1.0 INTRODUCTION

1.1 Background

Type-1 diabetes is a complex disease requiring insulin administration in conjunction with a meal plan that takes into consideration social, economic and logistical factors when deriving an individual plan to achieve optimal diabetes control. Short-term goals are to ensure normal growth and development, while minimizing complications such as hypoglycaemia and hyperglycaemia. Long-term goals are to achieve blood sugars as close to the normal range as possible to prevent or delay devastating long-term complications.

1.1.1 Average blood sugar levels

The diabetes control and complication trial (DCCT) proved that improved diabetes control through achieving and maintaining average blood sugars as close to the non-diabetic range as possible was effective in delaying the onset and slowing the progression of diabetic retinopathy, nephropathy and neuropathy.[1] Average blood glucose levels are assessed using glycosylated haemoglobin (HbA1c), considered the "gold standard" for measurement of glycaemic control. Glucose molecules react with haemoglobin during the 120 day life span of a red blood cell, forming glycosylated haemoglobin. This is an irreversible process which is time and concentration dependant. The HbA1c level is proportional to average blood glucose concentration over the previous 2-3 months. The American Diabetes Association (ADA) recommends a target HbA1c of less than 7%.[2]

1.1.2 Glycaemic variability

Further analysis of DCCT trial data suggested that average blood sugar level achieved was not the only factor responsible for driving diabetes complications. Investigators assessed the relationship of HbA1c values during the trial and during the 9 year follow up period and compared them to the risk of retinopathy progression. The data found that those treated in the conventional arm of the study with 1 or 2 injections per day had a 2.5 fold higher rate of complications than those achieving the same level of control in the intensive arm using multiple daily injections or with insulin infusion pumps. The authors suggested that reductions in blood glucose fluctuations with more intensive management was responsible for the reduced complications seen even when the same average level of blood sugar control was achieved. More recent evidence supports these previous findings that blood glucose variability, characterized by extreme glucose excursions, is a predictor of diabetic complications, independent of HbA1c levels.[3, 4] Further support for the toxic effects of wide glucose fluctuations (high glycaemic variability) comes from a study which found that apoptosis in human umbilical endothelial cells was accelerated in cells exposed to fluctuations in glucose versus those maintained at stable low or high glucose levels.[5] In vitro studies have shown that retinal capillary pericytes that have been cultured in high glucose environments undergo apoptosis when there is a rapid drop in glucose concentration.[6] This provides evidence of how increased glycaemic variability is mechanistically linked to the development of certain diabetic complications.[6, 7]

1.1.3 Postprandial blood glucose (PPBG)

Rapid glucose excursions are mainly due to meal ingestions and controlling these fluctuations following a meal is important in maintaining good glycaemic control and in limiting glucose

variability. “Postprandial blood glucose” refers to plasma glucose concentrations after eating. “Postprandial glucose excursions” are defined as the change in glucose concentration from before to after a meal and “incremental glucose area”, as the area under the glucose curve that is above the pre-meal value. In non-diabetic individuals glucose concentrations start to rise 10 minutes after the start of a meal in response to the absorption of carbohydrates. The postprandial profile is determined by carbohydrate absorption, insulin and glucagon action and hepatic and peripheral tissue glucose metabolism.[8] The peak glucose concentration depends on several factors including the carbohydrate load and glycaemic index (GI) of the carbohydrates consumed, but will rarely exceed 8mmol/l in non-diabetic individuals and occurs at approximately 60 minutes after the start of the meal.[8] Patients with type 1 diabetes have a higher and more prolonged postprandial glucose excursion due to abnormalities in insulin, glucagon and amylin secretion, hepatic and peripheral glucose uptake and suppression of hepatic glucose production.[8] It has been shown that postprandial glycaemia contributes more than fasting glycaemia to HbA1c especially at lower HbA1c levels and that control of postprandial glycaemia is essential for obtaining an HbA1c goal of less than 7%.[9] In a recent study of patients with type 2 diabetes it was shown that postprandial, but not fasting, blood glucose was an independent risk factor for cardiovascular events.[10] Even in well-controlled patients it has been shown that postprandial hyperglycaemia is a stronger predictor of cardiovascular disease than elevations of fasting blood glucose.[11] Thus, postprandial blood glucose concentrations which contribute significantly to average blood glucose measurements may well be an independent risk factor for other diabetic complications and need to be targeted independently of the mean blood sugar as measured by the HbA1c.

1.2 Diet in diabetes

Dietary carbohydrate is the primary determinant of meal related blood glucose excursions. Carbohydrate impact on blood glucose depends on the rate at which the carbohydrate is digested and absorbed (glycaemic index) and upon the overall load of carbohydrate consumed. A tighter match between insulin action and carbohydrate delivery will improve blood glucose control while reducing the occurrence of both hyper and hypoglycaemia.

Dietary management in diabetes consists of 2 major components. The first component is to provide the recommended daily intake of energy, macronutrients and micronutrients. The second is to manipulate the diet in such a way that these nutrients are supplied while minimising glucose exposure and fluctuations.

1.2.1 Carbohydrate metabolism

Normoglycaemia is maintained in healthy individuals when consuming a carbohydrate containing meal because of the coordinated effects of multiple hormones and gut peptides. The cephalic phase primes the gut and pancreas to accommodate the pending meal. Gut peptides, predominantly GLP-1 (glucagon-like peptide-1), are released which augment endogenous insulin production, delay gastric emptying and act on the central nervous system as an acute appetite suppressant.[12] Insulin and amylin are co-secreted from the pancreatic beta cells where insulin functions to suppress glucagon release, reduce hepatic glycogenolysis and gluconeogenesis and stimulate hepatic, adipocyte and skeletal muscle glucose uptake.[12] Amylin has an additional effect on delaying gastric emptying, allowing for a slower absorption of digested carbohydrates and buying time for insulin to store incoming glucose.[12] This co-ordinated effort keeps postprandial blood glucose

values to less than 8mmol/l and minimises the time spent with glucose above baseline.

In the absence of endogenous insulin, amylin and GLP-1, ingested carbohydrate is more rapidly absorbed and glucagon is inadequately suppressed leading to an attenuated suppression of hepatic glucose output.[12] Because insulin is injected into the periphery and hence into the systemic circulation, the normal portal: systemic insulin ratio of 10:1 is reduced to 1:1 leading to greater postprandial glucose elevation, delayed peak insulin action, elevated and prolonged postprandial glucose exposure and greater risk of delayed hypoglycaemia.[13]

Injected subcutaneous insulin forms dimers and then hexamers which delay the entry of insulin into the systemic circulation.[14, 15] This delay is somewhat overcome with insulin analogues but is still much slower than endogenous insulin action. This delay allows carbohydrate to enter the circulation at a rate exceeding the insulin action and postprandial blood sugar elevations are the result.

1.2.2 Carbohydrate counting

Carbohydrate counting is a method that matches carbohydrate intake with insulin delivery and has been shown to offer an advantage in terms of greater reduction in HbA1c than standard meal planning.[16] Most people with diabetes on intensive regimens use insulin to carbohydrate ratios using either "Carb" counts (1 Carb = 15g carbohydrate) or carbohydrate gram counts to match insulin delivery to carbohydrate content of meals and snacks. Each individual will have a different Carb ratio based on how much insulin they need to inject to cover each Carb consumed. Carb counting offers greater flexibility and freedom but is more demanding on the patient. Theoretically

carbohydrate counting could reduce glycaemic variability by better matching carbohydrate intake with insulin dose. The ADA recommends that the post-prandial peak glucose level should be less than 10mmol/l and 4 hours post-meal blood sugars should have returned to the pre-meal level without overshooting and causing hypoglycaemia. They currently recommend prandial insulin dosing to be based on the total carbohydrate content of the meal, but consideration should be given to the glycaemic index (GI) and glycaemic load of the meal.[17]

Dietary advice for diabetes has changed dramatically over the last century. Initial dietary management in the pre-insulin era centered on Allan's starvation diet- a low carbohydrate, low calorie diet.[18] After the discovery of insulin the carbohydrate content and calorie load were normalized. Today the recommendation is to follow a balanced diet consisting of approximately 50% carbohydrate content, 30% fat and 20% protein.[19] Within this carbohydrate recommendation further options for dietary management include carbohydrate counting and the use of low GI foods.

1.2.3 Glycaemic index (GI)

Glycaemic index is a measure of how rapidly glucose is released from ingested foods and arrives in the blood stream. Initial studies compared 50g portions of various carbohydrates with 50g of glucose. Blood samples were taken for 2 hours after the ingestion of carbohydrate. The area above the fasting glucose concentration was calculated and expressed as a percentage of the area obtained after the ingestion of 50g of glucose; the higher the area under the curve, the higher the GI of a food.[20] GI was originally thought to be an inherent property of food but has since been found to be influenced by many determinants.[20] Some of the factors that determine the GI of a particular food include the portion size of the carbohydrate consumed, the particle size and form of the

carbohydrate, the ripeness of fruits, any processing of the food such as grinding, rolling or pressing, the preparation or cooking method including the amount of water used, the time of cooking and the type of heat utilized and the presence of dietary fiber.[20,21,22] Certain characteristics of subjects also affect the resultant GI obtained for certain foods. These include age, sex, body fatness, glucose tolerance status, dose and timing of insulin, degree of diabetes control and fasting blood glucose level at the time of the test.[21]

Fat influences the GI of a meal by delaying gastrointestinal transit and increasing the time for carbohydrate absorption.[20,21] Both fat and protein increase insulin secretion without augmenting glucose concentrations.[20] Most evidence shows that the amount of fat and protein needed to have significant effect on GI are large compared with that normally eaten.[21]

Low GI diets are commonly prescribed to limit the rapidity of blood sugar rise after meals but have at best shown HbA1c reductions of 0.43% over standard diabetes diets.[23] Most day to day meal plans equate to an intermediate GI diet. This was demonstrated in a meta-analysis of low GI diets where even in spite of the study stratifying participants into low, intermediate and high GI diet groups, by the end of the study period there was regression towards intermediate GI meal content in both the low and high GI dietary groups.[23] This highlights one of the major limitations of the low GI diet- that being long term compliance. In addition to this there is wide variability of GI in certain foods even of similar category; there is difficulty in calculating GI in certain foods and lack of decent food labeling. Another limitation of low GI diets is that the modern analogue insulin's have a shorter onset and duration of action which may lead to carbohydrate to insulin mismatching and increased blood glucose variability. Recent studies have looked at optimal coverage of low and high GI meals. In a study in children it was found that postprandial glucose excursion was

significantly lower for the low GI meal compared with the high GI meal. The investigators proved again that pre-meal insulin dosing is essential for reducing post prandial glycaemic excursions and that pre-meal analogue insulins have a marginal benefit over regular insulin for low GI meals.[24]

Since the days of Allen's starvation diet and Joslin's low carbohydrate diet in the 1920's there has been support for the use of low carbohydrate diets and these have proven to be safe in the intermediate term and could offer an additional method of minimizing glycaemic variability.

1.3 Insulin treatment

The goal of modern intensive insulin therapy is to as closely as possible match a physiological insulin profile and thereby achieve good glycaemic control.

There are 3 components to insulin replacement

1) Prandial (bolus) insulin: insulin injected to emulate the endogenous insulin response to food. The dose is calculated according to the carbohydrate ratio.
2) Basal insulin: background insulin emulating the constant insulin release that regulates lipolysis and hepatic glucose production.
3) Corrective –dose insulin: insulin given to rectify episodes of hyperglycaemia and return blood glucose back into the target range. The dose will depend on the individual's sensitivity factor. This is the amount that 1 unit of insulin will decrease their blood glucose level.

Insulin regimens need to be individualized with regard to "food-dose", "corrective-dose" and basal insulin requirement.

The first insulin used in humans was 'impure' bovine insulin. In the 1930's protamine zinc insulin was developed. The addition of protamine and zinc slowed the release of insulin and created 'long-acting' insulin. In the 1950's NPH (neutral protamine Hagedorn) and 'lente' insulins were developed. They were bound to protamine and zinc respectively to prolong their duration of action.[15] By the 1980's purified porcine and then human recombinant DNA insulin (regular insulin) was produced. With these insulins antibody reactions and immune-mediated lipoatrophy was virtually eliminated.[15] Finally in the 1990's analogue insulins came onto the market. Insulin analogues are insulins that have been genetically engineered to alter their amino acid sequences thereby changing their pharmacodynamic and pharmacokinetic properties but still maintaining the same or improved action that human insulin offers with regards to glycaemic control.[15]

There are 2 groups of insulin analogues. The first are rapid acting analogues. These insulins have been designed for rapid absorption and they work faster than regular insulin and are therefore used as bolus insulin. The second group consists of long acting analogues. They are designed for slow release and are used as basal insulins.

Insulin Lispro (Humalog®) was the first rapid acting analogue developed and is of recombinant DNA (rDNA) origin. The penultimate lysine (B29) and proline (B28) residues on the c-terminal end of the β-chain have been reversed which alters binding of this portion of the insulin molecule. This conformational change does not alter receptor binding, but blocks formation of insulin dimers and hexamers.[15] This increases the amount of active monomeric insulin available and speeds absorption. Insulin Aspart (NovoRapid®) is also of rDNA technology. B28 proline has been replaced by a negatively charged aspartic acid residue. Its receptor affinity is similar to human

insulin but the formation of hexamers is prevented.[15] In Insulin Glulisine (Apidra®) the amino acid asparagine at B3 has been replaced by lysine and the lysine at B29 by glutamic acid. It has similar pharmacodynamics and pharmacokinetics to Insulins Lispro and Aspart.[15] The equivalent dose of analogue insulin will reach twice the maximal concentration in half the amount of time compared with regular insulin. The onset of action of the rapidly acting analogues is 5-15 minutes. Their peak action is within 30-90 minutes and their effective duration is 4-6 hours. They are all approved for use in a regular syringe, an insulin pen or in a continuous subcutaneous insulin infusion pump.[15]

Insulin Glargine (Lantus®) is a long acting analogue, where glycine is substituted for asparagine at A21 and two additional arginine molecules are added at B30. This causes a shift in the isoelectric point from pH 5.4 toward pH 7. This decreases the solubility of the molecule at physiological pH and causes it to precipitate in the subcutaneous tissue where it forms a depot from which insulin is slowly released.[15] A small amount of insulin is immediately available and the remaining sequested insulin is slowly released supplying basal insulin requirements. Insulin Glargine has little peak compared with NPH. The onset of action is 2-4 hours and effective duration 20-24 hours, allowing for once daily administration without the risk of dose stacking.

Detemir (Levemir®) is an acylated derivative of human insulin with a neutral pH. It binds to albumin through a fatty acid chain that is attached to lysine at residue B29.[15] The binding to albumin results in a "depot" of bound detemir that is gradually released as free detemir thereby prolonging the duration of action. Insulin Detemir also has less variability in absorption when compared to NPH and therefore more predictability. It has a shorter action time than insulin Glargine and therefore often has to be given twice a day for complete basal coverage.

Because rapid acting analogues are absorbed more quickly than soluble human insulins they can be injected immediately before meals instead of 20-30 minutes before. They also approximate a physiological profile more closely. The analogues therefore provide more flexible treatment regimens and improved lifestyle while lowering the risk of hypoglycaemia and improving blood sugar control.[25, 26, 27] They offer less variability in absorption and less intra and inter-patient variability. They are all approved for use in a regular syringe, an insulin pen or in a continuous subcutaneous insulin infusion pump.

The rapid acting analogues have been shown to offer better control of postprandial hyperglycaemia in diabetic children and adolescents when compared with soluble human insulin.[28] A study done with insulin aspart (one of the rapid acting analogues) showed improved glycaemic control by not only improving early postprandial hyperglycaemia but by also reducing episodes of hypoglycaemia.[29]

1.4 Insulin pump therapy

Insulin pump therapy, also known as continuous subcutaneous insulin infusion (CSII), was introduced in the 1970s but only became widely used in the early 1990s. An ever increasing percentage of patients with type 1 diabetes are choosing insulin pumps to manage their diabetes.

The pump contains a cartridge that is pre-filled with rapid acting insulin, most often an analogue. This is connected to a catheter that is inserted into the subcutaneous tissue. The pump delivers a predetermined continuous infusion that covers basal insulin requirements (basal rate). Bolus doses

of insulin are given with meals and as corrective doses to bring the blood glucose back into range. CSII offers numerous advantages over injection therapy in that basal insulin delivery can be adjusted to optimize fasting blood sugar control and mealtime boluses are easier to give and have improved bioavailability. Use of CSII has been shown to lower HbA1c levels, decrease glycaemic variability and lower fasting glucose levels.[30] This improvement in glycaemic control is associated with fewer diabetic and metabolic complications.[30] CSII has been shown to specifically improve postprandial glucose excursions in children.[31] The effect of reduced variability and greater predictability in day-to-day absorption of insulin compared with multiple daily insulin injections results in better blood glucose stability. These effects are as a result of a smaller subcutaneous insulin depot and the sole use of rapid-acting conventional human insulin or insulin analogues without the need for long-acting insulins. Episodes of severe hypoglycaemia are reduced by up to 4-fold with CSII when compared with multiple daily injection (MDI) therapy.[30] Improved quality of life is one of the most often cited reasons for starting CSII therapy as it offers flexibility in meal choices and the ability to delay or skip meals. Exercise participation is easier and the number of injections is reduced.

An additional feature built into modern "smart" pumps is the "bolus calculator". This calculator aids the patient in bolus insulin dosing by recommending and calculating doses according to a predetermined algorithm. These pumps calculate the food dose of insulin when the amount of carbohydrate about to be consumed is entered into the pump. They also correct for high or low glucose levels using the patient's sensitivity factor. This is initially determined using a formula: 100 divided by the patient's total daily dose of insulin. This is the amount that the glucose will decrease by in mmol/l when given 1 unit of insulin. Thereafter, the sensitivity factor is adjusted according to the clinical response seen when using this calculated figure. There are therefore 2

components to a pre-meal dose calculation:

1. The food dose is calculated according to a preset carbohydrate to insulin ratio. This ratio is initially determined using a formula: 500 divided by the total daily dose of insulin. This is the amount of carbohydrate in grams that is covered by 1 unit of insulin. This ratio is changed depending on the clinical response seen when using this calculated figure.

   For example, for every 10 grams of carbohydrate entered into the bolus calculator 1 unit of insulin will be delivered. This dosage calculation is based upon a presumed linear relationship between carbohydrate load and insulin delivery. In this example, for 40 grams of consumed carbohydrate 4 units of insulin will be delivered.

2. The correction dose is calculated by calculating the difference between the current entered blood glucose and the predetermined target (thus providing the amount the blood glucose needs to be reduced by). This figure is then divided by the sensitivity factor (the amount 1 unit of insulin will drop the blood sugar by). For example, if the target blood sugar is 5mmol/l, the sensitivity factor is 1u: 5mmol/l, the current blood glucose reading is 15mmol/l and the patient is about to eat 40g of carbohydrate:
   Corrective dose = (current blood glucose- target blood glucose)/sensitivity
   = (15-5)/5
   = 2 units
   This is added to the food dose of 4 units to give a total meal bolus of 6 units.

The type of prandial bolus can also be varied. With a normal or standard bolus the insulin is infused rapidly. A square wave bolus will infuse the same amount of insulin over an extended

period as determined by the patient. With a "dual wave" bolus a percentage of the total insulin will be infused immediately and the remaining portion over a longer time period. Square or dual wave boluses are preferred for meals with a high fat content as fat delays the absorption of carbohydrate. These types of boluses also work better for patients with gastroparesis and when eating over a prolonged period of time. In a study looking at the effect of glycaemic index and bolus type on postprandial blood sugars in children on insulin pump therapy it was shown that a dual wave bolus best covers low GI meals.[32]

1.5 Continuous Glucose Monitoring System

Tools have become available for measuring glycaemic variability. The Medtronic Minimed Continuous Glucose Monitoring System® (CGMS) is a device that measures subcutaneous glucose levels every 10 seconds and records the average value every 5 minutes. It provides mean amplitude of glycaemic excursion (MAGE) analysis of the frequently sampled glucose measures. A thin plastic tube is inserted into the subcutaneous tissue and can be worn up to 6 days. This is connected to the sensing device. Data is transmitted wirelessly to the insulin pump where glucose levels can be viewed in real-time. See appendix 1.

It is often assumed that if self-monitored blood glucose (SMBG) levels performed before meals and at bedtime are in range together with an HbA1c at target that overall glycaemic control is good. A study looking at 56 children with diabetes who wore a CGMS for 3 days found that despite good HbA1c levels and preprandial glucose values at target, 70% of participants' experienced postprandial hyperglycaemia and nocturnal hypoglycaemia not detected with routine SMBG.[33] They showed that wearing a CGMS provides a means to optimize basal and bolus insulin

replacement.[33] It has also been shown that the glucose area under the CGMS profile correlates directly and independently with HbA1c values.[34] The use of CGMS has been shown to decrease HbA1c levels after 3 months of use, has a high accuracy when compared with SMBG and was better than SMBG in detecting asymptomatic hypoglycaemia and postprandial hyperglycaemia.[35]

1.6 Summary

Since the publication of the DCCT trial intensified insulin therapy has become the gold standard for managing diabetes and forms the backbone of most modern insulin regimens.

The majority of modern insulin regimens in type 1 diabetes utilize some form of carbohydrate counting to match bolus meal time insulin to the amount of carbohydrate eaten either in the form of fixed carbohydrate to insulin ratios or incorporated into scales that factor in both a corrective dose and the meal carbohydrate load. Alternatively fixed insulin doses are coupled with a recommendation to fix carbohydrate load at each meal and snack. In effect all of these regimens work on a premise that the relationship between carbohydrate intake and injected bolus insulin is linear.

Previous euglycaemic clamp studies where insulin infusion rates were adjusted to maintain a stable serum glucose level in adult patients challenged with increasing oral carbohydrate loads, found a linear relationship between insulin infused and carbohydrate load.[36] Studies such as this formed the basis of the linear carbohydrate to insulin relationship used in diabetes management.

However, there are significant differences in the pharmacokinetic and pharmacodynamic properties of subcutaneously injected bolus insulin versus intravenous continuous insulin

infusions. Our clinical experience leads us to hypothesize that a non-linear relationship exists between the amount of carbohydrate consumed and the insulin ratio required to cover it when using pre-meal bolus insulin. This clinical experience came from analysing blood glucose meter downloads of patients and seeing a trend of higher than expected postprandial blood sugars when these patients consumed high carbohydrate loads. No previous studies have been done using subcutaneously injected insulin to examine the relationship between the amount of carbohydrate consumed and the amount of insulin required to clear it.

1.7 Hypothesis

We hypothesize that as carbohydrate load increases the amount of insulin required increases exponentially rather than linearly. If this is the case, a new formula would need to be developed to allow the individual to accurately calculate the increased insulin required to cover for the prolonged absorption and greater carbohydrate delivery when consuming a greater carbohydrate load. This would help reduce postprandial hyperglycaemia and achieve blood glucose control closer to target. To test this hypothesis we used real-time continuous glucose monitoring to assess blood glucose exposure using fixed carbohydrate ratios with increasing carbohydrate loads.

2.0 METHODS

2.1 Study design

The study was an analytic observational study to test the aforementioned hypothesis.

2.2 Study population

Five type 1 diabetic adolescents and young adults already on insulin pump therapy with good control were invited to participate in the study. The participants were all patients attending The Centre for Diabetes and Endocrinology at Donald Gordon Medical Centre in Parktown Johannesburg. Ethics approval was obtained from the University of the Witwatersrand Human Research and Ethics Committee.

Inclusion criteria

Adolescents or young adults with type 1 diabetes

Stable HbA1c under 8%

On insulin pump therapy for at least 6 months

Stable carbohydrate to insulin ratios

Willingness to wear a CGMS for 5 days

Exclusion criteria

Gastroparesis or other condition that may impair carbohydrate absorption

2.3 Study procedure

Consent and assent were obtained from each participant. Participants, who had stable overnight and morning period glucose levels, indicating that their basal rates and carbohydrate to insulin ratios for these periods were optimal, were able to start the study on the same day. Participants who did not have stable glucose levels for these periods were given a CGMS to wear for 3 days so that their basal rates and carb ratios could be optimized. Breakfast was delayed till 10am on 2 days

to optimize overnight and morning basal rates as far as possible. Carb ratios were optimized by participants by eating a fixed carbohydrate load for breakfast and their insulin to carbohydrate ratios established.

A CGMS was inserted and calibrated for each participant on the first day. Participants continued doing regular SMBG checks and used 2 pre-meal glucose checks to calibrate their sensors on each day of the study.

For 5 consecutive days the participants ate, in random order, a pre-prepared breakfast consisting of varying amount of carbohydrate. Each participant consumed one meal that contained 30 grams of carbohydrate (2 Carbs), one of 60 grams (4 Carbs), one of 90 grams (6 Carbs), one of 120 grams (8 Carbs) and one of 150grams (10 Carbs). The different meals where made up by a dietician and consisted of increasing amounts of "Marie" biscuits, dried fruit squares and a drink made up with Nutren Diabetes® and Polycose®. See table 2.1 and 2.2 for nutrient summary. Nutren Diabetes® is a nutritionally complete meal replacement suitable for diabetics that is low in GI and low in calories. It is sugar, lactose and gluten free and is high in complex carbohydrates. Polycose® is a readily digestible carbohydrate for use when additional calories are required. Polycose® solutions have an osmolality approximately one-fifth that of pure glucose solutions of similar concentration are minimally sweet and mix readily with most foods and beverages. These specific foods were chosen as together they were small enough in volume, and included a beverage. This made consumption of a large amount of carbohydrate over a short period of time more manageable. The meals were made up with increasing but equal ratios of each of the above foods.

Table 2.1 Carbohydrate content of each meal

		g CHO/day	Nutren Diabetes (mls) 11g CHO/100mls + 9% glucose polymer	Fruit squares 2g CHO each	Marie biscuits 5g CHO each
% CHO			50%	33%	17%
Day 1 (g)	2 Carbs	30g	75mls (15g)	5 (10g)	1 (5g)
Day 2 (g)	4 Carbs	60g	150mls (30g)	10 (20g)	2 (10g)
Day 3 (g)	6 Carbs	90g	225mls (45g)	15 (30g)	3 (15g)
Day 4 (g)	8 Carbs	120g	300mls (60g)	20 (40g)	4 (20g)
Day 5 (g)	10 Carbs	150g	375mls (75g)	25 (50g)	5 (25g)

CHO = carbohydrate

Table 2.2 Fat and protein content of each meal

	Fruit squares Protein (g)	**Fruit squares Fat (g)**	**Marie biscuits Protein (g)**	**Marie biscuits Fat (g)**
Day 1	1.15	0.04	0.5	1
Day 2	2.3	0.08	1	2
Day 3	3.45	0.12	1.5	3
Day4	4.6	0.16	2	4
Day 5	5.75	0.2	2.5	5

Fruit squares = 2.3g protein/100g and 0.4g fat/100g

Marie biscuits (each) = 0.5g protein each and 1g fat each

The participants ate breakfast at the same time on each of the 5 study days. They gave themselves

a bolus of insulin 15 minutes before eating according to their predetermined carbohydrate to insulin ratios. They then did not eat anything for 6 hours after breakfast. Alcohol consumption was avoided for the 5 day period. Vigorous exercise was limited and testing did not take place during a menstrual period.

At the end of the 5-day study period the CGMS was removed and downloaded. The downloaded data included the participant's glucose readings every 5 minutes during the 5 days, the times that they administered the insulin boluses including the amount of insulin, the amount of carbohydrate that they consumed at specific times and the calibrations with their SMBG.

Each participant performed the 5 day study procedure twice so that inter and intra patient variability could be compared.

2.4 Data analysis

Medtronic Solutions® software was used to download the data for each participant. Of the 10 data sets (2 from each participant), 5 sets of data, one from each participant, were used in the final analyses. Reasons for data not being included in the analysis resulted from sensor failure and hypoglycaemic episodes. If a sensor lost connection with the pump for more than 15 minutes (3 data points), during the critical time period from before breakfast to 6 hours after the meal, then that participants data was excluded from the analysis. Likewise, if a participant had a blood glucose level less than 3, 5mmol/l or needed to correct an episode of hypoglycaemia before breakfast then that set of data was also excluded from the analyses. For an example of a Solutions® sensor download for a participant's 5 day study period see appendix 2.

For each meal for each of the final 5 sets of data the baseline glucose level of each participant was recorded. The glucose level was captured every 5 minutes from this baseline level, to the peak of glucose absorption and back to baseline. The change in glucose from baseline to each of these points was then calculated. Using the area under the curve formula (see appendix 3), the area under each glucose curve could then be calculated for each meal for each participant. This area represents the "incremental glucose area" or postprandial glucose exposure. The area under the curve for blood glucose excursion was compared for the 5 different meals. For an example of a participant data set see appendix 4.

The maximal change in glucose for each meal for each participant was documented, as was the time taken to reach this glucose level and the time taken for glucose level to return to baseline.

2.5 Statistical analysis

A straight line was fitted to the log data, allowing for possible differences between the 5 individual participants, using an Analysis of Covariance. See appendix 5 for Analysis of Covariance output. Predicted values were obtained from the fitted model. A p-value was obtained using the Analysis of Covariance (the significance of the fit of the line). A One-way randomized blocks design was used to determine the significance of the maximal change in glucose for each meal, the time to maximum glucose and the time taken for glucose to return to baseline. See appendices 6, 7 and 8 for One-way randomized blocks design output.

3.0 RESULTS

3.1 Participant demographics

The mean HbA1c of the participants was 7.1% with a range of 6.8% to 7.4%. Their average age was 21.4 years with a range of 14years to 30 years. The mean duration of diabetes was 8.4 years with a range of 4 to 20 years.

3.2 Study results

The maximal change in glucose level from baseline to peak glucose increased as carbohydrate load was increased (Table 3.1). The time taken for the glucose level to return to baseline following a carbohydrate load increased as the carbohydrate load increased (Table 3.2) and the time to maximum glucose increased as carbohydrate load increased (Table 3.3).

Table 3.1 maximal change in glucose in mmol/l

Carbs	2	4	6	8	10
Patient					
1	1.4	2.1	3.3	4.2	7.9
2	2.9	3.3	5.8	6.9	7.3
3	2.5	3.5	3.9	4.3	3.9
4	0.4	2.9	3.4	9.5	9.5
5	0.6	0.5	0.9	3.4	5.5
average	1.6	2.5	3.5	5.7	6.8
STDEV	1.1	1.2	1.8	2.5	2.2

Table 3.2 Time to baseline in minutes

Carbs	2	4	6	8	10
Patient					
1	105	85	115	155	185
2	105	80	150	140	180
3	115	125	160	150	245
4	35	105	280	140	385
5	70	80	165	130	175
average	86	95	174	143	234
STDEV	33.2	19.7	62.4	9.7	89.1

Table 3.3 Time to maximum glucose in minutes

Carbs	2	4	6	8	10
Patient					
1	70	45	50	40	80
2	35	45	80	70	70
3	55	55	100	65	100
4	15	55	95	90	250
5	30	70	35	60	70
average	41	54	72	65	114
STDEV	22	10	28	18	77

There was a highly significant increase ($p < 0.0002$) in the maximal change in glucose as carbs increased. There was also a significant increase ($p < 0.002$) in the time to baseline as carbs increased but no significant change (at the 5% significance level) in the time to maximum glucose as carbs changed.

The area under the curve (AUC) for each participant, for each meal was plotted. The area under the curve increased with increasing carbohydrate load and the increase was more exponential than linear, as is demonstrated in Figure 3.1.a and 3.1.b.

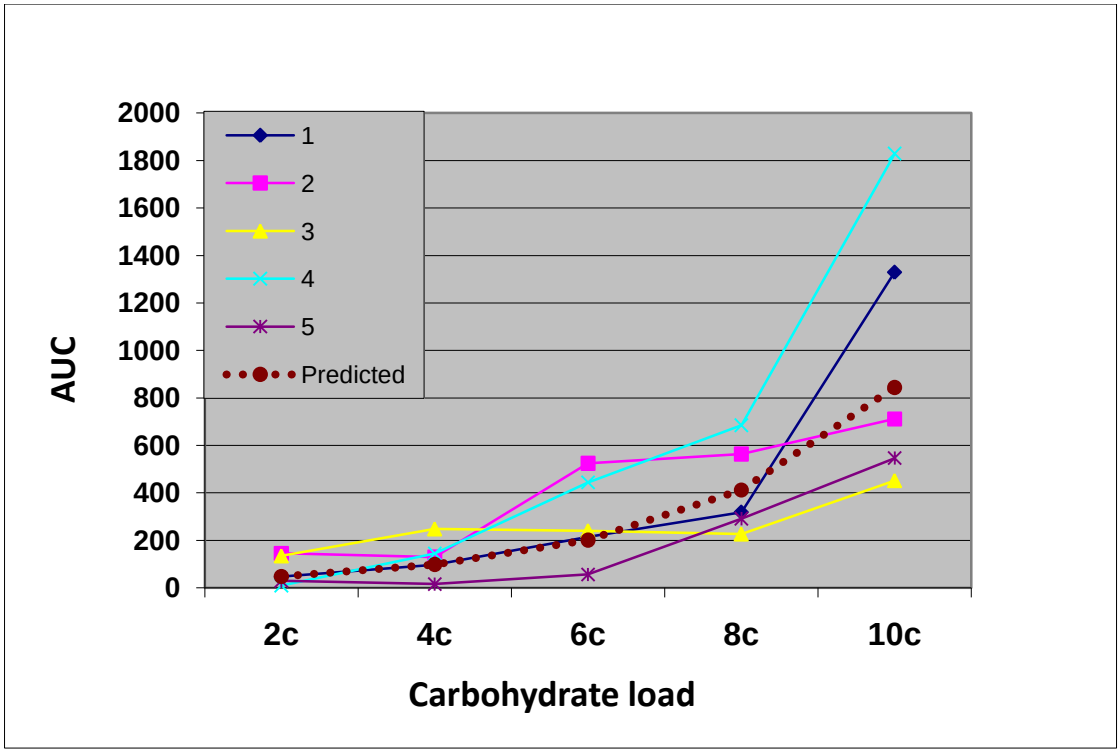


Figure 3.1.a Area under the curve for each meal for participants 1-5

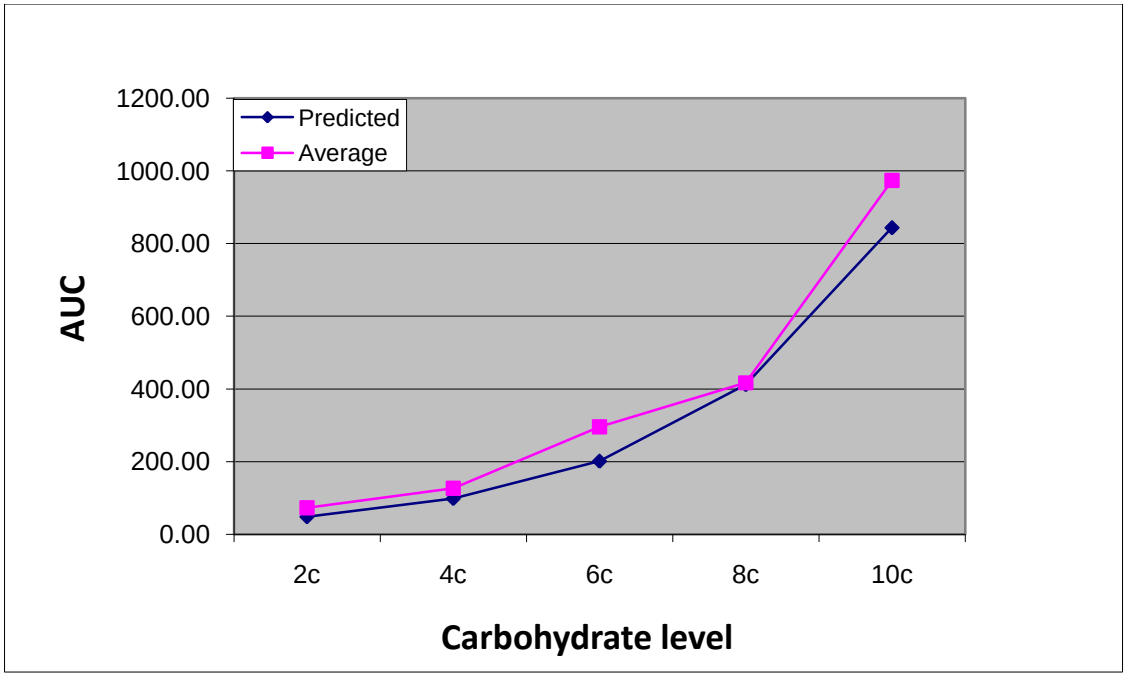


Figure 3.1.b Average AUC for each meal and predicted AUC for an exponential plot

The natural logarithm of the area under the curve, Ln(AUC), was plotted for each carbohydrate load for each participant as well as for the average of all 5 participants. Both graphs, particularly the average graph, showed a linear trend, confirming the exponential nature of the AUC trend. A straight line was fitted to the log data, distinguishing between the 5 participants in the model, via an Analysis of Covariance. The results did not reveal any significant differences between the participants, but the linear relationship between Ln(AUC) and carbohydrate load was highly significant ($p<0.0001$).

Predicted values from the fitted models are included in Figure 3.2 and 3.3. The fit to the average graph is particularly good. The predicted values were transformed back to the unlogged scale and included on the graph in Figure 3.1.

The analysis convincingly and highly significantly demonstrates that the AUC increases exponentially with increasing carbohydrate load over the range of carbohydrate loads tested.

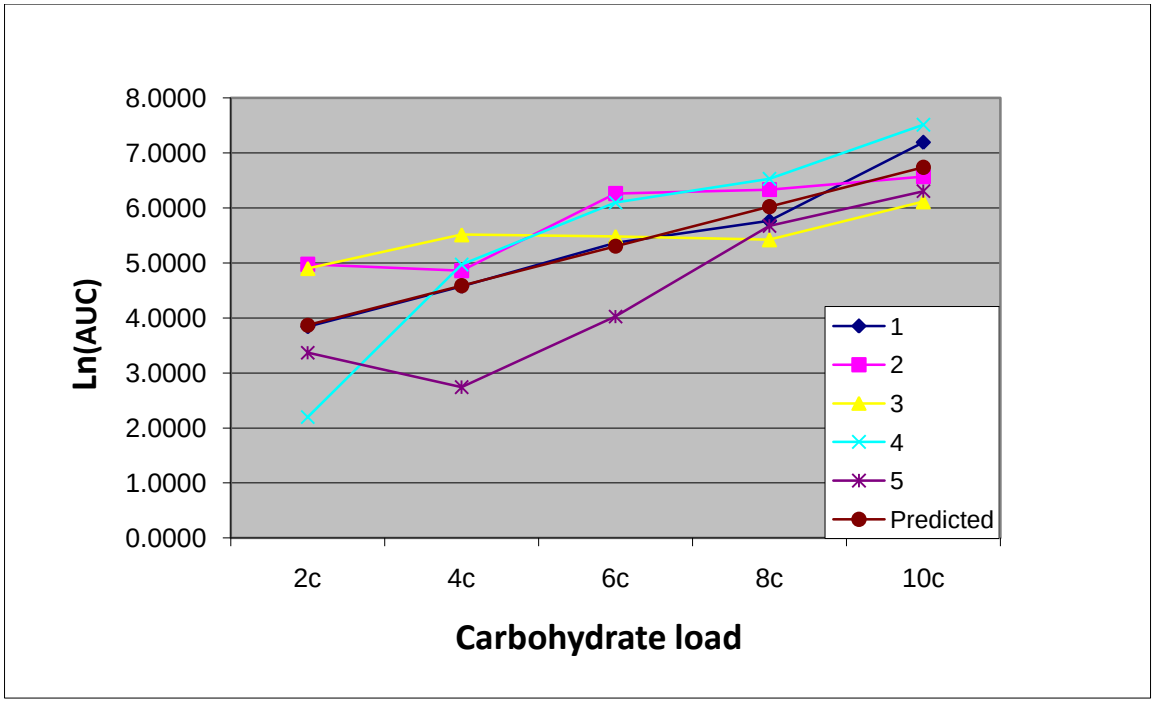


Figure 3.2 Ln(AUC) for each carbohydrate load for participants 1-5

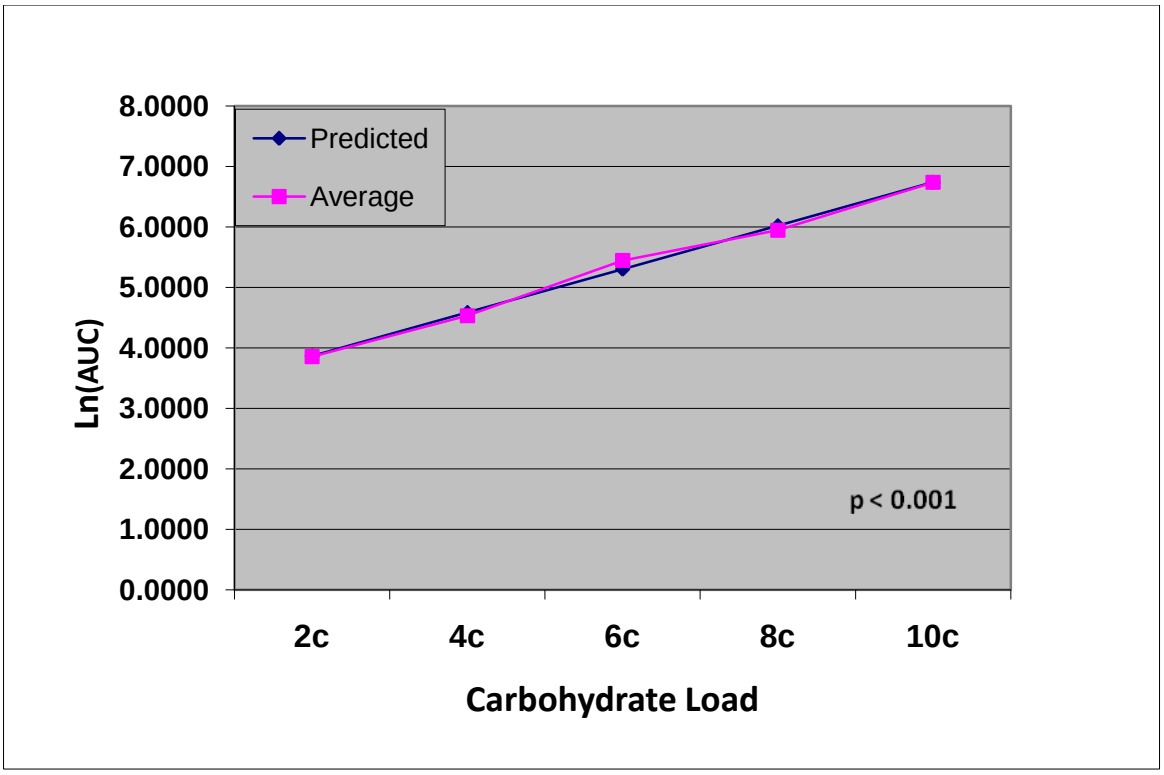


Figure 3.3 Ln(AUC) for average of all 5 participants and the predicted value

3.3 Descriptive results (see appendix 2)

The 2 and 4 carb meals showed a delay in time from eating to the start of the glucose rise and a smaller AUC suggesting that the ingested carbohydrates were met and matched by the injected insulin which was able to rapidly clear the absorbed glucose from the blood stream.

The 6, 8 and 10 carb meals showed an earlier rise in blood glucose suggesting that the carbohydrate load overwhelmed the insulin available for its clearance. The increase in time to maximum glucose from baseline, the greater peak and the delayed return to baseline supports the hypothesis that the injected insulin dose, calculated using a linear algorithm, was inadequate to meet and clear the absorbed carbohydrate.

Following the higher glucose peak of the larger carbohydrate meals (6-10 Carbs) the glucose level frequently dropped below baseline and often fell to the hypoglycaemic range (<3.5mmol/l). This delayed hypoglycaemia was possibly caused by a mismatch in the insulin peak and the carbohydrate peak. Following this drop in glucose level, which was untreated in the study participants, the glucose levels rebounded and remained high for up to 6 hours. This high, followed by a low, followed by a high shows a pattern of increasing glycaemic variability.

4.0 DISCUSSION

4.1 Insulin to carb ratios

By assuming that a linear relationship exists between the amount of carbohydrate consumed and the amount of insulin required to cover it, diabetics on injection therapy using carbohydrate

counting and diabetics on insulin pump therapy use this relationship to calculate the amount of insulin that they need to inject before meals (bolus insulin). This assumption was based on previous euglycaemic clamp studies using intravenously injected insulin.[36] If this assumption were correct then the relationship between the incremental glucose area (AUC) and carbohydrate consumed should be a flat line (there should be no increase in glucose exposure with increasing carbohydrate load) providing that the carbohydrate ratio was correct. We set out to test the hypothesis that the relationship between glucose AUC and carbohydrate load is not a flat line and hence the relationship between insulin and carbohydrate load is not linear.

The study showed that the relationship between glucose AUC and increasing carbohydrate load is in fact exponential, at least in the range of 2 to 10 Carbs. From this we can extrapolate that an exponential amount of insulin is needed to cover an increasing carbohydrate load. These findings have implications for diabetics using carb counting for insulin dosing. Subcutaneously injected insulin has different pharmacokinetic properties to intravenously injected insulin with regard to onset of action, peak action and duration of action and it is because of this that studies using intravenous insulin cannot be extrapolated to subcutaneously injected insulin.

4.2 HbA1c and glycaemic variability

The increase in postprandial blood sugars with the higher carbohydrate meals (6-10 Carbs) has great clinical significance for patients with diabetes. An increase in postprandial blood sugar contributes significantly to HbA1c levels.[9] These higher postprandial glucose excursions with subsequent falls in blood glucose to the hypoglycaemic range and the rebound hyperglycaemia contribute to increasing glucose variability. Both the increase in HbA1c and the increase in glucose

variability contribute to an increased risk of developing diabetic complications.[1, 3, 4]

4.3 Hypoglycaemia

The drop in glucose following the peak after the 6-10 Carb meal has significant implications for management of these postprandial glucose excursions. The increased insulin dose for the larger carbohydrate meals causes this late postprandial hypoglycaemia. A mismatch in the timing of action of the injected insulin and the glucose excursion is the probable cause of this unexpected finding. Insulin doses cannot just be increased exponentially to try and match the exponential increase in glucose excursion because the larger doses given as a single bolus before the meal will result in even more significant late postprandial hypoglycaemia.

4.4 Management strategies

There are a few possible management options for controlling the postprandial hyperglycaemia observed with larger carbohydrate meals.

4.4.1 Limiting carbohydrate intake

The first management option would be to limit carbohydrate intake at each meal to a maximum of 4 Carbs. This study shows that the glucose excursion after ingesting up to 4 Carbs can be limited by preprandial subcutaneous insulin using fixed insulin to carbohydrate ratios. Low carbohydrate diets have been tried previously in the management of patients with diabetes. A study looking at limiting carbohydrate intake to 70-90g (4, 5-6 Carbs) per day in patients with type 1 diabetes showed that 70-90g per day was a feasible long term option as part of their diabetes management.

The patients on this diet had improved glycaemic control and a significant reduction in HbA1c levels. There was also a lowering of the rate of symptomatic hypoglycaemia.[37]

4.4.2 Changing the timing of the pre-meal insulin

The second possible strategy in management would be to change the timing of the injected insulin. In the study, participants injected their pre-meal insulin 15 minutes before the meal. By injecting 20-30 minutes before the meal when eating higher carbohydrate loads could possibly limit the postprandial rise in glucose. Injecting earlier gives the insulin time for absorption so that there is a greater amount of insulin within the circulation before the absorbed carbohydrate overwhelms the insulin. By letting participants inject earlier, on non-study days but while wearing a CGMS, we anecdotally noticed that there was a lower rise in postprandial glucose levels even when consuming large carbohydrate loads. Other possibilities would be to change the pharmacokinetics of the injected insulin to accelerate its absorption. Heating the injection site has been shown to improve insulin absorption and reduce postprandial blood sugars. There are news devices, such as heating patches, which are being developed to assist with improving insulin pharmacokinetics. The InsuPatch®, a newly developed heating patch has been shown to have significant effect on the pharmacokinetics of insulin analogues and on PPBG.[38] They accelerate the time to maximum insulin absorption, increase the maximum insulin concentration, accelerate insulin clearance and decrease postprandial blood sugars.[38] Newer, faster insulins are also being developed that may hold promise for PPBG control.

4.4.3 Changing the glycaemic index of the carbohydrate

A third possible management strategy would be to alter the GI of the carbohydrates consumed. Studies in children have shown that postprandial glucose excursions are significantly lower for low GI meals when compared with high GI meals.[24] The delay in the glucose peak with a lower GI meal is better matched by the pre-meal insulin. However most diets consist of intermediate GI foods and the dietary restrictions of consuming only low GI meals can be limiting and objectionable. Furthermore, a delay in carbohydrate absorption from low GI meals coupled with large carbohydrate loads may be associated with delayed hyperglycaemia as carbohydrates are still being absorbed after the insulin has finished working.

4.4.4 Changing the insulin profile

The fourth possible strategy for management would be to change the profile of the injected insulin. Patients on insulin pump therapy have the option for changing the duration over which their pre-meal insulin is injected. Insulin can be injected using a "square wave" bolus where the total amount of insulin is infused over a set time period, for example, over 3 hours instead of all being injected at once. A second bolus type is a "dual wave" bolus where a percentage of the pre-meal dose can be injected up front and the remaining percent injected over a set time period. Both the percentage injected and the time period over which it is injected can be adjusted. Patients are usually advised to use these different bolus types when consuming meals with a high fat content as fat delays the absorption of carbohydrate. These bolus types better matches the absorption profile of the carbohydrate when eating food with a high fat content. These types of boluses also work better for patients with gastroparesis and when eating over a prolonged period of time. In a study looking at the effect of glycaemic index and bolus type on postprandial blood sugars in children on

insulin pump therapy it was shown that a dual wave bolus best covers low GI meals.[32] When eating carbohydrate loads of more than 4 Carbs a "dual wave" bolus would probably best cover the postprandial glucose excursion and by better matching the absorption profile would hopefully also prevent the late development of hypoglycaemia. Using a ratio of 60-70% insulin injected 20-30 minutes before the meal and 30-40% over the following 3-4 hours is postulated to best cover these high carbohydrate meals but further studies would have to be done to determine the ideal ratios and duration of insulin infusion.

To further prevent the late hypoglycaemia the basal rate insulin could be decreased for 4-6 hours following a high carbohydrate meal.

4.4.5 Changing the insulin

Patients on injection therapy obviously do not have the option of changing the bolus type or duration of insulin infusion. For these patients a further option would be to try human insulin instead of analogue insulin when eating a high carb meal. Human insulin (Actrapid®, Humulin R®) has a delayed onset of action and a longer duration of action when compared with analogue insulin. These insulins may better match the absorption profile of these high carb meals and prevent the postprandial hyperglycaemia but would need to be injected 30-60 minutes before the meal to allow for their slower onset of action. However, the late hypoglycaemia may still be a concern.

5.0 CONCLUSION

To prevent complications of diabetes patients need to reduce their average blood sugars, as measured by HbA1c, and their glycaemic variability to be as close to the non diabetic range as possible. Postprandial blood sugar levels contribute significantly to both the HbA1c and to glycaemic variability. PPBG accounts for the greatest contribution to HbA1c elevation the closer one comes to target HbA1c. It is therefore important to optimally manage and limit these post meal glucose excursions. Dietary carbohydrate is the primary determinant of these postprandial blood glucose excursions.

This study has shown that an increasing carbohydrate load leads to an exponential increase in postprandial glucose exposure when using fixed insulin to carbohydrate ratios, calculated using the assumption of a linear relationship. However, an exponential relationship exists between the amount of carbohydrate consumed and the pre-meal bolus insulin required to cover it. Late post prandial hypoglycaemia that follows carbohydrate loads greater than 60 grams limits using exponentially higher doses of insulin when consuming high carbohydrate loads.

Further studies need to be done looking at the optimal ratios of insulin needed for increasing carbohydrate loads, the duration and type of boluses needed to cover these high carbohydrate loads and the possibility of changing the linear equation used in current insulin pumps to one that would better cover the increase in post prandial glucose load with large carbohydrate meals. Options for limiting these postprandial glucose excursions include restricting carbohydrate intake, changing the timing of the pre-meal insulin, changing the GI of the carbohydrate consumed, changing the insulin delivery profile on the insulin pump and changing the type of insulin or insulin kinetics.

Further studies need to be done to assess the effectiveness of any of these interventions on postprandial hyper and hypoglycaemia.

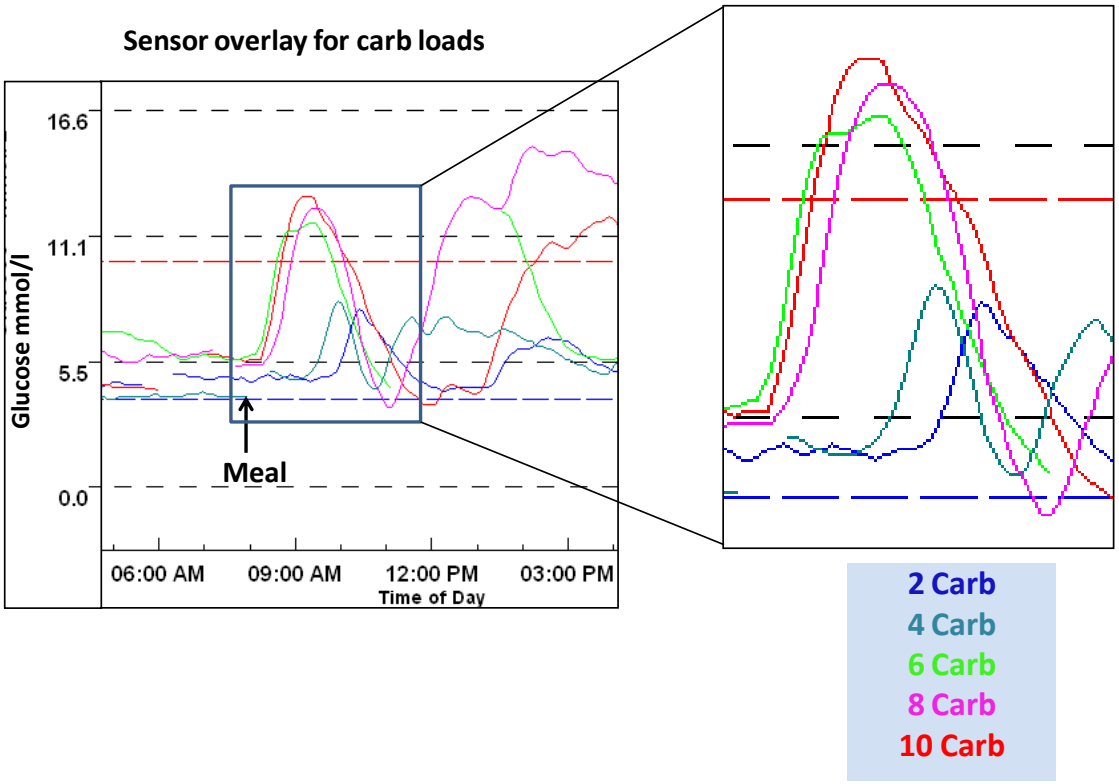
6.0 LIMITATIONS OF THE STUDY

The major limitation of this study was the small number of participants. The observational nature of the study however, allowed for a small number of subjects to test the study hypothesis. Sensor failure further limited the number of data sets available for analysis. Newer, more durable sensors will hopefully limit this problem in future studies.

Appendix 1

Medtronic MiniMed Paradigm® REAL-Time System - Courtesy Medtronic, Inc.

Appendix 2

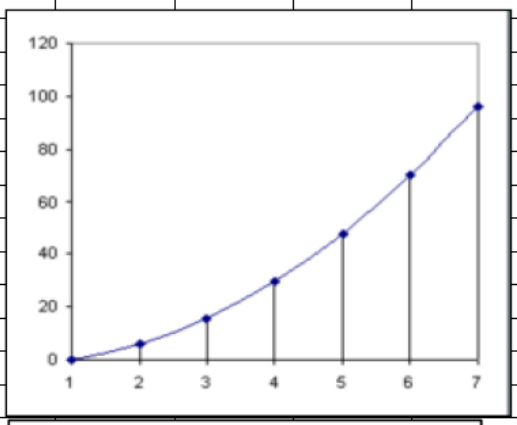

Sensor overlay for carb loads
16.6
11.1
5.5
0.0
Glucose mmol/l
Meal
06:00 AM
09:00 AM
12:00 PM
03:00 PM
Time of Day
2 Carb
4 Carb
6 Carb
8 Carb
10 Carb

Appendix 3

	A	B	C	D	E	F	G	H	I	J	K
1	**Area under the curve**										
2											
3	Trapezoid Rule										
4	x	y	trapezoid								
5	1	0	3								
6	2	6	11								
7	3	16	23								
8	4	30	39								
9	5	48	59								
10	6	70	83								
11	7	96									
12		area	218								
13											
14											
15											
16											
17											
18											
19											

=(B6+B5)/2*(A6-A5)

=SUM(C5:C10)



Trapezoid Rule
Divide curve into series of trapezoids, each with area=(average height)*(width). Sum the areas

Appendix 4

	actual glucose					change in glucose					area under curve				
time	2c	4c	6c	8c	10c	2c	4c	6c	8c	10c	2c	4c	6c	8c	10c
0	12.6	7	8.4	4.9	5.3	0	0	0	0	0	0	0	0	0.25	0
5	12.4	7	8.4	5	5.3	0	0	0	0.1	0	0	0.5	2.25	1.5	0.75
10	12.3	7.2	9.3	5.4	5.6	0	0.2	0.9	0.5	0.3	0	2	4.75	4	2.75
15	12.1	7.6	9.4	6	6.1	0	0.6	1	1.1	0.8	0	4	5.75	7.5	6.25
20	11.9	8	9.7	6.8	7	0	1	1.3	1.9	1.7	0	6.5	7.25	11.75	11
25	11.7	8.6	10	7.7	8	0	1.6	1.6	2.8	2.7	0	8.5	8.75	15.75	16
30	11.8	8.8	10.3	8.4	9	0	1.8	1.9	3.5	3.7	0	9.25	10.75	18.75	20.5
35	11.9	8.9	10.8	8.9	9.8	0	1.9	2.4	4	4.5	0	9.75	13	20.5	23.75
40	12.1	9	11.2	9.1	10.3	0	2	2.8	4.2	5	0	10.25	14.5	21	26.25
45	12.4	9.1	11.4	9.1	10.8	0	2.1	3	4.2	5.5	0.5	10.25	15.75	20.75	28.5
50	12.8	9	11.7	9	11.2	0.2	2	3.3	4.1	5.9	1.75	9.5	16.5	20	33
55	13.1	8.8	11.7	8.8	12.6	0.5	1.8	3.3	3.9	7.3	3.25	8.5	16.5	19	37.25
60	13.4	8.6	11.7	8.6	12.9	0.8	1.6	3.3	3.7	7.6	4.75	7	16.25	17.5	38.5
65	13.7	8.2	11.6	8.2	13.1	1.1	1.2	3.2	3.3	7.8	6	5.25	15.5	15.75	39
70	13.9	7.9	11.4	7.9	13.1	1.3	0.9	3	3	7.8	6.75	3.75	14.5	13.75	39
75	14	7.6	11.2	7.4	13.1	1.4	0.6	2.8	2.5	7.8	6.75	1.75	13.5	11.75	39.25
80	13.9	7.1	11	7.1	13.2	1.3	0.1	2.6	2.2	7.9	6.25	0.25	12	10.5	39.5
85	13.8	7	10.6	6.9	13.2	1.2	0	2.2	2	7.9	5	0	9.75	9.75	39.5
90	13.4		10.1	6.8	13.2	0.8		1.7	1.9	7.9	3.25	0	7.25	9.5	39.25
95	13.1		9.6	6.8	13.1	0.5		1.2	1.9	7.8	1.75	0	4.75	9.25	38.75
100	12.8		9.1	6.7	13	0.2		0.7	1.8	7.7	0.5	0	2.75	8.75	38
105	12.6		8.8	6.6	12.8	0		0.4	1.7	7.5	0	0	1.5	8	37.25
110			8.6	6.4	12.7			0.2	1.5	7.4	0	0	0.5	7.5	36
115			8.4	6.4	12.3			0	1.5	7	0	0	0	7.5	34.5
120				6.4	12.1				1.5	6.8	0	0	0	7.5	33.75
125				6.4	12				1.5	6.7	0	0	0	7	33.5
130				6.2	12				1.3	6.7	0	0	0	5.75	33.5
135				5.9	12				1	6.7	0	0	0	4.25	33.75
140				5.6	12.1				0.7	6.8	0	0	0	2.5	33.75
145				5.2	12				0.3	6.7	0	0	0	1	33.5
150				5	12				0.1	6.7	0	0	0	0.25	33.5
155				4.9	12				0	6.7	0	0	0	0	33.25
160					11.9					6.6	0	0	0	0	32.5
165					11.7					6.4	0	0	0	0	31.75
170					11.6					6.3	0	0	0	0	30.75
175					11.3					6	0	0	0	0	29.5
180					11.1					5.8	0	0	0	0	28.5
185					10.9					5.6	0	0	0	0	27.5
190					10.7					5.4	0	0	0	0	26.25
195					10.4					5.1	0	0	0	0	24.75
200					10.1					4.8	0	0	0	0	23.25
205					9.8					4.5	0	0	0	0	21.25
210					9.3					4	0	0	0	0	19
215					8.9					3.6	0	0	0	0	16.75
220					8.4					3.1	0	0	0	0	14.75
225					8.1					2.8	0	0	0	0	13.25
230					7.8					2.5	0	0	0	0	11.5
235					7.4					2.1	0	0	0	0	9.75
240					7.1					1.8	0	0	0	0	8.5
245					6.9					1.6	0	0	0	0	7.25
250					6.6					1.3	0	0	0	0	5.5
255					6.2					0.9	0	0	0	0	4
260					6					0.7	0	0	0	0	3.25
265					5.9					0.6	0	0	0	0	2.75
270					5.8					0.5	0	0	0	0	2.25
275					5.7					0.4	0	0	0	0	1.75
280					5.6					0.3	0	0	0	0	0.75
285					5.3					0	0	0	0	0	0
											46.5	97	214	318.5	1330

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.791400367
R Square	0.626314541
Adjusted R Square	0.610067347
Standard Error	0.817146193
Observations	25

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	25.740303	25.740303	38.54909013	2.46565E-06
Residual	23	15.3577417	0.6677279		
Total	24	41.0980447			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	3.150743138	0.383275538	8.220569345	2.68132E-08	2.357877284	3.943608992	2.357877284	3.943608992
carbs	0.358749934	0.057780961	6.208791358	2.46565E-06	0.239220909	0.478278959	0.239220909	0.478278959

Appendix 5

Maximal change in glucose in mmol/l

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Patient 1	5	18.9	3.78	6.467
Patient 2	5	26.2	5.24	4.138
Patient 3	5	18.1	3.62	0.472
Patient 4	5	25.7	5.14	17.133
Patient 5	5	10.9	2.18	4.867
Carbs 2	5	7.8	1.56	1.243
Carbs 4	5	12.3	2.46	1.488
Carbs 6	5	17.3	3.46	3.063
Carbs 8	5	28.3	5.66	6.343
Carbs 10	5	34.1	6.82	4.712

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Patients	31.7104	4	7.9276	3.5544	0.02943	3.0069
Carbs	96.6224	4	24.1556	10.8304	0.00019	3.0069
Error	35.6856	16	2.23035			
Total	164.0184	24				

Conclusion: Highly significant increase ($p < 0.0002$) in the maximal change in glucose as carbs increases

Appendix 6

Time to baseline in minutes

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Patient 1	5	645	129	1630
Patient 2	5	655	131	1530
Patient 3	5	795	159	2642.5
Patient 4	5	945	189	19967.5
Patient 5	5	620	124	2292.5
Carbs 2	5	430	86	1105
Carbs 4	5	475	95	387.5
Carbs 6	5	870	174	3892.5
Carbs 8	5	715	143	95
Carbs 10	5	1170	234	7930

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Patients	15076	4	3769	1.5637	0.2319	3.0069
Carbs	73686	4	18421.5	7.6430	0.0012	3.0069
Error	38564	16	2410.25			
Total	127326	24				

Conclusion: Significant increase ($p < 0.002$) in the time to baseline as carbs increases

Appendix 7

Time to maximum glucose in minutes

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
Patient 1	5	285	57	295
Patient 2	5	300	60	362.5
Patient 3	5	375	75	537.5
Patient 4	5	505	101	7967.5
Patient 5	5	265	53	370
Carbs 2	5	205	41	467.5
Carbs 4	5	270	54	105
Carbs 6	5	360	72	807.5
Carbs 8	5	325	65	325
Carbs 10	5	570	114	5930

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Patients	7704	4	1926	1.3494	0.2949	3.0069
Carbs	15294	4	3823.5	2.6789	0.0697	3.0069
Error	22836	16	1427.25			
Total	45834	24				

Conclusion: No significant change (at the 5% significance level) in the time to maximum glucose as carbs changes

Appendix 8

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