excursions?
3. What is the lability/variation of glycemic control?

This paper addresses these questions using some established methods and a novel algorithm and demonstrates their application in cohorts with and without diabetes.

## PATIENTS AND METHODS

The continuous glucose monitor utilized in this study was the Medtronic MiniMed (Northridge, CA) CGMS<sup>®</sup>. The methods involving monitor application and data retrieval have been described in detail elsewhere.<sup>8</sup> Limitations to the MiniMed CGMS include the in-

ability to measure interstitial tissue glucose values below 2.2 mmol/L or above 22.2 mmol/L. Data were downloaded using MiniMed Solutions version 1.7A.

CGMS recordings were obtained over 72-h periods from 10 children with type 1 diabetes (age range, 9.3–19.5 years) randomly chosen from the diabetes clinic at the Royal Children's Hospital (RCH), Melbourne, Victoria, Australia and from 10 healthy, adult controls without diabetes (age range, 30.0–46.5 years). Each CGMS trace was calibrated by a minimum of four finger-prick blood glucose measurements per 24-h period. A calibration point had to be carried out at least every 8 h for the data to be included in the analyses. Data cleaning entailed confirmation of regular calibration, identification of errors with paired sensor values, and review of missing data points or time points and was carried out using Stata<sup>™</sup> statistical software (Stata Corp., College Station, TX).

### *Representative nature of the CGMS trace*

To assess how representative a 72-h CGMS trace was for the average 3-month glycemic pattern of each patient we calculated the mean CGMS glucose value for the trace and the degree of inter-day glycemic variability. The mean CGMS glucose value was calculated as the arithmetic mean of glucose values within a given period.<sup>9</sup> HbA1c was assessed using the Bayer DCA 2000 immunoagglutination method (Calabria, Barcelona, Spain). Inter-day glycemic variation was assessed using the mean of daily differences (MODD). The absolute value of the difference between glucose values taken on two consecutive days at the same time was calculated; the MODD is the mean of these differences.<sup>10</sup> All readings of the trace where there was a reading 24 h previous were included in the calculation of the MODD.

### *Glycemic excursions*

To assess the amount of time of marked glycemic excursion over the duration of the trace, the percentage of time was calculated for three defined glucose ranges after data cleaning: low (CGMS value <4 mmol/L), normal (CGMS value 4–12 mmol/L), and high (CGMS value >12 mmol/L).

## Glycemic lability

When previous methods [M-value<sup>11</sup> and mean amplitude of glycemic excursions (MAGE)<sup>12</sup>] for assessing glycemic variation were evaluated, neither was found to be suitable for application to CGMS. A novel method, which describes intraday glycemic variation, has been devised by the authors. This measure has been named continuous overlapping net glycemic action (CONGA). For each observation after the first  $n$  hours of observations, the difference between the current observation and the observation  $n$  hours previous was calculated.  $CONGA_n$  was defined as the

standard deviation of the differences. Higher CONGA values therefore indicate greater glycemic variation. The choice of the time difference,  $n$ , will depend on the clinical question being addressed. We present results for CONGA1, CONGA2, and CONGA4, the time periods 1 h, 2 h, and 4 h, corresponding approximately to time between different activities in school, time between snacks, and time between meals. We also present results showing the relationship between CONGA values and the CONGA time period,  $n$ .

All formulae are listed in Table 1. Use of the Minimed CGMS at RCH has been approved by the RCH Human Research Ethics Committee.

TABLE 1. DEFINITION OF THE FORMULAE USED TO ASCERTAIN GLUCOSE VALUES

Name	Formula	
Mean glucose	$\frac{\sum_{t=t_1}^{t_k} GR_t}{k}$	$k$ = number of observations (number of glucose readings for a given individual)
Adjusted M-value	$M_{GR} + M_W$ <p>where</p> $M_{GR} = \frac{\sum_{t=t_1}^{t_k} \left  10 \times \log \frac{GR_t}{IGV} \right ^3}{n}$ <p>and</p> $M_W = \frac{G_{max} - G_{min}}{20}$	$M_{GR}$ = M-value for glucose readings $M_W$ = correction factor for $n < 24$ $IGV$ = ideal glucose value (arbitrary number) $G_{max}$ = maximum glucose reading $G_{min}$ = minimum glucose reading
"J"-index	$J = 0.324 \times (MBG + SD)^2$	$MBG$ = mean glucose levels $SD$ = standard deviation of glucose levels
MAGE	$\sum \frac{\lambda}{x}$ <p>if <math>\lambda &gt; v</math></p>	$\lambda$ = each blood glucose decrease from peak to nadir $x$ = number of valid observations $v$ = 1 SD of mean glucose for 24-h period
MODD	$\frac{\sum_{t=t_1}^{t_{k^*}}  GR_t - GR_{t-1440} }{k^*}$	$k^*$ = number of observations where there is an observation at the same time 24 h ago
CONGA $_n$	$\sqrt{\frac{\sum_{t=t_1}^{t_{k^*}} (D_t - \bar{D})^2}{k^* - 1}}$ <p>where</p> $D_t = GR_t - GR_{t-m}$ <p>and</p> $\bar{D} = \frac{\sum_{t=t_1}^{t_{k^*}} D_t}{k^*}$	$k^*$ = number of observations where there is an observation $n \times 60$ min ago $m = n \times 60$

Note that  $GR_t$  glucose reading at time  $t$  min after start of observations and  $t_i$  = time in minutes after start of observations of the  $i^{\text{th}}$  observation.



All calculations were performed using STATA version 8.0.

## RESULTS

For clarity the data are summarized according to the clinical questions posed.

*How representative are the CGMS data of average glycemic control within the same 3 months?*

The HbA1c values for the cohort with diabetes ranged from 6.6% to 9.9%, which showed them to have a wide range of metabolic control such as is evident in most diabetes clinics. Mean blood glucose was calculated as a measure of glycemic control within the CGMS trace. The data in Table 2 show a wide variety of mean CGMS glucose values in the cohort with diabetes (6.0–16.2 mmol/L) compared with the relatively narrow range in the cohort without diabetes (4.8–5.8 mmol/L).

Consistency between days on a CGMS trace was assessed using the MODD. The mean MODD value for the patients with diabetes was

4.3 (range 2.9–8.1), whereas the mean in the healthy controls was 0.8 (range 0.5–1.2). A MODD value less than 1.0 means that the pattern of the trace was very similar on each of the 3 days recorded for that individual.

*What is the amount of time of marked glycemic excursions?*

The amount of time of marked glycemic excursion was based on the percentage of time spent in the high or low glucose ranges. Table 2 shows that none of the healthy control traces recorded high CGMS values (>12 mmol/L) and only 0–20.5% of time was spent with low CGMS values (<4 mmol/L). None of the healthy controls experienced symptoms of hypoglycemia. By contrast, the traces from the patients with diabetes recorded percentage of time rates of 2.3–88.9% in the high range and 0–29.1% in the low range.

*What is the lability/variation of glycemic control?*

The lability of glycemic control within a CGMS trace was assessed by calculating the

TABLE 2. COMPARISON OF CONTINUOUS TRACES FROM CONTROLS WITHOUT DIABETES (ID 1–10) WITH THOSE FROM CHILDREN AND ADOLESCENTS WITH DIABETES (ID 11–20)

ID	Type 1 diabetes	Mean blood glucose	MODD	CONGA1	CONGA2	CONGA4	HbA1c	% of time		
								Hypoglycemic	Normal	High
Controls										
1	No	4.8	1.2	1.1	1.2	1.2	—	20.5	79.5	0.0
2	No	5.0	1.1	1.2	1.1	1.5	—	19.4	80.6	0.0
3	No	5.2	0.9	0.6	0.9	1.2	—	15.4	84.6	0.0
4	No	5.3	0.6	0.4	0.4	0.5	—	0.0	100.0	0.0
5	No	5.3	0.8	0.6	0.7	1.0	—	4.2	95.8	0.0
6	No	5.4	0.5	0.7	0.9	0.9	—	0.6	99.4	0.0
7	No	5.6	0.6	0.7	0.9	1.0	—	0.3	99.7	0.0
8	No	5.6	0.8	0.6	0.7	0.8	—	0.5	99.5	0.0
9	No	5.8	0.6	0.6	0.7	0.7	—	0.0	100.0	0.0
10	No	5.8	0.8	0.5	0.6	0.7	—	0.3	99.7	0.0
Diabetes										
11	Yes	6.0	3.2	2.0	2.9	4.0	6.6	29.1	68.6	2.3
12	Yes	8.0	3.6	2.4	3.3	3.5	7.0	15.4	67.3	17.3
13	Yes	8.5	2.9	2.5	3.3	3.8	8.2	4.5	82.5	13.0
14	Yes	8.8	3.7	2.1	2.6	2.9	7.9	1.9	84.1	14.0
15	Yes	9.2	8.1	2.4	4.1	6.1	7.8	20.2	49.1	30.7
16	Yes	10.8	5.4	3.2	5.0	7.3	7.6	9.2	57.1	33.7
17	Yes	12.6	4.3	2.9	4.4	5.4	8.4	0.8	50.1	49.1
18	Yes	12.8	4.2	1.7	2.6	3.6	9.9	0.0	38.4	61.6
19	Yes	13.5	3.9	2.7	3.9	4.9	9.7	0.0	41.7	58.3
20	Yes	16.2	4.1	2.6	3.5	4.4	9.0	0.0	11.5	88.5



CONGA for 1, 2, and 4 h time differences. The group mean CONGA1 value in the group with diabetes was 2.5 (range 1.7–3.2) compared with the healthy control group value of 0.7 (range 0.4–1.2). For CONGA2 and CONGA4, the differences between the two groups were even more marked: The mean CONGA4 value in the diabetes group was 4.6, compared with 1.0 in the healthy group. Figure 1 shows the relationship between CONGA value and time period use to calculate CONGA for each of the patients and controls. For the healthy controls, the time period used to calculate the CONGA has little effect on the CONGA value. For the patients with diabetes, however, CONGA generally increases as the time period increases. The largest increases are seen in those patients with large glycemic swings over the period of the trace. This is illustrated in Figure 2b and c, which show the CGMS trace and CONGA values for patients with diabetes having low and high intra-day glycemic variation, respectively. For comparison, the CGMS trace and CONGA values for a healthy control are shown in Figure 2a.

## DISCUSSION

The premise of our proposed approach to continuous glucose analysis is that there is no

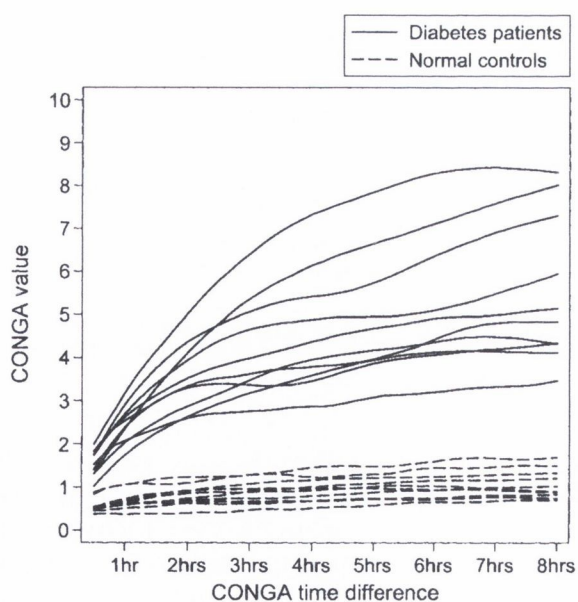


FIG. 1. Relationship between CONGA value and time period use to calculate CONGA for pediatric patients with diabetes (solid lines) and controls (dashed lines).

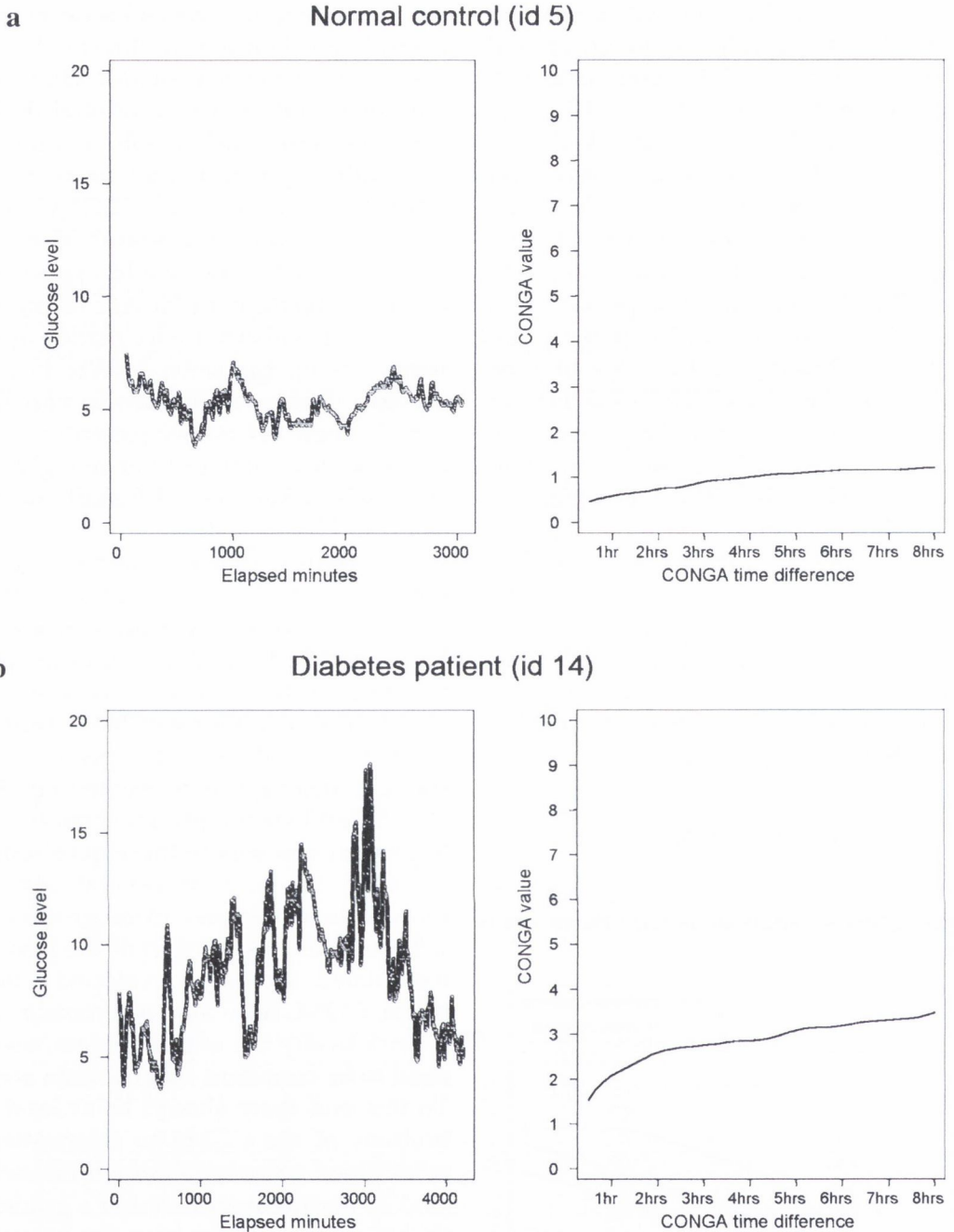
one measure that covers all aspects of glycemic control, and hence a multifaceted approach is warranted. CGMS provides clinically useful data over and above traditional diabetes outcome measures such as HbA1c and mean glucose values. Quantitative measures of inter-day (MODD) and intra-day (CONGA) glycemic variation can now be assessed. There is no clinical reason why one would expect these metrics to correlate with HbA1c or mean glucose values. Clinical experience readily attests to patients having the same HbA1c but markedly varying degrees of glycemic variation. Conversely, there can be two patients with the same degree of intra-day or inter-day glycemic variation who have very different mean glucose levels or HbA1c values.

Continuous glucose monitoring provides both qualitative and quantitative data. Hence analysis of CGMS data can be problematic. We have proposed an approach to the analysis of CGMS data that is based upon relevant hierarchical clinical questions: How representative are the data? What is the percentage of time spent in major glycemic excursions? How variable or labile is the glycemic control? In order to provide answers to these questions we have explored the use of several algorithms in CGMS data obtained from groups with and without diabetes. As part of the new approach formulated, we have developed a novel algorithm, CONGA, to assess glycemic variation.

Prior to any use of CGMS data, investigators need to be confident that the data are accurate. To this end there should be at least four calibrations of the CGMS to intermittent finger-prick blood glucose values within each 24-h period as per the manufacturer's guidelines. The CGMS software calculates the acceptability of the data by comparing the inputted blood glucose values with paired sensor readings.

*How representative are the CGMS data of average glycemic control within the same 3 months?*

This question is of prime importance as it refers to the credibility of the CGMS data when attempting to extrapolate the CGMS findings to a relevant clinical period. In order to ascertain how representative a CGMS trace is of any



**FIG. 2.** CGMS trace and CONGA values for (a) a normal control, (b) a pediatric patient with diabetes having low intra-day glycemic variation, and (c) a pediatric patient with diabetes having high intra-day glycemic variation.

given period we have elected to summarize the sensor values obtained and to assess inter-day variation of the trace for consistency. The two aspects of CGMS data we have chosen to use in these analyses are the mean CGMS glucose value and the MODD. The use of mean CGMS glucose assumes that the distribution of CGMS values is not skewed; the mean does not di-

rectly measure amplitude or variation of the CGMS trace. Boland et al.<sup>5</sup> have shown correlations between mean CGMS glucose values and HbA1c. Although the mean CGMS glucose value cannot account for glycemic variation it can potentially be utilized as a surrogate marker for HbA1c within trace analysis. Because of the small size of the cohort with dia-



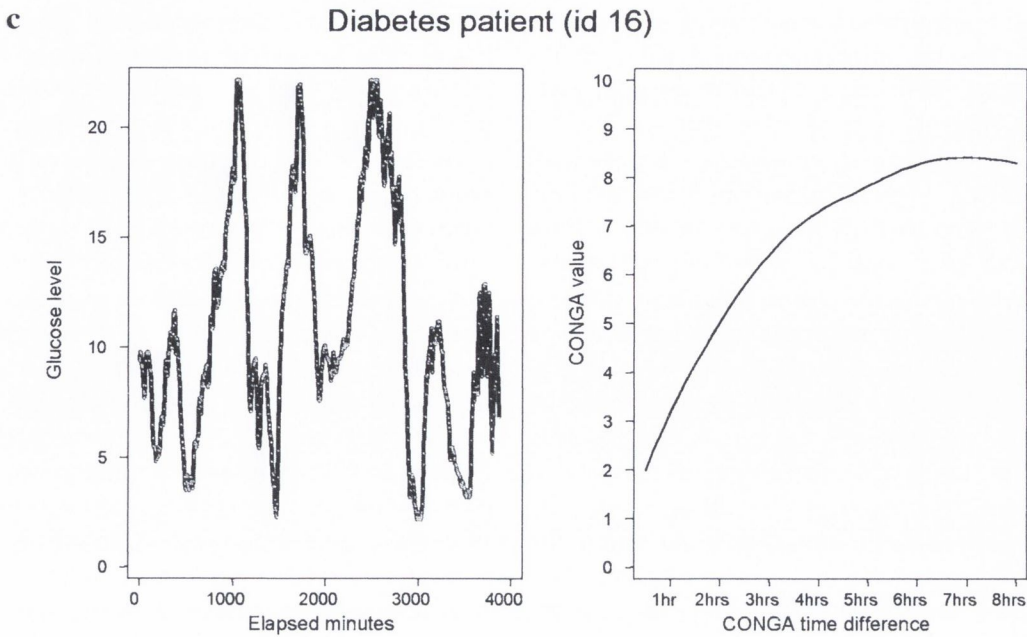


FIG. 2. (Continued)

betes assessed for this methods paper we did not attempt any statistical analyses comparing mean glucose values and HbA1c. Notwithstanding this we feel that the link between a 3-day CGMS recording and an independent measure of control such as HbA1c has already been established by Boland et al.<sup>5</sup> The clinical utility of CGMS is further enhanced by the integrated use of mean glucose data together with MODD, percentage of time in glyceemic ranges, and CONGA.

The MODD value was derived by Molnar et al.<sup>10</sup> in 1972. This value was designed to illustrate inter-day variation of blood glucose levels. Care has to be taken in adapting this formula for use with continuous monitoring as occasional CGMS time intervals can be lengthened or shortened by 1 min, altering the time difference and affecting the final MODD calculation. A high MODD score is indicative of a large glyceemic difference between days. MODD values have utility in that the degree of consistency in a CGMS trace can be assessed, and thus the degree to which observed daily patterns are ongoing and representative can be assessed. In our clinical experience, CGMS traces with high MODD values are indicative of irregular habits and require detailed contemporaneous lifestyle information prior to interpretation.

*What is the amount of time spent in marked glyceemic excursions?*

Assuming the CGMS data are representative, then an assessment of the amount of time experienced by an individual in various glyceemic ranges allows for a quantitative assessment of hypo- or hyperglycemia. Earlier CGMS studies have shown that occult hypoglycemia in childhood diabetes is a relatively common event.<sup>6,7</sup> In children, hypoglycemia may have adverse effects on cognitive function, potentially as significant as the long-term microvascular complications due to hyperglycemia.<sup>13-16</sup> Reliance only upon HbA1c and intermittent finger-prick testing may lead to a limited picture of overall metabolic control with little or no appreciation of glyceemic excursions or variation.<sup>9</sup>

To ascertain the effect of marked glyceemic excursions, the percentages of time spent in high and low glucose ranges were calculated. The percentage of time analyses proposed here have the advantage that CGMS data are summarized only in terms of time, an easily reproducible and comparable measure between subjects. Also, the percentage of time spent within various glyceemic ranges is a useful clinical concept that can be used to adjust therapy. Notwithstanding the clinical utility of percent-



age time, it is a limited measure of continuous CGMS data. The values obtained are sum totals and not indicative of whether the time spent at or below a particular glucose level is within one prolonged episode or consists of many shorter episodes. Subanalyses of percentage of time during various periods (night, school time, etc.) may be more helpful in specific clinical contexts. Other literature dealing with CGMS data has used area under or above the CGMS curve to describe the degree of glucose deviation above or below a certain threshold.<sup>6,17</sup> The method has been used to describe the incidence of hypo- and hyperglycemia and the effect of new therapies.<sup>6,17</sup> Researchers using this method have analyzed data using the whole area under the curve of each 24-h glucose profile within the range of the CGMS (2.2–22.2 mmol/L), and have evaluated both mean value of the area for 3 days and the sum of the areas for the same 3 days.<sup>6</sup> Similar to mean CGMS glucose values, a relationship has been shown between HbA1c and mean 3-day glucose area under the curve values.<sup>6</sup> Another group evaluating a new therapy employed the incremental area under the curve using the trapezoidal rule to calculate postprandial glucose excursions.<sup>17</sup> In the context of CGMS, areas under the curve analyses are problematic in that the curve is truncated at glucose values under 2.2 mmol/L and over 22.2 mmol/L. Thus the area under the CGMS curve does not distinguish between extremes in glucose levels (for example, values of 23 mmol/L and 40 mmol/L are treated equally).

In this limited study we found that patients and controls experienced similar percentage of times of CGMS readings below 4 mmol/L (6% vs. 8%, respectively). There are potentially two explanations for this. First, the normal range for blood glucose levels extends down to 3 mmol/L. Thus CGMS readings between 3 and 4 mmol/L can be viewed as normal for the control subjects. In this study a cutoff value of 4 mmol/L was chosen because in patients with diabetes this is a level at which patients frequently experience symptoms of hypoglycemia and the level at which our pediatric patients are counseled to take remedial action. The second reason pertains to the accuracy of interstitial tissue CGMS readings compared with blood

glucose at low glucose levels. In the control traces, the lowest recorded glucose level was 2.7 mmol/L. Out of a total of 6,488 5-min control readings, the glucose level was recorded between 2.7 and 3.0 mmol/L in only 35 occasions (0.5% of readings). The issue of sensor accuracy has been canvassed in the literature,<sup>18–20</sup> with discrepancies occasionally seen between interstitial tissue and blood glucose levels in detecting low glucose values. It is for these reasons that we are not advocating the use of the CGMS to assess the absolute degree of glycaemic excursions; rather, we are advocating the use of the CGMS to assess patterns of glycaemic excursions (i.e., percentage of time in and out of various glycaemic ranges). In addition, we do not refer to CGMS values as indicating hyper-, normo-, or hypoglycemia; rather we prefer the use of the terms high, normal, or low glucose values in order to reinforce the point that the CGMS is not measuring blood glucose levels per se.

#### *What is the lability/variation of glycaemic control?*

The importance of an understanding of the degree of variation/lability in glycaemic control in childhood and adolescence is yet to be fully established. Reporting of improved psychosocial outcomes seen in children receiving continuous insulin therapy suggests that glycaemic variation may be an important novel outcome measure in childhood diabetes.<sup>22</sup> Glycaemic variation incorporates the frequency of glycaemic excursions, including postprandial glycaemia—a phenomenon increasingly recognized to be of importance in overall metabolic control.<sup>23</sup> Finally, glycaemic variation may be a factor in determining the risk of severe hypoglycemia or diabetic ketoacidosis. Previous investigators have recognized the importance of trying to measure glycaemic variation and have devised algorithms for use in postprandial experimental models.<sup>11,12</sup> Examples of such analyses include M-values and MAGE. The M-value is a measure of the stability of the glucose metabolism in comparison with an ideal glucose value.<sup>9</sup> The M-value was first described in an attempt at quantitative analysis of postprandial blood glucose variation.<sup>11</sup> The M-value was originally de-



signed against a subjective standard of nine investigators' assessment of 72-h profiles from 20 patients with diabetes (six blood glucose measures per 24-h period) and has subsequently been extrapolated for use in the CGMS with little critical review.<sup>24</sup> The M-value is 0 in healthy controls, rising with increasing glycemic variation. In the context of diabetes, results are categorized as good (0–18), fair (19–31), or poor (>32) control.<sup>11</sup> Arbitrary cutoffs are required for administration of the formula with logarithmic transformation being required to increase the impact of hypoglycemic events on the index.<sup>11</sup> The original formula was later modified (Adjusted M-value; see Table 1)<sup>9</sup> for a higher arbitrary comparative glucose value when it was found that some cases the M-value was lower than that of a reference group of healthy individuals. When originally devised, the authors acknowledged that the M-value was limited by the number of glucose values measured. To adjust for this, subsequent authors added an appendix ( $M_w$ ) to the formula for calculations of 24 readings or fewer.<sup>11</sup> CGMS traces produce up to 288 readings in a day, and thus the appendix is now no longer applicable. As mentioned above, the M-value relies on selection of arbitrary glycemic reference points by the investigators. This introduces a bias effect that impedes the M-value's use in comparing separate studies that may use varying reference points. Further to this, hypoglycemia has a greater impact on the M-value than hyperglycemia, which limits the M value's usefulness as a true descriptor of glycemic variation.

In view of these disadvantages Wojcicki<sup>25</sup> proposed the "J"-index as an alternate formula for calculation of glycemic variation. The aim of this new index was to incorporate mean level and variability of glycemia utilizing one variable. The calculation summarized in Table 1 relates to glucose levels measured in mmol/L. This index has not been validated in continuous glucose monitoring and excludes hypoglycemia alarm states defined as severe hypoglycemia (<1.67 mmol/L) and continuous hypoglycemia (3 measurements <2.78 mmol/L).

The MAGE algorithm<sup>12</sup> was suggested in 1970 by the same investigators who devised MODD to quantitate glycemic excursion under controlled dietary/exercise conditions. The

MAGE algorithm was designed to calculate the peaks and nadirs encountered in a day, generating a value for the variation around a mean glucose value. MAGE values differ from M-values in that the reference point is a mean value rather than an arbitrarily chosen cutoff. The degree of variation is calculated according to the standard deviation of postprandial glycemic excursion.<sup>12</sup> Definition of glycemic peaks and nadirs is arbitrary or subjective,<sup>12</sup> this being the main factor limiting its use in ambulatory, non-controlled CGMS analyses. MAGE uses the pooled results of arbitrarily designated glycemic peaks (chosen by the investigators in a non-reproducible fashion), and ignores blood glucose swings, which are designated as insignificant by the person interpreting the data. When MAGE was first proposed, hourly blood glucose measures were assessed. By way of comparison, CGMS records 12 measures per hour. In continuous monitoring the distinction between peaks and nadirs is unclear compared with the original hourly measurements used when MAGE was devised. Thus MAGE analysis ignores a large percentage of CGMS data. Notwithstanding this, MAGE has recently been used in conjunction with the CGMS in a diet- and exercise-controlled cohort with diabetes.<sup>26</sup> In that study the authors defined the standard deviation according to the mean CGMS glucose value, and defined CGMS glucose peaks and nadirs manually in a way that we have been unable to reproduce. In addition, the diet and activities of participants in the CGMS study by Alemzadeh et al.<sup>26</sup> were controlled. The necessity for such restrictions limits the use of MAGE in non-controlled, ambulatory CGMS use. Our attempts to use the MAGE analysis with the CGMS data presented in this study proved unsuccessful as we found identification of significant CGMS peaks and nadirs for the MAGE calculation to be inconsistent between investigators. No mathematical system could be devised that reliably, objectively, and consistently identified clinically relevant glycemic peaks and nadirs required for this calculation to be used with the CGMS.

There exists no established "gold standard" of glycemic variation against which to compare the M-value, J-index, or MAGE. Thus on the largely empiric and practical grounds summa-



rized above we have concluded that the M-value, J-index, and MAGE are inappropriate tools to analyze continuous glycemic data in a non-controlled or ambulant setting. Given the shortcomings of these existing algorithms to assess glycemic variation we have devised the CONGA as a novel method of consistently and objectively expressing glycemic variation. The CONGA is defined as the standard deviation of the differences and measures the overall intra-day variation of glucose recordings. The CONGA does not require arbitrary glucose cutoffs, logarithmic transformation, chosen peaks and nadirs, or defined meal or exercise times. The CONGA calculation is an expression of the variation shown during normal, ambulatory activity, which is more useful in the assessment of a child with diabetes. The CONGA formula is based on the premise that normoglycemia inherently allows for little glycemic variation, whereas glycemic control in diabetes will result in greater variation in blood glucose levels. High CONGA values will therefore reflect increased glycemic excursions consistent with less stable control, and low CONGA values will reflect stable glycemic control. Our data demonstrate the dramatic difference in glycemic variation between people with diabetes and healthy controls. Whilst both distributions are centered around zero, a much wider range of differences is evident in the subjects with diabetes.

## CONCLUSIONS

The analysis of data produced from continuous glucose monitoring presents unique challenges. The wealth of data available from continuous glucose monitoring cannot be fully utilized with methods designed for intermittent glucose monitoring. In summary, the approach suggested for accurate CGMS analysis is depicted below according to what are the relevant clinical questions being asked of the data:

1. How representative are the CGMS data of average glycemic control within the same 3 months?
  - Mean blood glucose—is the value comparable to HbA1c?

- MODD analysis—is there inter-day consistency?
2. What is the amount of time spent in marked glycemic excursions?
    - Percentage of time within glycemic range analyses
  3. What is the lability/variability of glycemic control?
    - CONGA1, CONGA2, and CONGA4 analyses for intra-day variability

The measurement and analysis of continuous glucose readings have the potential benefit of increasing our understanding of glycemic control and generating a new independent outcome for diabetes, that of glycemic variation. CGMS can assist in this as both a quantitative and qualitative tool. The approach to CGMS interpretation advocated here allows for correlation of glucose values with HbA1c, an analysis of inter-day glycemic variation, description of amount of time spent within various glucose ranges, and finally an analysis of intra-day glycemic variation. Continuing advances in the production of continuous glucose monitoring should be paralleled by advances in the assessment of the data provided. The CONGA value, in particular, is a novel measure that is suitable for use in ambulant continuous glucose monitoring systems. Whether or not the CONGA value proves to be associated with other glycemic outcomes (hypoglycemia rates, risk of diabetic ketoacidosis, etc.) or behavioral variability is an area of ongoing enquiry. In the interim we propose that the CONGA analysis, measuring glycemic variation, should be an integral component in the assessment of CGMS data.

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**Appendix 5: Behavioural Assessment System for Children**

**Questionnaire.**



# BASC

Behavior Assessment System for Children

## Parent Rating Scales

Cecil R. Reynolds and Randy W. Kamphaus

### Instructions

On the pages that follow are phrases that describe how children may act. Please read each phrase and mark the response that describes how this child has acted over the last **six months**. If the child's behavior has changed a great deal during this period, describe the child's recent behavior.

Circle **N** if the behavior **never** occurs.

Circle **S** if the behavior **sometimes** occurs.

Circle **O** if the behavior **often** occurs.

Circle **A** if the behavior **almost always** occurs.

**Please mark every item.** If you don't know or are unsure, give your best estimate.

Before starting, please provide the information requested in the box at the top of the next page.

### How to Mark Your Responses

Use a sharp pencil or ballpoint pen; do not use a felt-tip pen or marker. Press firmly and be certain to **circle** completely the letter you choose, like this:

N (S) O A

If you wish to change a response, mark an X through it and circle your new choice, like this:

N (~~S~~) (O) A

AGS<sup>®</sup>

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A 0 9 8 7



First \_\_\_\_\_ Middle \_\_\_\_\_ Last \_\_\_\_\_  
 Birth date \_\_\_\_\_ Age \_\_\_\_\_  
 Day Year \_\_\_\_\_ Month Day Year \_\_\_\_\_  
 Grade \_\_\_\_\_  
 Male Other data \_\_\_\_\_

Your name \_\_\_\_\_  
 Sex:  Female  Male  
 First \_\_\_\_\_ Middle \_\_\_\_\_ Last \_\_\_\_\_  
 Relationship to child:  
 Mother  Father  Guardian  Other \_\_\_\_\_

- \_\_\_\_\_ or possessions with other children. N S O A
- \_\_\_\_\_ n parents. N S O A
- \_\_\_\_\_ us. N S O A
- \_\_\_\_\_ stracted. N S O A
- \_\_\_\_\_ that are not food. N S O A
- \_\_\_\_\_ ody likes me." N S O A
- \_\_\_\_\_ t thinking. N S O A
- \_\_\_\_\_ ye contact. N S O A
- \_\_\_\_\_ ent visits to the doctor. N S O A
- \_\_\_\_\_ making new friends. N S O A
- \_\_\_\_\_ N S O A
- \_\_\_\_\_ shifting gears from one task to another. N S O A
- \_\_\_\_\_ nimals. N S O A
- \_\_\_\_\_ out what parents think. N S O A
- \_\_\_\_\_ anguage. N S O A
- \_\_\_\_\_ s that are not there. N S O A
- \_\_\_\_\_ dge. N S O A
- \_\_\_\_\_ hen traveling in a car. N S O A
- \_\_\_\_\_ of humor. N S O A
- \_\_\_\_\_ nes. N S O A
- \_\_\_\_\_ peting with other children. N S O A
- \_\_\_\_\_ blems. N S O A
- \_\_\_\_\_ n denied own way. N S O A
- \_\_\_\_\_ dying. N S O A
- \_\_\_\_\_ thought over and over. N S O A
- \_\_\_\_\_ N S O A
- \_\_\_\_\_ hings. N S O A
- \_\_\_\_\_ riate table manners. N S O A
- \_\_\_\_\_ n problems. N S O A
- \_\_\_\_\_ s. N S O A
- \_\_\_\_\_ r children's things. N S O A
- \_\_\_\_\_ noises when playing. N S O A
- \_\_\_\_\_ N S O A

- 34. Complains when asked to do things differently. N S
- 35. Is critical of others. N S
- 36. Says, "I'm not very good at this." N S
- 37. Listens attentively. N S
- 38. Babbles to self. N S
- 39. Is sad. N S
- 40. Interrupts others when they are speaking. N S
- 41. Says, "please" and "thank you." N S
- 42. Complains about health. N S
- 43. Refuses to join group activities. N S
- 44. Sleeps with parents. N S
- 45. Adjusts well to changes in routine. N S
- 46. Blames others. N S
- 47. Says, "I'm afraid I will make a mistake." N S
- 48. Has a short attention span. N S
- 49. Rocks back and forth for long periods of time. N S
- 50. Pouts. N S
- 51. Cannot wait to take turn. N S
- 52. Responds when spoken to. N S
- 53. Complains of pain. N S
- 54. Is shy with other children. N S
- 55. Has a hearing problem. N S
- 56. Threatens to hurt others. N S
- 57. Gets very upset when things are lost. N S
- 58. Daydreams. N S
- 59. Says, "That's not fair." N S
- 60. "Shows off" when visitors are present. N S
- 61. Begins conversations appropriately. N S
- 62. Has ear infections. N S
- 63. Avoids other children. N S
- 64. Complains about being teased. N S
- 65. Is restless during movies. N S
- 66. Has allergic reactions. N S



# Remember:

How frequently each behavior occurs by circling

**S** — Sometimes    **O** — Often    **A** — Almost always

... to new teachers and caregivers.	N	S	O	A
...ers.	N	S	O	A
...asily when learning something new.	N	S	O	A
...of touch with reality.	N	S	O	A
...of being tired.	N	S	O	A
...much supervision.	N	S	O	A
...ts others.	N	S	O	A
...of strangers.	N	S	O	A
...fire.	N	S	O	A
...hings.	N	S	O	A
...children.	N	S	O	A
...out things that cannot be changed.	N	S	O	A
...e concentrating.	N	S	O	A
...rt self.	N	S	O	A
...nt to be alone" or "I like being alone."	N	S	O	A
...ctive.	N	S	O	A
...to help with things.	N	S	O	A
...of being cold.	N	S	O	A
...talk.	N	S	O	A
...ew surroundings.	N	S	O	A
...children names.	N	S	O	A
...kly.	N	S	O	A
...pset.	N	S	O	A
...ntrums.	N	S	O	A
...ks for help.	N	S	O	A
...of shortness of breath.	N	S	O	A
...parent in strange surroundings.	N	S	O	A
...parents when they are talking ne.	N	S	O	A
	N	S	O	A

100. Gets upset when left in a new situation without a parent or caregiver.	N	S	O	A
101. Talks back to parents.	N	S	O	A
102. Worries about what teachers think.	N	S	O	A
103. Gives up easily.	N	S	O	A
104. Says, "They're trying to get even with me."	N	S	O	A
105. Changes moods quickly.	N	S	O	A
106. Touches everything when shopping.	N	S	O	A
107. Smiles at others.	N	S	O	A
108. Uses medication.	N	S	O	A
109. Readily starts up conversations with new people.	N	S	O	A
110. Has toileting accidents.	N	S	O	A
111. Laughs.	N	S	O	A
112. Teases others.	N	S	O	A
113. Says, "It's all my fault."	N	S	O	A
114. Forgets things.	N	S	O	A
115. Has strange ideas.	N	S	O	A
116. Says, "I want to die" or "I wish I were dead."	N	S	O	A
117. Leaves seat during meals.	N	S	O	A
118. Encourages others to do their best.	N	S	O	A
119. Gets colds.	N	S	O	A
120. Is shy with adults.	N	S	O	A
121. Is a "good sport."	N	S	O	A
122. Orders others around.	N	S	O	A
123. Is fearful.	N	S	O	A
124. Sees things that are not there.	N	S	O	A
125. Whines.	N	S	O	A
126. Screams.	N	S	O	A
127. Congratulates others when good things happen to them.	N	S	O	A
128. Complains of dizziness.	N	S	O	A
129. Stands very close to family members when in public places.	N	S	O	A
130. Offers help to other children.	N	S	O	A
131. Fiddles with things while at meals.	N	S	O	A



Child's name \_\_\_\_\_ Your name \_\_\_\_\_  
First Middle Last First Middle Last  
 Date \_\_\_\_\_ Birth date \_\_\_\_\_ Age \_\_\_\_\_  
Month Day Year Month Day Year  
 School \_\_\_\_\_ Grade \_\_\_\_\_  
 Sex:  Female  Male  Guardian  Other \_\_\_\_\_  
 Relationship to child:  Mother  Father

### Instructions

Items on this form are phrases that describe how children behave. Please read each phrase and mark the response that best describes how this child has acted over the last **six months**. If the child's behavior has changed a great deal during this period, mark the child's recent behavior.

**Please mark every item.** If you don't know or are unsure, mark your best estimate.

Before starting, please provide the information requested in the box at the top of the page.

### How to Mark Your Responses

- Circle **N** if the behavior **never** occurs.
- Circle **S** if the behavior **sometimes** occurs.
- Circle **O** if the behavior **often** occurs.
- Circle **A** if the behavior **almost always** occurs.

Be certain to **circle** completely the letter you choose, like this: N **(S)** O A

If you wish to change a response, mark an X through it and circle your new choice, like this: N ~~(S)~~ **(O)** A

- Adjusts well to new teachers . . . . . N S O A
- Attempts to hurt others . . . . . N S O A
- Complains . . . . . N S O A
- Obeys directions . . . . . N S O A
- Runs back and forth for long periods . . . . . N S O A
- Runs away from home . . . . . N S O A
- Says, "I don't have any friends." . . . . . N S O A
- Does not wait to take turn . . . . . N S O A
- Runs to after-school activities . . . . . N S O A
- Says, "please" and "thank you" . . . . . N S O A
- Complains of shortness of breath . . . . . N S O A
- Quickly starts up conversations with people . . . . . N S O A
- Plays with fire . . . . . N S O A
- Shuts off . . . . . N S O A
- Is not serious . . . . . N S O A
- Is teased . . . . . N S O A
- Attempts to hurt self . . . . . N S O A
- Helps friends who are in trouble . . . . . N S O A
- Says, "I want to kill myself." . . . . . N S O A
- Remains seated during meals . . . . . N S O A
- Joins clubs or social groups . . . . . N S O A
- Encourages others to do their best . . . . . N S O A
- Complains of dizziness . . . . . N S O A
- Changes direction to avoid having to meet someone . . . . . N S O A

- 25. Dares other children to do things . . . . . N
- 26. Stutters . . . . . N
- 27. Says, "I'm afraid I'll hurt someone." . . . . . N
- 28. Is in trouble with the police . . . . . N
- 29. Cries easily . . . . . N
- 30. Throws tantrums . . . . . N
- 31. Uses medication . . . . . N
- 32. Congratulates others when good things happen to them . . . . . N
- 33. Complains of being cold . . . . . N
- 34. Hits other children . . . . . N
- 35. Has eye problems . . . . . N
- 36. Is easily soothed when angry . . . . . N
- 37. Teases others . . . . . N
- 38. Worries about what parents think . . . . . N
- 39. Forgets things . . . . . N
- 40. Repeats one activity over and over . . . . . N
- 41. Uses foul language . . . . . N
- 42. Says, "Nobody understands me." . . . . . N
- 43. Needs too much supervision . . . . . N
- 44. Is a "self-starter" . . . . . N
- 45. Has a sense of humor . . . . . N
- 46. Complains of pain . . . . . N
- 47. Avoids competing with other children . . . . . N
- 48. Gets upset when plans are changed . . . . . N
- 49. Argues with parents . . . . . N

Continue on the next page.



Says, "I get nervous during tests" or "Tests make me nervous." . . . . .	N S O A	93. Has allergic reactions . . . . .	N S O A
Is easily distracted . . . . .	N S O A	94. Shows fear of strangers . . . . .	N S O A
Picks at things like own hair, nails, or clothing . . . . .	N S O A	95. Breaks other children's things . . . . .	N S O A
Shows a lack of concern for others' feelings . . . . .	N S O A	96. Worries about what teachers think . . . . .	N S O A
Is easily frustrated . . . . .	N S O A	97. Complains about being unable to block out unwanted thoughts . . . . .	N S O A
Is restless during movies . . . . .	N S O A	98. Gets in trouble . . . . .	N S O A
Has lots of ideas . . . . .	N S O A	99. Says, "I want to die" or "I wish I were dead." . . . . .	N S O A
Volunteers to help with things . . . . .	N S O A	100. Has seizures . . . . .	N S O A
Vomits . . . . .	N S O A	101. Is usually chosen as a leader . . . . .	N S O A
Is shy with other children . . . . .	N S O A	102. Compliments others . . . . .	N S O A
Is a "sore loser" . . . . .	N S O A	103. Gets sick . . . . .	N S O A
Tries too hard to please others . . . . .	N S O A	104. Begins conversations appropriately . . . . .	N S O A
Daydreams . . . . .	N S O A	105. Is a "good sport" . . . . .	N S O A
Has to stay after school for punishment . . . . .	N S O A	106. Calls other children names . . . . .	N S O A
Is easily upset . . . . .	N S O A	107. Says, "I'm afraid I will make a mistake." . . . . .	N S O A
Fiddles with things while at meals . . . . .	N S O A	108. Completes work on time . . . . .	N S O A
Is good at getting people to work together . . . . .	N S O A	109. Plays in toilet . . . . .	N S O A
Uses appropriate table manners . . . . .	N S O A	110. Has been suspended from school . . . . .	N S O A
Has ear infections . . . . .	N S O A	111. Says, "Nobody likes me." . . . . .	N S O A
Has toileting accidents . . . . .	N S O A	112. Makes loud noises when playing . . . . .	N S O A
Makes frequent visits to the doctor . . . . .	N S O A	113. Will speak up if the situation calls for it . . . . .	N S O A
Adjusts well to changes in routine . . . . .	N S O A	114. Responds when spoken to . . . . .	N S O A
Is critical of others . . . . .	N S O A	115. Has difficulty breathing . . . . .	N S O A
Is afraid of dying . . . . .	N S O A	116. Avoids other children . . . . .	N S O A
Gives up easily when learning something new . . . . .	N S O A	117. Adjusts well to changes in family plans . . . . .	N S O A
Seems out of touch with reality . . . . .	N S O A	118. Argues when denied own way . . . . .	N S O A
Lies to get out of trouble . . . . .	N S O A	119. Says, "I'm not very good at this." . . . . .	N S O A
Complains about not having friends . . . . .	N S O A	120. Listens attentively . . . . .	N S O A
Interrupts others when they are speaking . . . . .	N S O A	121. Hears sounds that are not there . . . . .	N S O A
Is creative . . . . .	N S O A	122. Lies . . . . .	N S O A
Makes suggestions without offending others . . . . .	N S O A	123. Is sad . . . . .	N S O A
Has headaches . . . . .	N S O A	124. Climbs on things . . . . .	N S O A
Refuses to join group activities . . . . .	N S O A	125. Makes decisions easily . . . . .	N S O A
Shares toys or possessions with other children . . . . .	N S O A	126. Tries to bring out the best in other people . . . . .	N S O A
Complains about rules . . . . .	N S O A	127. Complains of heart beating too fast . . . . .	N S O A
Worries about things that cannot be changed . . . . .	N S O A	128. Clings to parent in strange surroundings . . . . .	N S O A
Completes homework from start to finish without taking a break . . . . .	N S O A	129. Is cruel to animals . . . . .	N S O A
Eats things that are not food . . . . .	N S O A	130. Worries about schoolwork . . . . .	N S O A
Gets into trouble in the neighborhood . . . . .	N S O A	131. Sees things that are not there . . . . .	N S O A
Changes mood quickly . . . . .	N S O A	132. Sleeps with parents . . . . .	N S O A
Is overly active . . . . .	N S O A	133. Says, "I'm so ugly." . . . . .	N S O A
Gives good suggestions for solving problems . . . . .	N S O A	134. Has a hearing problem . . . . .	N S O A
Politely asks for help . . . . .	N S O A	135. Is energetic . . . . .	N S O A
		136. Shows interest in others' ideas . . . . .	N S O A
		137. Has stomach problems . . . . .	N S O A
		138. Offers help to other children . . . . .	N S O A

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**Appendix 6: Raw data from all four time points of CGMS analysis.**

- **Individual subject data**
- **Cumulative data from glycaemic and metabolic variables**



**Appendix 6: Raw data from all 38 participants from each of the four timepoints of the study**

Patient	Run	Conga1	Conga2	Conga4	MODD	MBG	% time in low CGMS range	% time in normal CGMS range	% time in high CGMS range
1	0	2.50	3.80	5.50	3.80	12.90	0.00	43.00	57.00
1	1	2.22	2.90	3.30	2.40	9.93	0.00	72.28	27.72
1	2	3.06	4.60	5.50	4.10	10.87	0.18	65.54	34.28
1	3	3.23	4.60	5.00	2.60	11.22	0.00	60.93	39.07
3	0	2.10	3.10	5.00	17.40	11.10	36.00	21.00	43.00
3	1	2.65	4.30	6.00	5.10	11.47	9.94	39.66	50.40
3	2	2.32	3.60	4.30	6.10	11.37	3.56	44.15	52.29
3	3	2.80	4.10	5.70	6.80	12.94	4.25	30.65	64.28
4	0	3.43	5.10	5.50	4.30	13.20	0.00	42.00	58.00
4	1	2.85	4.00	4.90	3.90	13.66	0.00	32.51	67.49
4	2	2.71	4.40	6.40	7.50	14.05	0.00	39.00	61.00
4	3	3.00	4.70	6.40	4.30	9.91	6.92	60.84	31.19
5	0	3.30	4.70	5.70	8.90	14.00	11.00	24.00	65.00
5	1	3.68	5.40	7.50	0.00	13.49	6.24	31.52	62.24
5	2	3.77	5.90	7.90	6.30	12.69	7.95	37.75	54.30
5	3	2.69	3.90	5.50	4.80	12.77	4.25	37.57	57.84
8	0	3.23	4.30	5.00	6.00	11.80	1.00	59.00	41.00
8	1	2.25	3.70	5.50	4.80	9.39	0.00	73.82	26.18
8	2	3.11	4.70	6.60	7.80	11.35	5.86	55.50	38.65
8	3	3.22	5.20	7.30	5.70	11.08	5.75	48.32	43.09
10	0	3.85	5.60	6.90	6.40	12.20	7.00	42.00	51.00
10	1	2.68	3.40	4.30	5.00	11.22	0.52	53.88	45.60
10	2	2.12	3.40	5.20	3.60	9.20	15.36	55.23	29.40
10	3	3.07	3.90	4.70	4.60	8.94	8.42	71.81	18.13
11	0	3.56	5.30	6.00	4.60	7.90	28.00	56.00	16.00
11	1	3.08	4.10	5.00	3.90	10.83	11.82	48.92	39.26
11	2	2.76	4.00	5.10	3.60	10.21	5.99	57.27	36.73
11	3	1.82	2.50	3.20	3.20	11.33	0.00	64.58	35.42
12	0	4.14	6.00	7.60	5.30	11.20	11.00	45.00	45.00
12	1	3.28	5.10	7.10	3.80	9.48	24.37	39.87	35.76
12	2	4.14	6.40	8.20	5.60	12.74	8.78	37.39	53.82
12	3	3.95	6.20	8.50	4.40	10.91	13.25	32.57	46.03
14	0	3.93	6.50	8.70	6.20	11.20	11.00	47.00	42.00
14	1	3.92	5.70	6.90	5.50	11.48	6.63	51.10	42.27
14	2	2.41	3.80	4.60	5.30	10.00	3.69	62.66	33.65
14	3	3.51	5.50	6.50	5.20	10.15	6.75	57.20	32.20
15	0	2.87	4.40	6.40	2.30	8.40	20.00	60.00	20.00
15	1	3.14	4.70	6.40	4.60	15.12	0.00	29.06	70.94
15	2	2.51	3.80	5.00	5.40	14.87	0.00	25.93	74.07
15	3	3.58	5.40	7.30	6.20	12.41	7.42	39.63	52.52

**Appendix 6: Raw data from all 38 participants from each of the four timepoints of the study**

Patient	Run	Conga1	Conga2	Conga4	MODD	MBG	% time in low CGMS range	% time in normal CGMS range	% time in high CGMS range
18	0	3.17	4.80	6.50	5.00	14.80	0.00	27.00	73.00
18	1	2.85	4.00	4.70	5.20	13.66	0.00	39.61	60.39
18	2	2.55	3.50	5.20	8.10	10.78	5.54	62.38	32.08
18	3	2.56	3.80	4.40	3.30	15.16	0.00	29.35	70.65
20	0	2.88	4.10	5.40	4.10	10.40	6.00	57.00	37.00
20	1	2.67	3.90	5.30	4.20	10.34	3.37	64.92	31.71
20	2	2.14	2.70	3.50	4.30	11.25	0.00	60.02	39.98
20	3	2.12	3.10	3.90	2.70	6.94	11.08	75.12	8.89
21	0	3.43	4.80	5.10	4.70	17.70	1.00	6.00	93.00
21	1	2.87	4.50	6.10	6.20	9.49	25.17	37.48	37.35
21	2	2.79	4.70	6.20	5.80	11.61	1.92	49.26	48.82
21	3	2.02	2.60	3.60	2.90	16.58	0.00	5.41	94.59
23	0	3.55	5.50	7.10	4.40	8.90	13.00	67.00	20.00
23	1	2.49	3.50	3.80	3.20	8.52	0.00	89.09	10.91
23	2	2.61	3.70	4.30	3.60	10.09	0.00	73.00	27.00
23	3	2.96	4.60	5.70	3.70	11.01	0.25	63.35	36.32
24	0	2.72	4.30	5.80	8.00	9.20	29.00	39.00	32.00
24	1	2.09	3.10	4.30	4.10	10.61	5.29	59.29	35.42
24	2	1.61	2.40	3.10	3.60	8.69	1.62	87.89	10.50
24	3	2.44	3.80	5.80	3.70	9.19	6.83	66.01	25.93
25	0	3.59	5.40	6.60	3.30	8.40	20.00	53.00	27.00
25	1	2.87	4.60	6.70	4.10	8.09	29.57	44.59	25.84
25	2	3.20	5.00	6.90	5.90	13.87	1.13	35.69	63.18
25	3	2.92	4.30	6.30	5.30	13.98	0.50	32.26	67.20
26	0	2.72	3.30	4.70	4.30	6.90	31.00	59.00	10.00
26	1	3.75	5.50	7.20	4.70	11.50	8.54	43.31	48.15
26	2	3.49	4.60	5.80	6.60	11.78	3.71	51.27	45.02
26	3	3.10	4.30	5.20	5.00	9.60	5.42	62.80	28.92
28	0	2.81	4.10	5.50	5.50	10.60	11.00	52.00	37.00
28	1	2.31	2.90	3.50	3.10	11.50	0.00	65.68	34.32
28	2	2.06	3.00	4.10	4.30	9.85	1.11	69.77	29.12
28	3	2.37	3.50	4.60	2.80	11.66	0.83	51.96	46.78
29	0	3.01	4.40	6.30	4.90	11.60	6.00	54.00	4.00
29	1	2.92	4.10	5.40	4.40	7.85	14.76	67.91	17.33
29	2	3.31	4.90	6.70	5.90	10.63	14.61	45.97	39.42
29	3	3.57	5.10	6.30	3.90	10.56	3.17	59.93	35.50
30	0	4.32	6.20	8.30	6.80	12.20	14.00	31.00	56.00
30	1	2.07	3.00	3.70	4.10	9.24	2.81	77.40	19.79
30	2	2.25	3.20	4.10	7.20	9.61	17.35	42.42	40.23
30	3	3.50	4.60	5.50	4.20	9.91	2.50	73.37	23.47



**Appendix 6: Raw data from all 38 participants from each of the four timepoints of the study**

Patient	Run	Conga1	Conga2	Conga4	MODD	MBG	% time in low CGMS range	% time in normal CGMS range	% time in high CGMS range
31	0	2.97	4.50	6.30	3.50	12.50	2.00	37.00	61.00
31	1	2.81	3.90	4.90	4.00	11.80	2.79	48.56	48.65
31	2	3.15	4.20	5.10	3.40	12.27	2.46	45.08	52.46
31	3	3.95	6.40	8.90	3.90	10.23	15.67	38.07	39.28
32	0	2.99	3.90	4.40	3.80	12.70	0.00	47.00	53.00
32	1	3.02	4.60	5.40	4.10	10.42	3.88	64.00	32.13
32	2	3.64	5.40	6.70	5.60	12.14	4.33	50.57	45.10
32	3	2.57	2.90	3.40	3.10	11.08	0.00	63.79	36.21
33	0	2.65	3.80	4.20	3.20	10.20	1.00	69.00	30.00
33	1	3.72	5.20	6.70	5.70	12.94	5.98	34.85	59.16
33	2	2.30	3.20	3.80	2.60	7.84	1.64	91.33	7.03
33	3	2.46	3.90	5.40	3.20	6.38	31.75	54.06	11.95
35	0	2.63	4.10	5.40	4.60	11.10	2.00	55.00	43.00
35	1	2.36	3.50	4.90	3.70	12.18	0.00	48.11	51.89
35	2	3.27	5.10	7.30	5.10	9.49	14.36	56.92	28.72
35	3	2.81	4.50	6.10	7.40	15.14	6.08	23.27	70.09
36	0	3.25	5.40	7.80	2.60	12.80	1.00	51.00	47.00
36	1	2.44	3.70	5.20	4.70	13.21	0.00	42.66	57.34
36	2	2.90	4.70	5.90	2.80	14.94	0.00	23.28	76.72
36	3	2.78	4.40	6.10	4.50	13.94	0.00	32.92	67.08
37	0	3.06	4.90	7.40	6.60	10.50	12.00	53.00	35.00
37	1	2.63	4.20	5.90	6.00	8.92	18.92	56.65	24.43
37	2	2.95	4.40	6.30	6.10	12.71	16.53	23.32	60.14
37	3	3.67	5.70	7.60	6.30	12.06	10.33	34.47	49.87
38	0	3.65	5.70	7.90	7.10	10.40	10.00	56.00	35.00
38	1	2.71	4.00	5.40	4.10	9.92	5.68	62.66	31.65
38	2	3.37	5.30	7.80	5.90	13.20	5.31	37.08	57.61
38	3	3.16	5.10	7.70	5.80	9.98	6.83	56.08	33.07
39	0	3.07	4.50	5.30	4.30	9.80	9.00	60.00	31.00
39	1	3.50	5.40	7.50	3.50	8.79	15.40	64.18	20.41
39	2	2.16	3.10	4.50	5.20	13.02	0.00	44.77	55.23
39	3	3.55	5.60	8.30	4.50	10.35	14.25	38.58	39.72
41	0	3.90	6.10	6.90	5.60	10.50	5.00	63.00	32.00
41	1	2.99	4.70	6.10	4.10	10.10	6.26	64.76	28.98
41	2	3.23	5.00	6.70	4.00	10.49	7.88	58.91	33.20
41	3	3.11	5.00	7.00	5.00	11.24	14.33	40.32	43.74



**Appendix 6: Raw data from all 38 participants from each of the four timepoints of the study**

Patient	Run	Conga1	Conga2	Conga4	MODD	MBG	% time in low CGMS range	% time in normal CGMS range	% time in high CGMS range
42	0	2.68	3.30	4.10	5.00	9.30	0.00	88.00	12.00
42	1	2.70	4.50	7.00	6.50	10.00	0.00	77.70	22.30
42	2	2.99	4.70	6.20	3.40	14.49	0.00	23.97	76.03
42	3	2.88	4.30	6.20	5.20	13.48	4.25	33.82	61.56
43	0	2.49	3.70	4.80	3.50	9.20	8.00	70.00	22.00
43	1	2.74	4.20	5.60	5.00	10.27	9.29	58.83	31.88
43	2	2.16	3.30	4.80	2.30	9.30	1.28	78.26	20.46
43	3	1.90	2.50	2.80	1.30	13.61	0.00	21.05	78.95
45	0	3.93	5.60	7.70	4.80	11.40	7.00	47.00	46.00
45	1	3.55	5.10	6.30	3.80	11.74	4.69	44.68	50.63
45	2	2.89	4.60	6.20	5.00	10.13	6.27	61.25	32.48
45	3	2.39	3.60	5.30	4.80	12.78	0.00	45.28	54.72
46	0	3.29	5.00	6.80	3.90	9.00	17.00	56.00	27.00
46	1	4.06	5.90	7.20	5.90	13.68	1.14	37.94	60.91
46	2	3.08	4.70	6.10	3.60	12.16	1.18	58.73	40.09
46	3	2.89	4.10	5.30	4.40	10.55	5.58	57.67	36.24
47	0	3.13	4.80	6.70	4.40	10.50	12.00	47.00	40.00
47	1	1.86	2.90	4.00	5.10	11.52	0.00	61.30	38.70
47	2	2.19	3.10	3.80	4.80	12.94	0.00	43.46	56.54
47	3	2.32	3.30	4.20	3.60	12.74	0.00	46.01	53.99
48	0	3.27	4.90	5.90	5.20	10.30	9.00	57.00	35.00
48	1	3.09	4.70	4.30	5.70	11.96	0.00	55.90	44.10
48	2	3.47	5.20	7.20	5.40	12.60	12.31	28.39	59.30
48	3	2.82	4.30	6.50	4.00	13.89	0.33	37.16	62.37
49	0	3.26	5.10	6.30	4.50	9.00	11.00	70.00	19.00
49	1	3.72	5.60	6.00	5.10	9.62	16.65	49.66	33.69
49	2	3.47	5.50	7.90	3.80	12.80	1.07	50.30	48.64
49	3	3.58	5.30	6.80	6.50	12.58	8.83	35.04	53.13
51	0	3.01	4.50	5.60	5.80	12.80	0.00	43.00	56.00
51	1	2.30	2.90	3.80	2.70	11.80	0.00	50.69	49.31
51	2	2.76	4.00	5.60	3.90	11.81	0.26	56.68	43.06
51	3	2.58	3.40	4.60	4.00	12.60	0.17	45.63	54.12
52	0	5.70	5.50	6.70	2.40	10.20	15.00	51.00	33.00
52	1	4.20	6.30	8.50	3.50	9.00	29.66	44.49	25.86
52	2	3.24	5.10	7.00	7.20	13.41	12.57	27.07	60.37
52	3	2.14	3.30	4.90	6.20	12.27	0.00	44.66	55.34

**Appendix 6: Cumulative outcomes for each of the glycaemic and metabolic outcomes from each of the four timepoints of the study.**

Variable	Time	Mean	Minimum	Maximum	sd
HbA1c [%]	0	8.1	6.7	9.3	0.7
	1	8.6	6.7	10.8	0.9
	2	8.9	7.2	10.3	0.7
	3	8.5	7.1	10.1	0.8
Insulin dose [u/kg/day]	0	0.9	0.6	0.2	0.2
	1	0.9	0.6	1.3	0.2
	2	0.9	0.6	1.4	0.2
	3	0.9	0.6	1.3	0.2
BMI z-score	0	1.2	-1.3	3.3	0.9
	1	1.2	-0.9	3.3	0.8
	2	1.2	-1.0	3.2	0.8
	3	1.1	-1.1	3.1	0.8
Mean blood glucose	0	11.0	6.9	17.7	2.1
	1	10.9	7.9	15.1	1.8
	2	11.6	7.8	14.9	1.8
	3	11.6	6.4	16.6	2.1
Mean of daily differences	0	5.2	2.3	17.4	2.5
	1	4.4	0.0	6.5	1.2
	2	5.0	2.3	8.1	1.5
	3	4.5	1.3	7.4	1.3
Percent time in low glycaemic range	0	9.9	0.0	36.0	9.4
	1	7.1	0.0	29.7	8.8
	2	4.9	0.0	17.4	5.4
	3	7.8	0.0	34.0	8.9
Percent time in normal glycaemic range	0	50.1	6.3	88.2	15.3
	1	53.4	29.1	89.1	14.5
	2	50.5	23.3	91.3	17.0
	3	46.6	5.4	75.1	16.1
Percent time in high glycaemic range	0	40.0	10.4	93.1	17.3
	1	39.5	10.9	70.9	15.0
	2	44.7	7.0	76.7	16.4
	3	46.3	8.9	94.6	18.5
Continuous overall net glycaemic action1	0	3.3	2.1	5.7	0.6
	1	2.9	1.9	4.2	0.6
	2	2.9	1.6	4.1	0.6
	3	2.9	1.8	4.0	0.6
Continuous overall net glycaemic action2	0	4.8	3.1	6.5	0.8
	1	4.3	2.9	6.3	0.9
	2	4.3	2.4	6.4	0.9
	3	4.3	2.5	6.4	1.0
Continuous overall net glycaemic action4	0	6.2	4.1	8.7	1.2
	1	5.6	3.3	8.5	1.3
	2	5.7	3.1	8.2	1.4
	3	5.8	2.8	8.9	1.5

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**Appendix 7: Proportions of mixed insulins have little  
impact upon glycaemia in primary school-aged children.**



## Short Report

# Twice-daily variable insulin regimens: proportions of insulin types have little impact on glycaemic control in primary school-aged children

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### Abstract

**Aim** To ascertain the relationship between glycaemic outcome and proportions and timing of insulin admixture in a cohort of primary school-aged children who were receiving insulin in a twice-daily regimen.

**Methods** Children aged 4–10 years with Type 1 diabetes of > 2 years duration and on twice-daily variable insulin regimens were eligible for inclusion in this study, which took place over a 12-month period. Characteristics of insulin regimen [total daily dose (TDD), proportion of total daily dose given in the morning and proportion of the TDD given as intermediate-acting insulin] were compared with parameters of glycaemia including glycated haemoglobin (HbA<sub>1c</sub>) and continuous glucose monitoring measures (mean glucose, per cent time in various glycaemic ranges, and intra- and inter-day glycaemic variation).

**Results** Forty-nine children completed the study. Participants were all prepubertal at the start of the study and representative of the local diabetes population aged 4–10 years (mean age 8.2 years, mean duration of diabetes 3.5 years, mean HbA<sub>1c</sub> 8.1%). The mean TDD was 0.9 units/kg/day (range 0.6–1.3). The TDD, percentage of TDD given as intermediate-acting insulin and the percentage of TDD given as the morning dose were not associated with HbA<sub>1c</sub>, mean continuous glucose monitoring system glucose, per cent time in various glycaemic ranges or intra- and inter-day glycaemic variation.

**Conclusions** Insulin proportions in twice-daily, variable insulin regimens are not associated with any short- or medium-term glycaemic outcomes.

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**Keywords** continuous glucose monitoring, continuous overall net glycaemia variation, glycaemic variation, insulin

**Abbreviations** CGMS, continuous glucose monitoring system; CONGA, continuous overall net glycaemia variation; HbA<sub>1c</sub>, glycated haemoglobin; TDD, total daily dose (units/kg/day); MODD, mean of daily differences

### Introduction

Many primary school-aged children with Type 1 diabetes receive their insulin in a twice-daily regimen of variable admixtures of short- and longer-acting insulins [1–3]. A combination of regular and isophane insulins has traditionally been the most common regime, with analogue insulins being more recently incorporated. Diabetes care manuals and textbooks

have suggested proportions of short- to intermediate-acting insulins within these admixtures [4,5], with the aim being to avoid peaks and troughs of circulating insulin levels and to optimize glycaemic stability. Most texts recommend that the majority of insulin is given in the morning and that the majority of this should be given as intermediate-acting insulin [4–6]. The advent of continuous glucose monitoring allows the notion of improved glycaemic stability with such regimes to be tested. The purpose of this study was to ascertain glycaemia in a cohort of primary school-aged children who were receiving variable admixtures of short- and intermediate-acting insulins in a twice-daily regimen and to relate glycaemic outcomes to the proportions and timing of insulin admixture.

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## Patients and methods

All prepubertal children aged 4–10 years on 1 February 2003 with diabetes of greater than 2 years duration attending the Royal Children's Hospital, Melbourne and receiving twice-daily variable insulin regimes were eligible for inclusion in this study. Children were receiving regular diabetes care with routine 3-monthly clinic visits and glycated haemoglobin (HbA<sub>1c</sub>) measurements over a period of 12 months. For the purposes of this study they had additional continuous glucose monitoring [Continuous Glucose Monitoring System (CGMS); Minimed 405s®, Medtronic Systems, Northridge, CA, USA] performed for 3 days, with each clinic visit on four consecutive occasions. During the period of each CGMS trace, insulin doses were not adjusted. CGMS methodology has been previously described [7]. Clinical data collected at each clinic review included weight, details of insulin regimen and HbA<sub>1c</sub> (DCA 2000 immunoassay; Bayer Diagnostics Pty Ltd, Calabria, Barcelona, Spain). All children were under parental supervision for insulin injections. Data obtained from each 72-h CGMS trace were downloaded into Minimed Solutions version 1.7A. Data cleaning and analysis of the CGMS data was carried out as previously described [8,9]. CGMS data included: mean CGMS glucose, percentage time spent within glucose ranges defined as low (< 4.0 mmol/l), normal (4.0–12.0 mmol/l) and high (> 12.0 mmol/l), intra-day glycaemic variation as measured by continuous overall net glycaemic action (CONGA) and inter-day glycaemic variation as measured by the mean of daily differences (MODD). CONGA was calculated for time intervals of 1, 2 and 4 h. Data were pooled for all subjects. Outcomes were expressed as means and linear regression analysis was adjusted for repeated measures of glycaemic variation. Statistics were performed with Stata™9.0 statistics/data analysis system (Stata Corporation, College Station, TX, USA). Full ethics approval was given by the Ethics Committee of the Royal Children's Hospital.

## Results

### Subjects

Ninety-nine children (54 male, 45 female) were eligible for the study, with 52 (21 male, 31 female) consenting to participate. The majority of families who declined cited the time involved or the child's reluctance to undergo invasive CGMS testing as their reasons for non-participation. No differences were found between participants and non-participants for age [mean 8.2 years (range 4.3–10.3) vs. 7.7 years (range 3.2–9.9),  $P = 0.16$ ], mean duration of diabetes [3.5 years (range 2.1–7.2) vs. 4.0 years (range 2.1–8.1),  $P = 0.38$ ] and mean HbA<sub>1c</sub> 8.1% (range 6.4–11.4) vs. 8.1% (range 6.3–9.7),  $P = 0.55$ ]. Three children were recruited but were subsequently excluded during the study: two children switched to basal : bolus insulin regimens and a third child failed to obtain a valid CGMS trace after three attempts.

### Glycaemic outcomes

Data were obtained and pooled from 165 traces carried out over the time frame of the study from the 49 participants.

**Table 1** Summary of insulin dosage and glycaemic variables ( $n = 49$ )

Variable	Mean $\pm$ SD	Range
Total insulin dose (units)	29.1 $\pm$ 9.4	12.5–60
Insulin dose to weight (units/kg/day)	0.88 $\pm$ 0.16	0.56–1.3
Total intermediate acting insulin (units)	21.3 $\pm$ 7.0	9–40
Total short acting insulin (units)	7.8 $\pm$ 3.4	2–20
Total morning insulin dose (units)	20.1 $\pm$ 7.0	9–40
Total evening insulin dose (units)	9.0 $\pm$ 3.6	2–20
HbA <sub>1c</sub> (%)	8.5 $\pm$ 0.9	6.4–11.4
Mean CGMS glucose (mmol/l)	11.3 $\pm$ 2.0	6.4–17.7
MODD	4.8 $\pm$ 1.7	1.3–17.4
Percent time spent in low (< 4 mmol/l) CGMS range (%)	7.1 $\pm$ 7.9	0.0–36.0
Percent time spent in normal (4–12 mmol/l) CGMS range (%)	50.5 $\pm$ 15.3	5.4–91.3
Percent time spent in high (> 12 mmol/l) CGMS range (%)	42.4 $\pm$ 16.7	7.0–94.6
CONGA 1 hour	3.0 $\pm$ 0.6	1.6–5.7
CONGA 2 hour	4.4 $\pm$ 0.9	2.4–6.5
CONGA 4 hour	5.8 $\pm$ 1.3	2.8–8.7

Mean time per CGMS trace was 76.3  $\pm$  15.0 h prior to data validation and 72.3  $\pm$  16.4 h after data cleaning. Glycaemic variables are summarized in Table 1.

### Insulin regimens

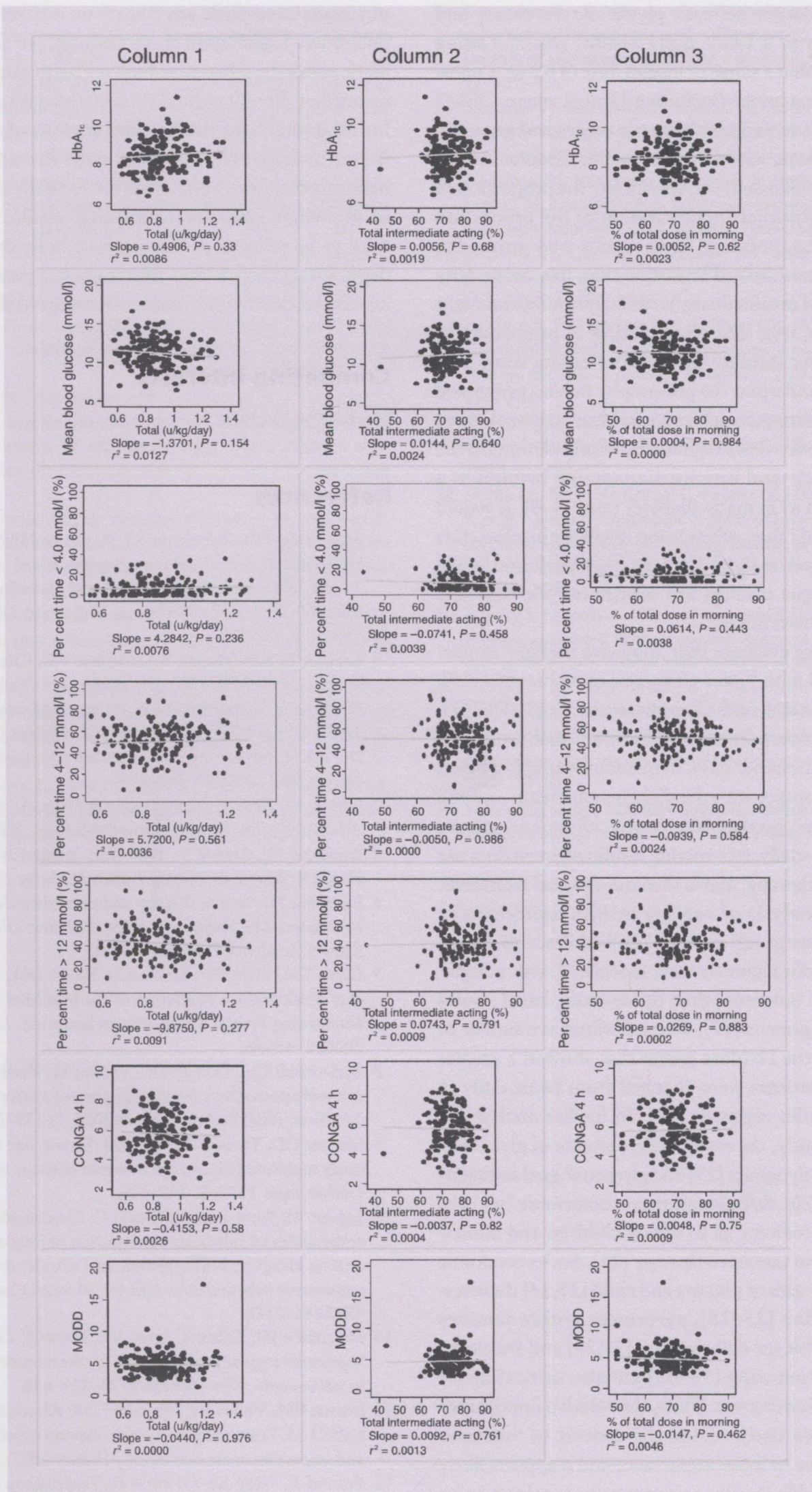
Insulin was administered in twice-daily combinations of intermediate-acting (isophane) and short-acting (either regular or analogue) insulins. Short-acting regular and analogue insulins were used in 73 and 27% of patients, respectively, in the morning insulin and in 32 and 68% of patients, respectively, in the evening insulin. The mean total daily insulin dose (TDD) of the study group was 0.9 units/kg/day (range 0.6–1.3). The mean proportion of the total daily insulin dose given in the morning was 69% (range 50–90). Morning insulin consisted of a mean of 74% (range 60–93) intermediate-acting insulin combined with a mean of 26% (range 7–40) short-acting insulin. A similar pattern was seen in evening insulin with a mean of 73% intermediate-acting (range 0–100) insulin given in combination with a mean of 27% short-acting insulin (range 0–100). Insulin dosages are further summarized in Table 1.

The relationship between intercurrent HbA<sub>1c</sub> and CGMS data and TDD, the percentage of the TDD given as intermediate-acting insulin and the percentage of the TDD given as the morning insulin were assessed. Linear regression analysis did not demonstrate that TDD, percentage of the TDD given as intermediate-acting insulin or the percentage of the TDD given as the morning insulin were associated with HbA<sub>1c</sub> or any of the CGMS measures of glycaemic outcome (Fig. 1).

## Discussion

Data presented in this study have shown that, in sequentially assessed children with Type 1 diabetes aged 4.3 to 10.3 years,





**FIGURE 1** Linear regression analysis comparing outcomes of glycaemia with total insulin dosage, balance of insulin dosage and insulin duration. Column 1: association of glycaemic outcomes with relative total daily dose. Column 2: association of glycaemic outcomes with proportion of total daily dose given in the morning. Column 3: association of glycaemic outcomes with proportion of total daily dose given as intermediate-acting insulin.



there was no association between glycaemic outcomes and insulin proportions in a twice-daily insulin regimen using isophane insulin. Within a range of insulin dose of 0.6–1.3 units/kg/day, glycaemic outcomes (including HbA<sub>1c</sub>, mean CGMS glucose, per cent time in various glycaemic ranges and intra-day and inter-day glycaemic variability) were not associated with the proportions of insulin delivery, specifically the proportion of insulin given as intermediate-acting insulin or the proportion of insulin given in the morning. These results were surprising and appear counter-intuitive. The notion that, in a twice-daily regimen, a balance of insulin doses between morning and night and intermediate-acting and short-acting is important in determining glycaemic stability has been current for some time and to some extent underpins the philosophy behind pre-mixed insulins [10,11]. Attempts to achieve a stable pharmacokinetic insulin profile in a twice-daily regimen by manipulations in the proportions of short- and intermediate-acting insulins is a clinical goal referred to in many diabetes texts [4–6]. It would appear from this study that, when using isophane insulins, this goal is illusory. Whether these findings are also found when longer-acting analogue insulins are substituted for isophane insulins is yet to be determined.

There is cumulative evidence that ‘intensive therapy’ in paediatrics is associated with better glycaemic outcomes [12–15]. In the Diabetes Control and Complications Trial (DCCT), ‘intensive therapy’ incorporated an intensive insulin regimen (more than two injections per day), frequent measuring of blood glucose, increased contact with the diabetes care team and the setting of glycaemic goals [16]. Many clinicians assume therefore that a twice-daily, free-mixing insulin regimen does not constitute ‘intensive therapy’ and is thus sub-optimal treatment. In a comparative analysis of varying insulin regimens in an international Hvidoere group study of 2269 adolescents, twice-daily free-mixed insulin regimens were associated with marginally better glycaemic outcomes than thrice-daily, basal : bolus and insulin-pump regimens [17]. These findings are similar to a previous study of the Hvidoere group that showed a greater mean HbA<sub>1c</sub> after patients were changed from twice-daily to four-times-daily insulin regimens [18]. In further analyses of the 2007 Hvidoere study, the major determinants of glycaemic outcome were family dynamics [19] and glycaemic goal setting by diabetes care teams [20]. Self-management competence has been found to be a key predictor as to which children and adolescents will benefit from intensive therapy [21]. Socio-economic status [22], mental health of parents and child [23,24] diabetes-related family conflict [25–28], agreement within families as to who is responsible for diabetes care [19,29] and frequency of blood glucose monitoring [30–32] are also critical determinants of glycaemic outcome. Thus, the relative importance of the insulin regimen itself, within the context of ‘intensive therapy’ appears to be of lesser importance and it appears likely that twice-daily, variable insulin regimes may continue to be utilized in many centres for some time.

This study has shown that the details of insulin proportions in twice-daily, variable regimens using isophane insulins are

not associated with any short- or medium-term glycaemic outcomes. Based upon these findings, we should not be dogmatic about the inherent values of a particular fixed regimen (for example a 70 : 30 regimen compared with a 50 : 50 regimen) for all patients. In the broader context of intensive therapy, these findings are consistent with the notion that insulin regimens themselves do not appear to be the major determinants of glycaemic outcome. Individual insulin therapies always need to be tailored to the needs of individual patients and a dogmatic approach that insists upon a prescribed balance of insulin proportions appears to be misguided.

### Competing interests

Nothing to declare.

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## Appendix 8: Hyperglycaemia and Externalizing Behaviour in children with type 1 diabetes.

*[The following text is extremely faint and largely illegible. It appears to be the main body of a research paper or report, containing sections such as Introduction, Methods, Results, and Discussion. The text is too light to transcribe accurately.]*



# Hyperglycemia and Externalizing Behavior in Children With Type 1 Diabetes

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**OBJECTIVE** — Anecdotal, parents report behavioral changes in their diabetic children who have fluctuating blood glucose levels. This study aimed to test associations between intercurrent glycemia and child behavior in an ambulant setting.

**RESEARCH DESIGN AND METHODS** — Prepubertal children attending the Royal Children's Hospital, Melbourne, Australia, with type 1 diabetes received glycemic assessment and simultaneous behavioral assessment on two occasions 6 months apart. Subjects wore a continuous glucose monitor over a 72-h period, and parents completed the Behavior Assessment System for Children at the two study time points.

**RESULTS** — There was a high correlation between intra-individual externalizing and internalizing behavior scores ( $r = 0.88$ ,  $P < 0.001$  and  $r = 0.81$ ,  $P < 0.001$ , respectively) at the two time points. Mean blood glucose (MBG) was significantly associated with the mean externalizing behavior score ( $\beta = 1.7$  [95% CI 0.6–2.8], adjusted  $r^2 = 0.088$ ). Percentage of time in the normal ( $r = -0.2$  [-0.3 to -0.5], adjusted  $r^2 = 0.068$ ) and high ( $r = 0.2$  [0.07–0.3], adjusted  $r^2 = 0.089$ ) glycemic ranges were significantly associated with the mean externalizing behavior score. For every 5% increase in time in the normal glycemic range, there was a decrease in the externalizing behavior score of 1.0, and for every 5% increase in time in the high glycemic range there was an increase in the externalizing behavior score of 1.0. There was no significant association between MBG and the mean internalizing behavior score.

**CONCLUSIONS** — Externalizing behaviors were associated with intercurrent glycemic status. These findings underscore the importance of understanding the mechanisms of this association and how it might impact ultimate diabetes outcomes.

Diabetes Care 30:2211–2215, 2007

Parents of children with type 1 diabetes often report that they can detect elevations in their child's blood glucose due to changes in outward behavioral patterns. These reports, however, are entirely anecdotal, and to date, there has been little direct inquiry in this phenomenon. In particular, both the existence and causality of the association have yet to be confirmed. Much of the literature that exists on the impact of behavior on diabe-

tes or vice versa relates to long-term effects on poor metabolic control (1–4) or to transient changes during acute severe episodes of hypoglycemia (5,6). Recently, studies have identified impairment of cognitive performance and mood in adults with either type 1 or type 2 diabetes (7,8). Poor metabolic control has also been associated with disengagement and negativity in adolescents with type 1 diabetes (9). High levels of externalizing behaviors (e.g., aggression,

overactivity, conduct problems) at diagnosis are strongly predictive of externalizing behaviors in adolescence (10), and these behaviors, in turn, are predictive of poor metabolic control and mental health problems in middle-aged adults (11).

The purpose of this study was to test for any possible association between childhood behavior and intercurrent glycemia. To address direct parental observations of behavior, we elected to conduct this study in a noncontrolled ambulant context using continuous glucose monitoring techniques and standardized parent-report behavioral questionnaires.

## RESEARCH DESIGN AND METHODS

This study was conducted prospectively in 2003 and 2004 over a 6-month period and was approved by the Human Ethics Research Committee at the Royal Children's Hospital (RCH), Melbourne, Australia.

Primary school-aged children attending the diabetes outpatient clinic of RCH with >2 years duration of type 1 diabetes aged 5–10 years on 1 February 2003 were invited to join this study. The RCH clinic cares for ~1,350 children and adolescents with type 1 diabetes. This group is demographically representative of the community in Melbourne, Australia. Children were recruited sequentially over a 3-month period during their routine attendance at the outpatient clinic and received continuous glycemic and simultaneous behavioral assessment on two occasions 6 months apart.

## Continuous glycemic assessment

Each child wore a continuous glucose monitor (Minimed, Northridge, CA) (12) over a 72-h period at the two study time points. This procedure has been previously described elsewhere (12). After each 72-h recording period, data were downloaded (using CGMS Gold software) and cleaned to remove invalid data. Invalid data were defined as occasions where there were less than four calibrations from finger-prick blood glucose readings in a 24-h period or a calibration was not carried out within an 8-h period. Data were summarized using an algorithm previously reported by this group (13). Mean blood glucose (MBG) was cal-

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**Abbreviations:** BASC, Behavior Assessment System for Children; CGMS, continuous glucose monitor system; MBG, mean blood glucose; RCH, Royal Children's Hospital.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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## Hyperglycemia and externalizing behavior

culated as was percentage of time spent in low (continuous glucose monitoring system [CGMS] <4 mmol/l), normal (4–12 mmol/l), and high (>12 mmol/l) glucose ranges.

### Behavioral measures

On both occasions when CGMS was applied, parents were asked to complete the Behavior Assessment System for Children (BASC) (14). This is a standardized, validated, parent-report instrument that takes ~20 min to complete on each occasion and reflects the child's typical behavior over the previous 6 months. To minimize the risk that parental ratings are influenced by factors extraneous to the child's actual behavior, parents are asked to report on the occurrence and frequency of specific behaviors and not on their perceptions and feelings regarding the child's behavior (e.g., "Does your child hit other children almost always? Often? Sometimes? Never?" rather than "Is your child aggressive?"). Analyses of BASC data were carried out using BASC Enhanced ASSIST (version 2.0), which generates summary T-scores (mean of 50 and SD of 10) standardized for age and sex for externalizing and internalizing behavior. Externalizing behavior comprises hyperactivity, aggression, and conduct scores, whereas internalizing behavior comprises anxiety, depression, and somatization scores. High scores indicate greater psychopathology.

### Statistical analysis

The degree of agreement between intra-individual measurements on the two occasions was evaluated using the correlation coefficient ( $r$ ). Linear regression was used to evaluate the relationship between behavior scores and glycemic measurements.

**RESULTS** — A total of 42 children (27 female and 15 male) aged 5–10 years consented to be in the study. At the time of recruitment, this cohort was representative of the total RCH clinic population aged 5–10 years (mean age 8.0 vs. 7.6 years for the study cohort vs. the overall 5- to 10-year-old clinic cohort, respectively,  $P = 0.25$ ) and A1C levels (8.2 vs. 8.1%,  $P = 0.60$ ). All patients completed the assessment according to the protocol. Preliminary analyses showed that the relationship between dependent and independent variables at the two observational time points was similar such that data from both time points were pooled providing 84 glycemic and behavioral paired datasets.

Table 1—Characteristics of the study cohort at baseline and 6 months

	Baseline	6 months
<i>n</i>	42	42
Age (years)	8.3 ± 1.4	8.9 ± 1.4
Sex (male/female)	15/27	15/27
Total daily insulin dose (units · kg <sup>-1</sup> · day <sup>-1</sup> )	0.87 ± 0.2	0.9 ± 0.2
A1C (%)	8.6 ± 0.8	8.5 ± 0.8
Glycemic measures		
MBG (mmol/l)	10.8 ± 1.8	11.6 ± 2.1
Percentage of time in low glycemic range	7.4 ± 8.6	6.9 ± 7.9
Percentage of time in normal glycemic range	53.7 ± 14.0	47.1 ± 15.6
Percentage of time in high glycemic range	38.9 ± 14.9	46.0 ± 17.8
Behavioral variables		
Externalizing behavior score	48.6 ± 9.3	48.1 ± 11.3
Internalizing behavior score	54.2 ± 12.2	52.9 ± 14.0

Data are means ± SD.

Sample characteristics are shown in Table 1. Forty patients were receiving insulin in a twice-daily mixing regime, two patients were receiving insulin in a 3–4 injection regime, and zero patients were receiving insulin pump therapy. The mean number of valid hours per CGMS trace was 73.9 h. Glycemic and behavioral data were normally distributed.

### Glycemic data

**MBG.** The overall MBG value was 11.2 ± 2 mmol/l (mean ± SD). The intra-individual correlation of MBG values between the two time points studied (0 and 6 months) was not significant ( $r = 0.04$ ,  $P = 0.83$ ). In individual patients, MBG was moderately correlated with intercurrent A1C ( $r = 0.43$  [95% CI 0.24–0.59],  $P < 0.001$ ).

**Percentage of time in various glycemic ranges.** The overall mean percentage of time spent in the low (<4 mmol/l), normal (4–12 mmol/l), and high (>12 mmol/l) CGMS ranges were 7.1 ± 8.2, 50.4 ± 15.1, and 42.4 ± 16.7%, respectively, and there were poor correlations of the intra-individual measures at the two time points (low,  $r = 0.14$ ,  $P = 0.36$ ; normal,  $r = 0.33$ ,  $P = 0.03$ ; and high,  $r = 0.10$ ,  $P = 0.53$ ).

### Behavioral data

**Externalizing behavior scores.** There was a high correlation between intra-individual externalizing behavior scores at the time points studied ( $r = 0.88$ ,  $P < 0.001$ ) (Fig. 1A). In view of this, data from the two time points were pooled to calculate an overall mean externalizing behavior T-score (48.3 ± 10.3).

**Internalizing behavior scores.** There was high correlation between intra-

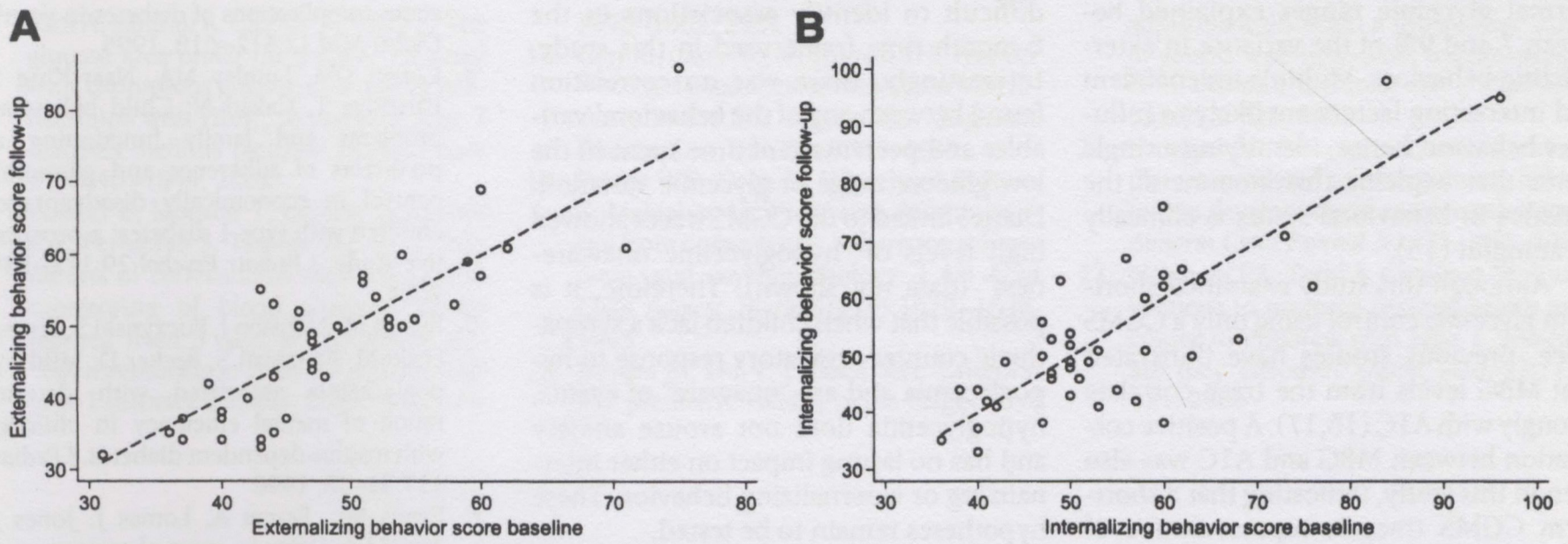
individual internalizing behavior scores across the two time points studied ( $r = 0.81$ ,  $P < 0.001$ ) (Fig. 1B). Using pooled data from both time points, the overall mean internalizing behavioral T-score was 53.5 ± 13.1.

### Associations between glycemic measures and behavior scores

**MBG and behavior scores.** MBG was significantly associated with the mean externalizing behavior score (regression coefficient = 1.7 [95% CI 0.6–2.8], adjusted  $r^2 = 0.088$ ) (Fig. 2A). This indicates that on average, for every 1 mmol/l rise in MBG, there was a concomitant rise of 1.7 in the externalizing behavior score, and variation in the MBG explained 8.8% of the variance in the group mean externalizing behavior score. There was no significant association between MBG and the mean internalizing behavior score.

**Percentage of time in various glycemic ranges and behavior scores.** Percentage of time in the normal ( $r = -0.2$  [95% CI -0.3 to -0.5], adjusted  $r^2 = 0.068$ ) and high ( $r = 0.2$  [0.07–0.3], adjusted  $r^2 = 0.089$ ) glycemic ranges were significantly associated with the mean externalizing behavior score (Fig. 2B and C). These data indicated that for every 5% increase in time in the normal glycemic range, there was a decrease in the externalizing behavior score of 1.0 and that for every 5% increase in time in the high glycemic range, there was an increase in the externalizing behavior score of 1.0. Variation in either percentage of time in the normal or high glycemic ranges explained 6.8 and 8.9% of the variance in the overall mean externalizing behavior score, respectively. There were no significant associations between percentage of time in the low gly-





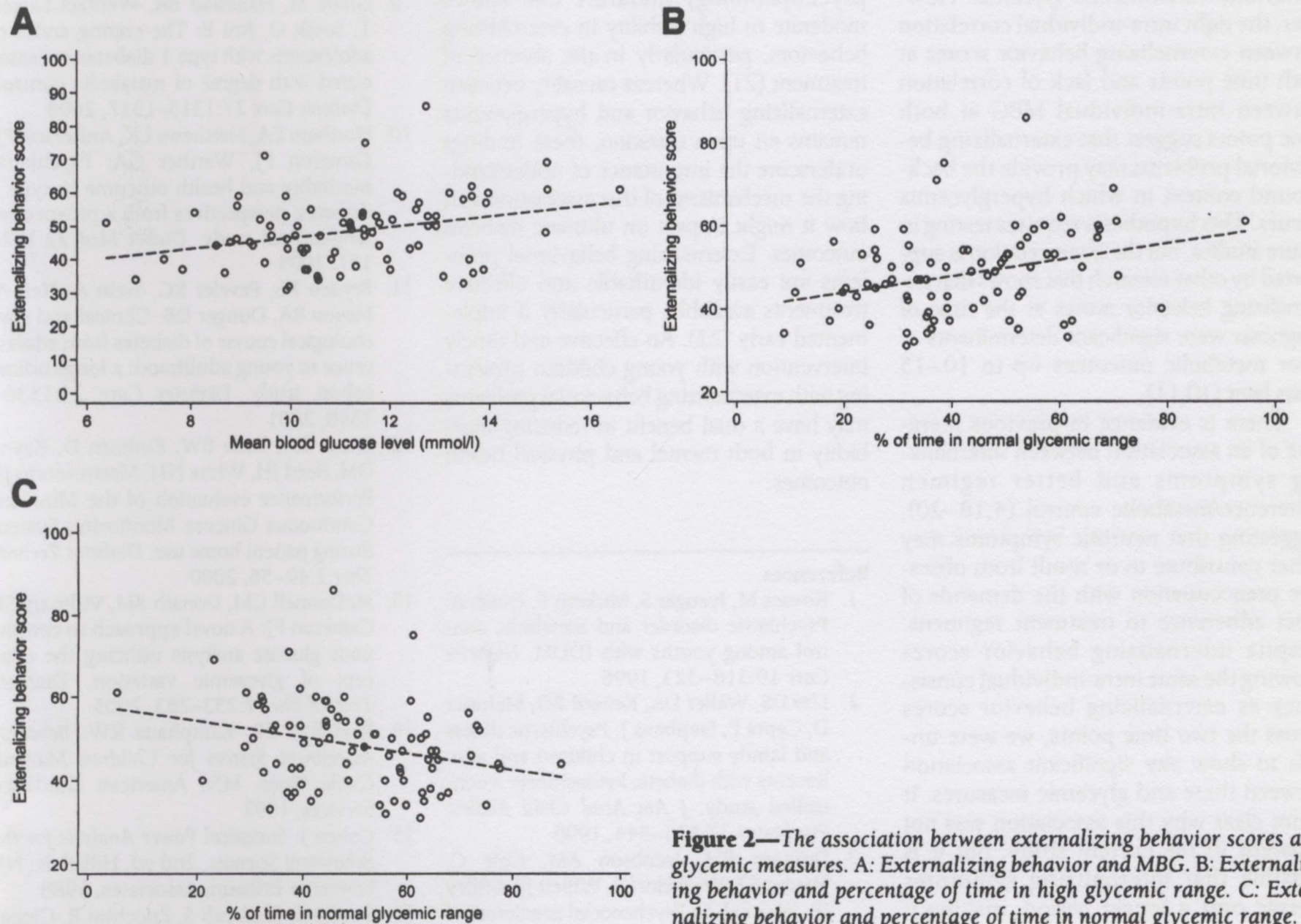
**Figure 1**—Intra-individual association of behavioral measures at baseline and 6 months. A: Externalizing behavior scores. B: Internalizing behavior scores.

cemic range and externalizing behavior scores, and significant associations were not found between internalizing behavior scores and the percentage of times in the normal, high, or low glycemic ranges.

**CONCLUSIONS**— In this study of a primary school-aged cohort of children

with type 1 diabetes, we found that higher MBG values, increased percentage of time in the high glycemic range, and decreased percentage of time in the normal glycemic range were all associated with higher externalizing behavior scores. Our findings are noteworthy in that we have demonstrated consistency in the relationship be-

tween the three glycemic measures and externalizing behavior, with higher MBG and percentage of time in high glycemic ranges being associated with more behavioral problems and higher percentage of time in the normal glycemic range being associated with fewer problems. Overall, MBG and percentage of time in high and



**Figure 2**—The association between externalizing behavior scores and glycemic measures. A: Externalizing behavior and MBG. B: Externalizing behavior and percentage of time in high glycemic range. C: Externalizing behavior and percentage of time in normal glycemic range.



normal glycemic ranges explained between 7 and 9% of the variance in externalizing behaviors. Multiple independent and interacting factors are likely to influence behavior; hence, identifying a single factor that explains this amount of the variance in behavioral status is clinically meaningful (15).

Although this study examined short-term glycemic control using only a CGMS trace, previous studies have illustrated that MBG levels from the trace correlate strongly with A1C (16,17). A positive correlation between MBG and A1C was also seen in this study, indicating that a short-term CGMS trace is representative of long-term metabolic control. This is also consistent with previously reported associations between high A1C levels and high externalizing behavioral scores (10, 11).

Externalizing behaviors identified on the BASC include hyperactivity, aggression, and conduct disorders. These behaviors are similar to those identified anecdotally by parents as being associated with higher blood glucose levels. In this study, we were unable to determine causality in the association between the behavioral variables and glycemia. However, the tight intra-individual correlation between externalizing behavior scores at both time points and lack of correlation between intra-individual MBG at both time points suggest that externalizing behavioral problems may provide the background context in which hyperglycemia occurs. This hypothesis requires testing in future studies, but the interpretation is supported by other research that shows that externalizing behavior scores at the time of diagnosis were significant determinants of poor metabolic outcomes up to 10–15 years later (10,11).

There is evidence in previous literature of an association between internalizing symptoms and better regimen adherence/metabolic control (4,18–20), suggesting that neurotic symptoms may either contribute to or result from obsessive preoccupation with the demands of strict adherence to treatment regimens. Despite internalizing behavior scores showing the same intra-individual consistency as externalizing behavior scores across the two time points, we were unable to show any significant association between these and glycemic measures. It is not clear why this association was not apparent in the current study, but it is possible that internalizing symptoms emerge over a longer period, making it

difficult to identify associations in the 6-month time frame used in this study. Interestingly, there was no correlation found between any of the behavioral variables and percentage of time spent in the low glucose range or glycemic variation. Diaries linked to the CGMS traces showed high levels of “hypoglycemic unawareness” (data not shown). Therefore, it is possible that when children lack a sympathetic counter-regulatory response to hypoglycemia and are “unaware” of events, hypoglycemia does not arouse anxiety and has no lasting impact on either internalizing or externalizing behavior. These hypotheses remain to be tested.

This is the first report of a study examining behavior and intercurrent glycemia using CGMS in an ambulant noncontrolled setting. Our findings provide some support for parental observation of an association between externalizing behavioral problems and intercurrent hyperglycemia. As such, our findings are consistent with the previously recognized association between behavioral problems and longer-term metabolic control, as reflected in A1C levels. The current findings are also consistent with the developmental psychopathology literature that shows moderate to high stability in externalizing behaviors, particularly in the absence of treatment (21). Whereas causality between externalizing behavior and hyperglycemia remains an open question, these findings underscore the importance of understanding the mechanisms of this association and how it might impact on ultimate diabetes outcomes. Externalizing behavioral problems are easily identifiable and effective treatments available, particularly if implemented early (22). An effective and timely intervention with young children presenting with externalizing behavioral problems may have a dual benefit in reducing morbidity in both mental and physical health outcomes.

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**Appendix 9: Social consumption of alcohol in adolescents with Type 1 diabetes is associated with increased glucose lability, but not hypoglycaemia.**

and F. J. Cameron

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**Introduction**

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# Social consumption of alcohol in adolescents with Type 1 diabetes is associated with increased glucose lability, but not hypoglycaemia

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## Abstract

**Aims** To determine the effects of social consumption of alcohol by diabetic adolescents on glycaemic control.

**Methods** Fourteen (five male) patients aged > 16 years were recruited from the diabetes clinic at the Royal Children's Hospital. The continuous glucose monitoring system (CGMS) was attached at a weekend when alcohol consumption was planned for one night only. For each patient, the 12-h period from 18.00 h to 06.00 h for the night with alcohol consumption (study period) was compared with the same period with non-alcohol consumption (control period) either 24 h before or after the alcohol study night. Thus, each subject was his/her own control. Glycaemic outcomes calculated from continuous glucose monitoring included mean blood glucose (MBG), percentage of time spent at low glucose levels (CGMS < 4.0 mmol/l), normal glucose levels (CGMS 4.0–10.0 mmol/l) and high glucose levels (> 10.0 mmol/l) and continuous overall net glycaemic action (CONGA).

**Results** The mean number of standard alcohol drinks consumed during the study period was 9.0 for males and 6.3 for females. There was no difference in percentage of time at high and normal glucose levels in the study and control periods. During the control period, there was a higher percentage of time with low glucose levels compared with the study period ( $P < 0.05$ ). There was an increased level of glycaemic variation during the study time when compared with the control period.

**Conclusions** In an uncontrolled, social context, moderately heavy alcohol consumption by adolescents with Type 1 diabetes appears to be associated with increased glycaemic variation, but not with low glucose levels.

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**Keywords** adolescence, alcohol, glycaemic control

**Abbreviations** CGMS, continuous glucose monitoring system; CONGA, continuous overall net glycaemic action; MBG, mean blood glucose; RCH, Royal Children's Hospital

## Introduction

Adolescents with Type 1 diabetes frequently engage in risk-taking activities [1]. Amongst these activities is the social

consumption of alcohol, frequently as underage drinkers [2]. Whilst the effects of alcohol consumption upon glycaemia have been well described in a controlled setting [3–6], little is known about the impact on glucose levels of alcohol consumption by adolescents within an ambulant, social context. The purpose of this project was to utilize continuous glucose monitoring to study the impact of social alcohol consumption on glycaemic control in a group of alcohol-using adolescents.

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## Patients and methods

This study was approved by the Human Ethics Research Committee of the Royal Children's Hospital (RCH). That approval was contingent upon the fact that the investigators should not be seen to encourage underage drinking in adolescents. Consequently, we only approached adolescents who we knew were drinking socially and, despite our previous counselling, elected to continue to drink alcohol on a semi-regular basis. We recruited 22 adolescents with Type 1 diabetes from the RCH diabetes clinic. The adolescents were considered eligible only if > 16 years old and parental/patient consent was obtained. HbA<sub>1c</sub> (Bayer DCA 2000 immunoagglutination method, Calabria, Barcelona, Spain) was measured, and diabetes duration and insulin doses were recorded.

The MiniMed continuous glucose monitoring system (CGMS) was attached to the study patients over a weekend period. Patients were required to have an alcohol-free period for at least 24 continuous hours during the weekend trace period. A diary was kept of activities during the trace period (insulin injections, meal, snacks, dancing, alcohol consumption, sport). There was no change in insulin doses between study and control periods. In the evening when alcohol was consumed, patients were asked to recall how many and what type of drinks were consumed and how inebriated they became. Patients recall of alcohol consumption was converted to 'standard drinks' (one standard drink contains the equivalent of 12.5 ml 100% alcohol) using The Australian Alcohol Guidelines [7]. CGMS data was recorded between 18.00 and 06.00 h on the evening when alcohol was consumed (the study period) and between 18.00 and 06.00 h on the evening when no alcohol was consumed (the control period).

CGMS data were only analysed if there had been regular calibrations with intermittent capillary blood glucose readings at a maximum of 8-h intervals. Each CGMS trace was qualitatively and quantitatively analysed using mean glucose values, per cent time in glycaemic ranges and continuous overlapping net glycaemic action (CONGA) [8]. CONGA values were calculated to assess glycaemic variation over 1-, 2- and 4-h intervals. Low glucose values were defined as CGMS values < 4 mmol/l, normal glucose values when CGMS values were 4–10 mmol/l and high glucose values when CGMS values were > 10 mmol/l. Each patient acted as their own control with study periods and control periods being compared. Inter-individual values were grouped for comparison.

Differences between study and control periods were analysed using paired *t*-tests. Analyses were done in Stata [9].

## Results

### Patients

Of the 22 subjects recruited, eight were excluded because their CGMS traces did not have sufficiently frequent calibration points with intermittent capillary measures of blood glucose. Of the 14 subjects remaining, we were able to obtain study period data on 14 patients and matched control period data on only 12 patients. The study period occurred on the night prior to the control period in nine subjects. There were five

males and nine females. The mean age was 18.5 years (range: 17.4–19.5). The mean duration of diabetes was 9.4 years (range: 3–16.3). Six of our subjects took four insulin injections per day and eight took two injections daily. The mean insulin dose was 1.1 units/kg/day (range: 0.7–1.8), and the mean HbA<sub>1c</sub> was 9.6% (range: 8.2–10.8).

### Activities during the study period

Thirteen subjects had dinner before drinking and only one subject did not consume any food before going out. Three subjects 'danced a lot' and six subjects went dancing but did not dance a lot. Ten subjects had something to eat after drinking.

### Alcohol consumption during the study period

The mean number of alcohol drinks consumed on the study night was 9.0 (range 3–16) for males and 6.3 (range 3–14) for females. All the females consumed pre-mixed sweetened alcohol drinks (5% alcohol), with only one consuming beer and one consuming wine. Four of the males consumed mixed spirits, one mixed spirits and beer and one beer only. Forty per cent of the males had more than seven standard drinks during the study and 67% of the females had more than five drinks. In total, 80% of the subjects had pre-mixed sweetened alcohol drinks at some point during the study period. Forty-three per cent of the subjects reported that they became inebriated and 14.3% consumed alcohol to the point where they became physically sick. None of the subjects lost consciousness or took recreational drugs during the study period.

### Comparative CGMS data between study and control periods

There was no significant difference between the overall mean glucose levels of patients when comparing study and control periods (Table 1; *P* = 0.43). Similarly, there were no significant differences in the amount of time spent with either normal or high glucose values between study and control periods (Table 1). A larger proportion of time was spent with low glucose values during the control period when compared with the study period (1.9 vs. 16.8%, *P* = 0.03). A significantly larger degree of glycaemic variation was seen in the CONGA values in the study period when compared with the control period (Table 1). The difference in CONGA values were consistent and independent of whether glycaemic variation was assessed over 1-, 2- or 4-h intervals.

## Discussion

It has long been recognized that a prohibitionist approach is usually ineffective when counselling adolescents who engage in risk-taking behaviours [10]. Many centres today, ourselves included, have instead adopted a harm minimization approach in dealing with such behaviours. An important component



Outcome measure	Study period mean value	Control period mean value	Mean difference between study period and control period (95%CI)	P-value
Blood glucose levels (mmol/l)	11.8	10.6	1.2 (-2.1, 4.4)	0.43
Per cent time low glucose	1.9	16.8	-14.9 (-28.1, -1.8)	0.03
Per cent time high glucose	57.8	58.6	-0.8 (-27.3, 25.8)	0.95
Per cent time normal glucose	40.3	24.6	15.7 (-4.5, 35.8)	0.12
CONGA1*	2.7	2.1	0.6 (0.2, 1.0)	0.006
CONGA2*	4.3	3.2	1.1 (0.3, 1.9)	0.01
CONGA4*	5.5	3.7	1.8 (0.4, 3.1)	0.01

\*CONGA calculated at 1-, 2- and 4-h intervals. CONGA $n$  is the standard deviation of different glucose measures  $n$  hours apart for the duration of the CGMS trace.

Table 1 CGMS outcomes, study and control periods

of counselling using a harm minimization approach is that the information provided be credible and reflective of 'real' or 'lived' circumstances. Continuous glucose monitoring provides a technique whereby the glycaemic consequences of various behaviours can be documented in an ambulant or non-artificial setting.

Adolescents with Type 1 diabetes frequently consume alcohol in a social context [11]. Alcohol is known to inhibit the gluconeogenic pathway, to inhibit lipolysis, impair glucose counter-regulation and blunt hypoglycaemia awareness [3,4]. Previous studies in young adults with Type 1 diabetes have shown that moderate consumption of alcohol in the evenings without concomitant food intake may cause hypoglycaemia the following morning [5]. Consumption of alcohol after a meal, however, has shown no similar adverse effects on glucose [6]. It is reasonable to assume, therefore, that alcohol consumption may be a significant risk factor for hypoglycaemia in adolescents with Type 1 diabetes [5].

Studies of the glycaemic effects of alcohol consumption in an ambulant adolescent/young adult population can be difficult. This is because such behaviours are uncontrolled, often spontaneous and usually in the context of other social activities (parties, dancing, etc.). In order to ensure that we only reported accurate CGMS data during these activities, capillary blood glucose calibration was considered vital and those patients who failed in this regard were excluded from analysis. Just over 60% of the patients recruited were able to successfully wear and calibrate a CGMS unit during these activities. Given that patients who experience hypoglycaemic symptoms are more likely to perform capillary self measures of blood glucose, we feel that it is unlikely that those patients excluded from the analysis had a greater frequency of hypoglycaemia than those patients reported.

We were unable to record our subjects' alcohol consumption in a contemporaneous fashion and hence were reliant upon their recall. It is possible that their remembered patterns of consumption were not entirely accurate. This potential inaccuracy should not be seen as a weakness of this study, as we only set out to determine patterns of glycaemia in adolescents engaging in spontaneous and uncontrolled alcohol consumption.

We neither specified the type nor the amount of alcohol to be consumed (our ethical approval was contingent on this not occurring). The data as to amount of alcohol consumed have been included for descriptive purposes only.

The results of this study show that alcohol consumption by adolescents in a social context is associated with a greater degree of glycaemic variation and less time spent with low glucose values than evenings where no alcohol is consumed. Whilst the second of these findings appears counter-intuitive, there may be several possible explanations. Firstly, the vast majority of our study group ate a meal prior to going out and ate upon their return before going to bed. These are practices that we have instilled as harm minimization strategies to avoid alcohol-induced hypoglycaemia in our clinic. Secondly, most of the alcohol consumed was as pre-mixed spirit and sweetened, carbonated beverages. Finally, alcohol consumption was only associated with vigorous exercise (dancing) in a minority of our study group. All of these factors could have combined to negate the hypoglycaemic effects of alcohol.

In a previous study of glycaemia during alcohol consumption in adult men [5], hypoglycaemia occurred most often 10–12 h after wine consumption when the evening before ended at 23.00 h. We analysed our data to see if a similar phenomenon occurred in this study and found that the per cent of time spent with CGMS readings < 4 mmol/l between 06.00 and 12.00 h on the morning after the study period (i.e. the morning after the drinking night) was only 1.1%. Notwithstanding the fact that our cohort frequently consumed alcohol later than 23.00 h, the factors that impacted upon glycaemic control during the study night appear to have carried over to the 'morning after'.

The findings in this study highlight the importance of ambulant testing. It is important to note that the findings of the group studied here may not be seen in adolescents who drink non-sweetened alcoholic drinks or in those adolescents with better underlying metabolic control. Whilst alcohol consumption in isolation may reasonably be thought to cause hypoglycaemia, alcohol consumption by adolescents in the context of meals, sweetened mixers and little activity did not result in more hypoglycaemia than an alcohol-free evening. Whether the increase in glycaemic variation seen on an evening



of alcohol consumption has negative clinical outcomes remains an area for further investigation.

### Competing interests

CMM was a Novo Nordisk research fellow. FJC received fees for speaking at conferences and funds for research from Novo Nordisk.

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