

The effect of protein and fat meal content on the insulin requirement of type 1 diabetic children

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Mini-dissertation submitted in partial fulfilment of the
requirements for the degree Master of Science in Dietetics at
the Potchefstroom Campus of the North-West University

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April 2017

ACKNOWLEDGEMENTS

I thank God for the ability to study, for the people set on my path and for His grace at every step of this journey.

I would like to thank the following people:

- Prof Marlien Pieters, my study promotor. What an example of a person, leader, brilliant mind and researcher. I sincerely thank her for her patience, insight and exceptional understanding throughout this study.
- Dr JC van Dyk, for his motivation, support with patient recruitment, sharing his expertise with me on a daily basis and, most of all, showing me to work and learn with passion.
- Dr Robin Dolman and Dr Maricke Cockeran for valuable input in study design and statistical analysis.
- Amanda Matthee for the language editing.
- My husband, Johan, for all his support and prayers, and my parents for teaching me perseverance.

Finally, thank you to every child and parent who participated in this study. Nothing would have been possible without parents who understand the value of research.

ABSTRACT

BACKGROUND AND AIMS

In type 1 diabetes, post-prandial hyperglycaemia remains a major challenge. Determining meal bolus insulin is mainly dependent on carbohydrate counting and the carbohydrate content of a meal. Recent studies have shown this method to be ineffective at times. Also, it has been proven that the fat and protein contents of meals demand insulin as well. The aim of this study was to determine the true post-prandial glycaemic response and total insulin need for mixed meals with known, constant carbohydrate content but different fat and protein contents, using insulin pump therapy and continuous glucose monitoring (CGM) in children with type 1 diabetes.

RESEARCH DESIGN AND METHODS

A total of 22 participants aged four to 17 years with type 1 diabetes on insulin pump therapy took part in this home-based, cross-over, randomised controlled trial. They were given two meals at dinner time on different nights. Both meals had identical carbohydrate content but one was a low-fat, low-protein (LFLP) meal and the other a high-fat, high-protein (HFHP) meal. CGM and finger prick testing were done for 10 hours post-meal, with correction bolus insulin given every two hours if required.

RESULTS

The HFHP meal required significantly more insulin than the LFLP meal, namely eight times more post-meal correction insulin (1.2 vs. 0.15 units) and 1.3 times (30%) more total meal insulin (3.48 vs. 2.7 units). The HFHP meal increased the duration of digestion (364 vs. 185 min) and led to a significantly larger area under the blood glucose response curve (AUC) (198 vs. 46.3). Protein and fat both influenced total meal and correction insulin requirements, and a correction of 1 unit insulin for every 8g protein and 1 unit for every 4g fat, in a mixed meal, was observed. Insulin requirement and glucose responses were, however, also influenced by patient characteristics, independent of the meals. The participants' total correction insulin requirements were

significantly influenced by the duration of diabetes and their total daily insulin use (units/kg). Peak CGM and AUC were influenced by duration of diabetes and total daily insulin use (units/kg) as well as HbA1c (AUC only). In addition, a significant interaction was noted between the test meals and duration of diabetes in terms of peak sensor glucose value ($p=0.014$) and between duration of diabetes ($p=<0.0001$), total daily insulin use (u/kg) ($p=0.003$) and HbA1c ($p=0.003$) in terms of AUC. The difference in peak CGM and AUC between the two test meals was larger in individuals who have had diabetes for longer and those with a higher total daily insulin use.

CONCLUSION

Children with type 1 diabetes on insulin pump therapy require more insulin over a longer period of time when consuming mixed meals than the insulin requirement calculated with current regimens. HFHP meals required insulin up to six hours post-meal, while LFLP meals required insulin up to three hours post-meal. Fat required double the amount of correction insulin compared to protein. However, the amount of additional insulin required is influenced by duration of diabetes and total daily insulin use.

KEYWORDS

carbohydrate, protein and fat, type 1 diabetes, glucose, insulin infusion systems

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LIST OF ABBREVIATIONS

ANOVA – analysis of variance

AUC – area under the curve

BG – blood glucose

BMI – body mass index

CC – carbohydrate counting

CFP – carbohydrate, fat and protein

CGM – continuous glucose monitoring

CHO – carbohydrate

CI – confidence interval

CSII – continuous subcutaneous insulin infusion (insulin pump therapy)

FFA – free fatty acids

FPRM – fat-protein rich meal

FPU – fat protein unit

GAD – glutamic acid decarboxylase 65 autoantibodies

GI – glycaemic index

HbA1c – glycated haemoglobin

HF – high fat

HFHP – high fat high protein

HLA – human leukocyte antigen

HP – high protein

IA2 – insulinoma antigen 2

IAA – insulin autoantibodies

ISPAD – International Society for Pediatric and Adolescent Diabetes

LF – low fat

LFLP – low fat low protein

LP – low protein

MDI – multiple daily injections

SD – standard deviation

SM – standard meal

SMBG – self monitoring of blood glucose

WHO – World Health Organisation

ZnT8 – zinc transported 8 autoantibodies

CHAPTER 1 – INTRODUCTION

1.1 Background information

Type 1 diabetes, previously known as juvenile onset diabetes, is a form of insulin dependent diabetes. Recent studies have shown a great increase in type 1 diabetes among children and young people, with an estimated 500 000 children younger than 15 years worldwide living with type 1 diabetes (Patterson *et al.*, 2014). There is no known cure or prevention for type 1 diabetes at this stage, and therapy includes lifetime management of exogenous insulin delivery either by injection or by subcutaneous insulin infusion, also known as pump therapy. Other aspects of daily care include self-monitoring of blood glucose (SMBG) with capillary blood testing and blood glucose meters, the use of continuous glucose monitoring (CGM) systems for some, managing diet and carbohydrate counting to calculate bolus insulin requirements and managing activity levels. Long-term monitoring of overall glycaemic control with the aim of preventing long-term micro and macro vascular complications is also done (Hanas, 2007).

Forms of insulin delivery by injection include using insulin analogues or using long-acting insulin in combination with short-acting insulin, also known as multiple daily injections (MDI), or giving continuous short-acting insulin via an insulin pump.

Current practices for determining meal-time bolus insulin, whether on MDI or continuous subcutaneous insulin infusion (CSII) or pump therapy, involve advanced or Level 3 carbohydrate counting. However, this and other methods of carbohydrate counting assume that only carbohydrates affect the post-prandial glucose rise in subjects with type 1 diabetes. Many studies have shown that additional aspects other than carbohydrates, related to the composition of the meal, affects post-prandial glycaemia. Some of these aspects can include the other macronutrients as well as the fiber and glycaemic index of the meal. (Kordonouri *et al.*, 2012; Lodefalk *et al.*, 2008; Pańkowska *et al.*, 2012; Smart *et al.*, 2013; Wolpert *et al.*, 2013) Some of these factors may in their own right, through different mechanisms, require additional meal insulin (Wolpert *et al.*, 2013).

Different meals have different macronutrient compositions which affect digestion and result in different post-prandial glucose profiles. This specifically applies to kilojoule-rich meals high in fat and/or protein in combination with carbohydrates, where a prolonged hyperglycaemic state has been noted three to four hours after ingestion of the meal, often accompanied by insulin resistance (Kordonouri *et al.*, 2012). For this, insulin pumps offer the option of a dual-wave or multi-wave bolus, or a square or extended bolus for insulin delivery. The dual-wave or multi-wave and square or extended types of boluses are also known as prolonged boluses as the time during which the bolus is delivered can be set over hours.

The use of only normal or standard boluses and carbohydrate counting, where bolus insulin is only determined based on the carbohydrate content of the meal, has been shown ineffective to optimise post-prandial blood glucose levels for mixed meals (Chase *et al.*, 2002; Kordonouri *et al.*, 2012). An elevated post-prandial blood glucose level is known to influence HbA1c levels and has been indicated to be as important as fasting hyperglycaemia in relation to long-term diabetes related complications such as retinopathy, nephropathy, atherosclerotic disease and mortality (Bell, 2001; Chase *et al.*, 2002; Hanefeld & Temelkova-Kurktschiev, 2002). The need to improve post-prandial glycaemia has been emphasised by the Diabetes Intervention Study that showed high post-prandial glucose levels are associated with an elevated all-cause mortality rate and decreased insulin sensitivity (Bell, 2001; Hanefeld & Temelkova-Kurktschiev, 2002).

1.2 Rationale for this study

For many patients with type 1 diabetes, post-prandial glucose rises are one of the major challenges in diabetes care and contribute greatly to glucose variability and overall glycaemic control (Chase *et al.*, 2002; Heptulla *et al.*, 2008). Some studies have indicated that even in the presence of a near normal HbA1c, late complications can still develop and glucose variability may play a key role (Danne *et al.*, 2006).

Hence, it is imperative to adjust nutritional therapy and carbohydrate counting advice to improve the post-prandial hyperglycaemia that many patients experience. Prolonged boluses – such as the square or extended bolus or dual-wave or multi-wave bolus features – have been developed by insulin pump companies in an attempt to address this issue. However, current known methods used to better determine how to set these boluses – such as the Carbohydrate, Fat and Protein (CFP) counting developed by Pańkowska *et al.* (2012) – are even more complex than advanced carbohydrate counting and may be impractical to implement in the day-to-day living with type 1 diabetes and even more so in the paediatric population. Also, CFP counting has resulted in significantly more episodes of hypoglycaemic events post-prandially (Kordonouri *et al.*, 2012). Although the value of these developed methods should not be dismissed, determining the post-prandial blood glucose profiles in children with type 1 diabetes could help to give insight in how to determine an optimal dual or extended bolus that is specific to the age of the child and macronutrient content of the meal.

Insulin requirements for mixed meals may also be influenced by various factors inherent in the patient. One such factor might be age. For example, gastro-intestinal maturity could influence digestion time, and higher levels of reproductive hormones in teenagers can result in insulin resistance, which means that both factors will influence the post-prandial glucose profile. Danne *et al.* (2006) compared different insulin pump therapy practices among children and adolescents and found that children of different ages have different basal insulin requirements as well as different prandial insulin requirements. However, it does not seem as if any other studies have explored the relation between age and prandial insulin requirements of children with type 1 diabetes. Factors such as gender, weight, current insulin use, duration of diabetes and current glycaemic control may also influence prandial insulin needs, and are therefore worth investigating.

The *2014 Guide for nutritional management in children and adolescents with diabetes* developed by the International Society for Pediatric and Adolescent Diabetes (ISPAD) states that there is evidence to suggest that dietary fat and protein have an impact on

post-prandial glucose irrespective of carbohydrates. The guide also states that trials are needed, specifically randomised controlled trials aimed at developing methods to better manage post-prandial hyperglycaemia after fat- and protein-rich meals (ISPAD Consensus Guidelines, 2014).

The rationale behind this study was to investigate the insulin requirements of, and post-prandial glycaemic response to, a low-fat, low-protein (LFLP), and high-fat, high-protein (HFHP) carbohydrate-containing meal in children with type 1 diabetes in an attempt to prevent post-prandial hyperglycaemia in future. Data from this study will aid in developing nutritional recommendations for insulin dosaging in type 1 diabetes.

1.3 Research aim

The aim of this study was to determine the true post-prandial glycaemic curve and total insulin need for high-fat, high-protein meals in combination with carbohydrates in children with type 1 diabetes (4 years to 17 years and 11 months).

1.4 Research objectives

This study aimed to:

- Determine the effect of adding fat and protein to carbohydrates in the form of a high-fat, high-protein mixed meal on the post-prandial glucose profile of children with type 1 diabetes.
- Use the post-prandial glucose profiles collected in this study to determine the prandial insulin requirement of children with type 1 diabetes for a high-fat, high-protein meal based on the amount and timing of insulin given as bolus corrections that were needed in the hours post-prandially.
- Identify participant characteristics that may influence the post-prandial glucose response to a high-fat, high-protein meal.

1.5 Structure of this mini-dissertation

This mini-dissertation is presented in article format. Technical aspects were applied according to the postgraduate guidelines of North-West University (NWU). Language

formatting was done by a competent editor following the language format and referencing style as stipulated in the Manual for Master's and Doctoral Studies of 2013 of NWU. This study consists of three chapters.



Chapter 1 provides background information on the study and includes the rationale for the study. The aims and objectives, list of research members and their contributions, and the outline of the mini-dissertation are also presented in this chapter.

Chapter 2 provides a literature review of type 1 diabetes in children, its current treatments and recommendations for determining insulin dosages for meals. The chapter touches on insulin pump therapy and different methods of meal insulin delivery available in pump therapy. The effect of different macronutrients on glycaemia in type 1 diabetes is investigated, and studies in which different types of insulin boluses were used in an attempt to improve post-prandial hyperglycaemia following high-protein and/or high-fat meals are discussed. This has highlighted the urgent need for randomised controlled trials to explore this field in order to develop better methods of determining insulin dosages for meals.

In Chapter 3, the following the article is presented: "Protein and fat meal content increase insulin requirement in children with type 1 diabetes – role of duration of diabetes". The article contains all the information regarding the study. This article will be submitted for publication in the journal of the International Society for Pediatric and Adolescent Diabetes (ISPAD), named *Pediatric Diabetes*. The article is presented in the technical style stipulated by the journal and is written according to the word count restrictions of the journal.

1.6 Contributions of members of the research team

Table 1.1: Members of the research team and their contributions to the study

Initials, surname and signature*	Affiliation	Role in this study
Ms M. van der Hoogt 	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Part-time MSc student, protocol writing, funding obtained for study, data collection, data processing, statistical analysis, interpretation of results and writing up of the literature and data.
Prof. M. Pieters 	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Supervisor, Study design and planning, data interpretation, statistical analysis
Dr. R. Dolman 	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Co-supervisor, study design and planning
Dr. J.C. van Dyk 	Life Groenkloof Hospital, Pretoria, South Africa	Study design, planning and execution, subject recruitment
Dr. M. Cockeran 	Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, South Africa	Statistical guidance and analysis
<p>*With my signature I declare that I approved the above-mentioned article, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby consent that it may be published as part of the mini-dissertation of Maryke van der Hoogt.</p>		

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CHAPTER 2 – LITERATURE REVIEW

2.1 Introduction

Diabetes mellitus is a multifaceted metabolic disorder characterised by chronically elevated blood glucose levels resulting from defective insulin secretion and/or impaired insulin action. Diabetes mellitus is broadly classified into type 1 diabetes, which is characterised by absolute insulin secretion deficiency, and type 2 diabetes, which is a result of both resistance to insulin action and insufficient insulin secretion (Craig *et al.*, 2014).

In young people, type 1 diabetes is the most common form of diabetes, and those of Caucasian background are affected more (Craig *et al.*, 2014). In the majority of Western countries, 90% of children and adolescents who have diabetes will have type 1 diabetes with 80 000 children under the age of 15 years developing type 1 diabetes annually worldwide (Patterson *et al.*, 2014). The incidence rates of type 1 diabetes vary significantly between different countries and different ethnic groups. Currently, the highest incidence rates are found in Finland with 60 to 65 children under 14 years per 100 000 diagnosed each year, while Northern Europe and Canada have the second and third highest incident rates respectively (Patterson *et al.*, 2014).

Unfortunately, type 2 diabetes is also on the rise among the youth, although reliable statistics are not available (Craig *et al.*, 2014). Currently, there is no proven intervention to prevent or delay the onset of type 1 diabetes (Couper *et al.*, 2014).

Therapy for type 1 diabetes includes lifetime management of exogenous insulin delivery either by injection or by subcutaneous insulin infusion, also known as pump therapy. Other aspects of daily care include self-monitoring of blood glucose (SMBG) with capillary blood testing and blood glucose meters, the use of continuous glucose monitoring (CGM) systems for some, managing diet and carbohydrate counting to calculate bolus insulin requirements and managing activity levels (Hanas, 2007). Current practice internationally is to quantify the carbohydrate content of a meal or 'carb

count' the meal and then decide on the dosage of insulin for the meal or the meal insulin bolus (Smart *et al.*, 2013).

Although available insulin and the amount of carbohydrates are considered to be among the most important factors influencing post-prandial glucose, many studies have indicated that other factors – such as the type of carbohydrate, the glycaemic index of the meal, and the fat, fibre and protein content of the meal – play an important role in contributing to delayed post-prandial hyperglycaemia and should be considered when trying to optimise post-prandial glucose levels (Kordonouri *et al.*, Lodefalk *et al.*, 2008; Lodefalk & Åman, 2010; Pańkowska *et al.*, 2012; Smart *et al.*, 2014; Wolpert *et al.*, 2013). There is also evidence that dietary fat and increased free fatty acids can impair insulin sensitivity and elevate glucose production from the liver (Wolpert *et al.*, 2013). The use standard insulin boluses and carbohydrate counting alone, where bolus insulin is only determined by the carbohydrate content of the meal, has been shown ineffective to optimise post-prandial blood glucose levels for mixed meals (Chase *et al.*, 2002; Kordonouri *et al.*, 2012).

An elevated post-prandial blood glucose level is known to influence HbA1c levels and has been indicated to be as important as fasting hyperglycaemia in relation to long-term diabetes-related complications such as retinopathy, nephropathy, atherosclerotic disease and mortality (Bell, 2001; Chase *et al.*, 2002; Hanefeld & Temelkova-Kurktschiev, 2002). The need to improve post-prandial glycaemia has been emphasised by the Diabetes Intervention Study which showed that high post-prandial glucose levels are associated with an elevated all-cause mortality rate and decreased insulin sensitivity (Bell, 2001; Hanefeld & Temelkova-Kurktschiev, 2002). For many type 1 diabetes patients, post-prandial glucose rises are one of the major challenges in diabetes care as these rises contribute greatly to glucose variability and overall glycaemic control (Chase *et al.*, 2002; Heptulla *et al.*, 2008). Some studies have indicated that even in the presence of a near normal HbA1c, late complications can still develop and glucose variability may play a key role (Danne *et al.*, 2006). It is therefore imperative to adjust nutritional therapy and carbohydrate counting advice to improve the post-prandial hyperglycaemia that many patients experience.

Prolonged meal insulin boluses where insulin delivery is spread over time instead of being delivered all at once at the start of the meal have been developed by insulin pump companies in an attempt to address the issue of post-prandial hyperglycaemia. Currently, there are no guidelines on how to use these prolonged boluses (Heinemann, 2009; Olinder *et al.*, 2009).

Some studies have tested the effect of using these boluses and some have attempted to develop other methods for insulin dosage determination – such as carbohydrate, fat and protein (CFP) counting developed by Pańkowska *et al.* (2012).

Unfortunately, these methods are even more complex than advanced carbohydrate counting and may be impractical to implement in the day-to-day lives of people with diabetes, and even more so in the paediatric population. Also, CFP counting has resulted in significantly more episodes of hypoglycaemic events post-prandial (Kordonouri *et al.*, 2012). Although the value of these developed methods should not be dismissed, determining the post-prandial blood glucose profiles in children with type 1 diabetes of all age groups could provide insight into how to better determine an optimum dual or extended bolus that is specific to the age of the child and macronutrient content of the meal.

The 2014 consensus guide for nutritional management in children and adolescents with diabetes, developed by the International Society for Pediatric and Adolescent Diabetes (ISPAD), states that there is evidence to suggest dietary fat and protein have an impact on post-prandial glucose irrespective of carbohydrates. The guide also states that trials are needed, specifically randomised controlled trials aimed at developing methods to better manage post-prandial hyperglycaemia after fat-rich and protein-rich meals (ISPAD Consensus Guidelines, 2014).

The aim of this study is to determine the post-prandial glucose profile of children with type 1 diabetes for meals with a known, constant carbohydrate content but different fat and protein contents, using continuous glucose monitoring (CGM) or sensor technology in an attempt to develop simple guidelines for determining the insulin requirement of fat

and protein and the optimal type of insulin bolus for meals of mixed macronutrient content.

This literature study will cover the following aspects: the causes of type 1 diabetes, digestion in children with type 1 diabetes, current therapies for type 1 diabetes, insulin pump therapy and continuous glucose monitoring, insulin bolus delivery through pump therapy, lack of guidance on the use of prolonged insulin boluses, dietary factors affecting insulin requirements and, finally, a summary of different insulin bolus delivery methods.

2.2 Causes of type 1 diabetes mellitus

Type 1 diabetes is an autoimmune condition resulting in complete insulin deficiency which is different from type 2 diabetes and other forms of monogenic diabetes where some endogenous insulin production is still present (Craig *et al.*, 2014). Diabetes is broadly categorised into four groups:

- Group 1: This group consists of type 1 diabetes where insulin-producing beta cell destruction leads to absolute insulin deficiency. This group consists of two sub-groups, namely type 1 A, immune mediated, and type 1 B, of idiopathic cause.
- Group 2: Type 2 diabetes is characterised by a range of insulin deficiencies with or without insulin resistance.
- Group 3: This group includes insulin insufficiency or complete deficiency caused by a) genetic defects of beta cell function, b) genetic defects in insulin action, c) diseases of the exocrine pancreas, d) endocrinopathies, e) drug or chemical induced diabetes, f) infection, g) uncommon forms of immune-mediated diabetes, and h) other genetic syndromes associated with diabetes.
- Group 4: This group consists of gestational diabetes, which refers to the development of diabetes during pregnancy (Craig *et al.*, 2014).

Most cases of paediatric diabetes are type 1, caused by autoimmune-mediated beta cell destruction. The aetiology of type 1 diabetes is heterogeneous. The destruction of the insulin-producing beta cells in the pancreas can be immune mediated or idiopathic. The majority of children with type 1 diabetes present with the autoimmune type 1 A diabetes.

Susceptibility to developing this type of diabetes is determined by a number of genes, of which the human leukocyte antigen (HLA) genotype is the largest contributor to the risk. Individuals who have the HLA marker and who are therefore genetically predisposed to develop autoimmune type 1 A diabetes, and who come into contact with environmental triggers leading to pancreatic beta cell destruction may present with clinical symptoms of type 1 diabetes when 90% of their beta cell mass has been destroyed (Craig *et al.*, 2014).

Environmental triggers may be infective or chemical. In terms of chemical triggers, enterovirus has been highlighted, but congenital rubella and cytomegalovirus have also been implicated as common triggers. Drug-induced or chemical-induced diabetes can also occur. Substances involved in potentially triggering the autoimmune process leading to diabetes include pentamidine, nicotinic acid, glucocorticoids, thyroid hormone, diazoxide, beta adrenergic agonists, thiazides, dilantina and alpha interferon. Conditions such as gestational diabetes and genetic syndromes such as 'Stiff-man' syndrome, Down syndrome, Turner syndrome, Klinefelter syndrome, Wolfram syndrome, Friedreich's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria and Prader-Willi syndrome have also been linked to the development of diabetes (Craig *et al.*, 2014).

Type 1 diabetes typically presents in stages, starting from the asymptomatic preclinical phase which develops into the established chronic condition phase with possible long-term complications (Couper *et al.*, 2014). Typical clinical symptoms of type 1 diabetes include polydipsia, polyuria, weight loss, weight loss even in the presence of polyphagia, fatigue, enuresis, impaired growth and blurry vision (Craig *et al.*, 2014). When an infant younger than six months shows symptoms of type 1 diabetes, molecular genetic testing is advised to diagnose monogenic diabetes and to determine the specific subtype of neonatal diabetes. This is done as type 1 diabetes is rarely seen between the ages of zero and six months. In age groups over six months, a child who presents with clinical signs of diabetes should receive diabetes autoantibody tests first. If the autoantibody tests are negative, molecular genetic testing can be done (Craig *et al.*, 2014).

2.3 Diagnosis of type 1 diabetes mellitus

Diagnosis of type 1 diabetes follows after presentation of the above-mentioned clinical symptoms or a hyperglycaemic crisis which is a plasma glucose level ≥ 11.1 mmol/L, or a fasting level of ≥ 7 mmol/L. When an oral glucose tolerance test is done, a two-hour post-load glucose value of ≥ 11.1 mmol/L is used to diagnose diabetes and when HbA1c (glycosylated haemoglobin) is used a value above 6.5% indicates diabetes (Craig *et al.*, 2014). However, caution should be taken when only these glucose-related measures are used to diagnose diabetes. A person with diabetes could in some instances have a normal HbA1c level at diagnosis, or a non-diabetic person can present with hyperglycaemia in the indicated ranges in the event of acute infections, trauma and/or circulatory or other types of severe stress (Craig *et al.*, 2014).

The preliminary diagnosis should therefore be confirmed by the presence of one or more diabetes-associated autoantibodies such as glutamic acid decarboxylase 65 autoantibodies (GAD), or other autoantibodies like tyrosine phosphate-like insulinoma antigen 2 (IA2), insulin autoantibodies (IAA) and also b-cell specific zinc transported 8 autoantibodies (ZnT8) (Craig *et al.*, 2014). If a child therefore presents with the clinical symptoms of type 1 diabetes and a hyperglycaemic status, but has no autoantibodies, it is considered to be type 1 B or idiopathic diabetes.

2.4 Digestion in subjects with type 1 diabetes mellitus

Digestion in healthy, non-diabetic subjects consists of several stages. Following the consumption of carbohydrates, there is an increase in serum glucose resulting in a rapid rise in pancreatic insulin production, which is followed by a cephalic reaction, enhanced storage of glucose in the muscles and a rapid suppression of glucose production from the liver which is triggered by increased circulating insulin levels (Heinemann, 2009). The result is a limitation of the post-prandial glycaemic rise. Endogenous insulin production in healthy non-diabetic individuals is therefore in part dependent on the rate of glucose absorption from the gut. However, in patients with diabetes, post-prandial hyperglycaemia is not as easily managed as the patients no longer produce insulin and are dependent on exogenous insulin, the dosage of which, for a certain meal, has to be

predetermined and delivered before consumption. It is also limited by the pharmacological properties of the specific exogenous insulin given, unlike in non-diabetic subjects where endogenous insulin production is constantly adapting according to the glycaemic response of the meal. A distinct difference in digestion and glycaemic response between subjects with diabetes and those without, is the presence of pre-existing hyperglycaemia on gastric emptying. Pre-existing hyperglycaemia before a meal is a common phenomenon in type 1 diabetes but unseen in non-diabetics. Hyperglycaemia has the effect of increased gut sensations resulting in delayed gastric emptying; this again affects perceptions of satiety and other abdominal symptoms. Chronic, prolonged hyperglycaemia may also result in irreversible gut dysmotility which affects the rate at which food exits the gut (Lodefalk & Åman, 2010).

Additional factors specific to subjects with diabetes may also have an effect on post-prandial glycaemic excursions. These include the pharmacological properties and amount of circulating exogenous insulin whether it be basal, injected or available or 'active' insulin from the previous bolus given, the site of injection or insulin pump infusion set, the balance between the start of the meal and time of the bolus, the type of meal and the preparation method of the meal including GI, the amount of carbohydrates, and the bolus taken and glucose absorption variability in the gut due to the possibility of gastroparesis in long-standing or poorly controlled diabetes (Heinemann, 2009). This list is most probably not complete, and the presence and amount of other macronutrients like fat and protein, and the age and degree of gastrointestinal maturity of a child might also play a role. (Heinemann, 2009).

2.5 Current treatment for type 1 diabetes mellitus

Providing exogenous insulin is crucial for the survival of children with type 1 diabetes. Insulin can be delivered by injecting insulin with a needle, usually in the form of a specialised injecting pen, by using a port in some instances or by using an insulin pump which delivers insulin continuously over 24 hours. Insulin pump therapy is also known as continuous subcutaneous insulin infusion (CSII).

Different types of insulin are available for use, which consist mainly of premixed or biphasic types of insulin, long-acting insulin and short or rapid-acting insulin.

It is recommended to not use premixed insulin in the paediatric group, but to rather aim for optimal glycaemic control and insulin replacement as close to normal physiological insulin as possible (Danne *et al.*, 2014). Both the insulin pen (in the form of multiple daily injections (MDI) by insulin pens) and insulin pump therapy can be used to achieve this. Using premixed insulin leads to more day-to-day variability in absorption compared to using long-acting insulin in conjunction with short or rapid-acting insulin – with variability in absorption affecting glycaemic control negatively. The use of long-acting insulin combined with rapid-acting insulin also provides more freedom in terms of dietary restrictions (Danne *et al.*, 2014). The aim should be adequate insulin to cover the basal requirements over 24 hours as well as meal-time insulin, the dosage of which should be based on the current blood glucose level and, according to current practice, also the carbohydrate content of the meal.

In 2004, the American Diabetes Association recognised carbohydrate as the most important determinant of post-prandial glycaemia (Bell *et al.*, 2015). However, various studies have shown that considering only the carbohydrate content of a meal should not be the only determinant of meal-time insulin, that high-fat and/or high-protein meals increase post-prandial glycaemia and that carbohydrate counting with standard or normal boluses is not sufficient to prevent this rise in post-prandial glycaemic levels (Bell *et al.*, 2015; Kordonouri *et al.*, 2012; Lee *et al.*, 2004; Lodefalk *et al.*, 2008; Neu *et al.*, 2015; Smart *et al.*, 2013; Wolpert *et al.*, 2013). Hence, there is a definite need to investigate the effect of fat and protein on glycaemic excursions post-meal and to develop insulin regimes that account for the effects of all macronutrients, not just carbohydrates, in children with type 1 diabetes.

2.5.1 Insulin pump therapy

The past decade of paediatric diabetes treatment has seen a shift from mixed or biphasic insulins toward multiple daily injections (MDI) and continuous subcutaneous insulin infusion (CSII) therapies, and an increase in the use of CSII or insulin pump

therapy (Danne *et al.*, 2014). The major benefit of CSII above MDI in children with type 1 diabetes is that CSII allows more precise insulin dosing due to smaller increments. Insulin pumps can deliver basal dosages in 0.025 unit increments compared to insulin pens which mostly only deliver in full units with the exception of one or two insulin companies that provide 0.5 unit injecting pens. A second benefit of CSII is multiple dosing without the pain of injections. Thirdly, different bolus options for meals including prolonged type boluses are available to better fit the composition, digestion and resulting glycaemic response of specific meals. The fourth major benefit is that hourly adaptation of basal rates according to the physiological basal insulin need is possible, which can help to reduce hypoglycaemic events (Danne *et al.*, 2014). Basal rates differ greatly between individuals. Factors such as age, growth hormone levels and cortisol levels can influence basal insulin requirements. In addition, stress, illness, activity and menstrual cycles can also influence the basal glycaemic response and thus the basal insulin need (Danne *et al.*, 2014). In MDI, only one or sometimes two shots of steady basal dosage insulin are injected. This is usually in the form of long-acting insulin like Levemir or Lantus (Optisulin). CSII, on the other hand, is preferred over MDI as up to 48 different basal rates and increments as small as 0.025 units per hour can be delivered, better catering to this large variability of basal insulin need, especially in growing children.

Compatible insulin pump models also offer the advantage of connecting wirelessly to a continuous glucose monitoring device, known as CGM or sensor technology.

2.5.2 Continuous glucose monitoring

As mentioned previously, the current blood glucose value is a determinant in calculating insulin requirements, whether it be on MDI where correction dosages for hyperglycaemic levels are calculated manually, or through the 'bolus wizard' feature on an insulin pump. To obtain the current blood glucose value, blood glucose testing or finger pricks are performed a few times daily. Self-monitoring of blood glucose via finger prick tests multiple times a day is a vital part of optimising glycaemic control. Another form of glucose measurement is continuous glucose monitoring (CGM). CGM does not measure blood glucose but rather the glucose value in the interstitial fluid compartment.

Thus, CGM and blood glucose tests are not the same and will rarely provide the exact same glucose value at one point in time. As self-monitoring of blood glucose cannot happen every minute or hour of the day, CGM can identify fluctuation and patterns in glucose values that would have been overseen if only a few blood glucose tests were done during a day (Langendam *et al.* 2012).

There are two kinds of CGM systems, namely real-time systems and retrospective systems (Langendam *et al.*, 2012). A real-time system is one that measures interstitial glucose values continuously and displays it on a screen for the patient. For example, the sensor measures the glucose in the interstitial fluid every five minutes, providing 288 readings in a day. Every five minutes, the value becomes visible on the pump screen, and glucose trend curves are developed and available for the patient to see as it is happening. The sensor is therefore connected to the insulin pump, for example the Enlite Sensor which is connected to the Medtronic Veo or 640G pump, or the Dexcom sensor which is linked to a partnering company's insulin pump.

Retrospective CGM systems refer to those systems where a sensor is worn for a set amount of time, usually five to 14 days, and the data in the sensor is downloaded after completion of the time period. In this case, the patient is not aware of the sensor's interstitial glucose values while wearing the sensor. Retrospective sensors are not necessarily connected to an insulin pump and can be worn by patients on MDI as well. Examples of retrospective sensors available in South Africa are the Medtronic Ipro and the Abbott Libre Pro Ambulatory CGM. A drawback of sensor technology is the considerable cost involved for patients who wear sensors permanently. Cost is also one of the factors preventing many centres and countries from making increased use of CGM technology.

CGM technology in combination with insulin pump therapy adds to diabetes management as the data collected in the form of glucose patterns and trends play a significant role in making therapeutic decisions about an individual's insulin dosages. It also helps to prevent episodes of low blood glucose and it gives insight into the effect of specific foods or meals on glycaemic levels in the hours following the meal. Hence, this

data can help to better predict how to use prolonged boluses (Kaufman & Westfall, 2012).

2.5.3 Insulin delivery in insulin pump therapy

An insulin pump offers three options to deliver bolus or meal-time insulin: the normal or standard bolus, the dual-wave or multi-wave bolus and the square wave or extended bolus (Figure 2.1).

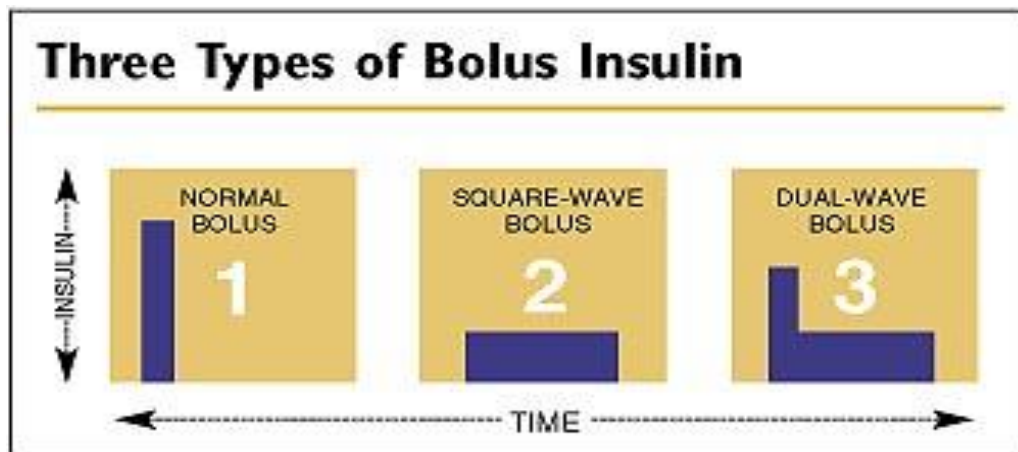


Figure 2.1: Three types of insulin meal boluses available on insulin pumps

The normal or standard bolus is a method of bolusing where the total insulin dose calculated by the pump is given immediately, usually within three minutes (Heinemann, 2009; Olinder *et al.*, 2009). The dual-wave or multi-wave boluses and square or extended types of boluses are also known as prolonged boluses as the time in which the bolus is delivered can be set over hours.

A dual- or multi-wave bolus refers to a method of bolusing where some of the calculated insulin is given as a standard or normal bolus and the rest over an extended period of time which can be chosen by the patient. A square or extended wave is where the entire calculated dose is delivered over a longer period of time (Olinder *et al.*, 2009).

The rationale behind prolonged boluses that can be adapted at every meal by the patient is that the glycaemic excursion of each meal is different. Different meals have different nutrient compositions which affect digestion and result in different post-prandial

glucose profiles. This applies specifically to calorie-rich meals high in fat and/or protein in combination with carbohydrates, where a prolonged hyperglycaemic state has been noted for three to four hours after ingestion of the meal, often accompanied by insulin resistance (Kordonouri *et al.*, 2012). For this purpose, insulin pumps offer the option of a dual- or multi-wave bolus, or a square or extended bolus for insulin delivery. Unfortunately, no guidelines exist on how exactly to use these boluses to improve post-prandial hyperglycaemia.

2.5.4 Lack of guidance on the use of prolonged type of boluses

A number of studies have shown that the current use of only normal or standard boluses in combination with or based on carbohydrate counting, where bolus insulin is determined by the carbohydrate content of the meal only, is ineffective to optimise post-prandial blood glucose levels for mixed meals (i.e. meals containing high amounts of protein and fat in combination with the carbohydrate load) (Chase *et al.*, 2002; Kordonouri *et al.*, 2012). Post-prandial hyperglycaemia remains important in type 1 diabetes care as it contributes to elevated HbA1c levels and thereby increases the risk for various long term diabetes complications (Bell, 2001; Chase *et al.*, 2002; Hanefeld & Temelkova-Kurktschiev, 2002). The need to improve post-prandial glycaemia has been emphasised by the Diabetes Intervention Study that showed high post-prandial glucose levels to be associated with an elevated all-cause mortality rate and decreased insulin sensitivity (Bell, 2001; Hanefeld & Temelkova-Kurktschiev, 2002).

The correct use of a dual or square wave bolus instead of a standard bolus leads to additional benefits such as fewer night-time hypoglycaemic events in children previously prone to hypoglycaemic events during sleep, and the elimination of the need for bed-time snacking as a result of mismatching insulin to the true post-prandial blood glucose profile (Chase *et al.*, 2002). This is particularly relevant for meals that contain either fat or protein or both. Still, there are no guidelines (other than CFP counting) to help guide patients or parents on how to initiate prolonged boluses or how to consider the fat and protein content of a meal, together with advanced carbohydrate counting, in order to deliver the optimal bolus for a specific meal and to improve post-prandial blood glucose profiles.

Type 1 diabetes care requires various additional daily tasks from children. For many of them, carbohydrate counting or advanced carbohydrate counting alone poses a challenge. For this reason, an easier method than CFP counting is required to determine what type of bolus to use for a specific meal and how to set it. Simple, safe and valid guidelines are needed to promote the use of dual-wave and square-wave boluses in children of all age groups to reduce the negative effect of prolonged elevated blood glucose levels after mixed meals.

2.6 Dietary factors affecting insulin requirements

Current practices for determining meal-time bolus insulin whether on MDI or CSII involve advanced carbohydrate counting. However, this, and other methods of carbohydrate counting, assumes that only carbohydrates affect the post-prandial glucose rise in subjects with type 1 diabetes. Although available insulin and the amount of carbohydrates are considered to probably be the most important factors influencing post-prandial glucose, various studies have indicated that other factors – such as the type of carbohydrate, the glycaemic index of the meal, and the fat, fibre and protein content of the meal – also play an important role in delaying post-prandial hyperglycaemia and should be considered when trying to optimise post-prandial glucose levels (Lodefalk *et al.*, 2008; Kordonouri *et al.*, 2012; Pańkowska *et al.*, 2012; Smart *et al.*, 2014; Wolpert *et al.*, 2013). There is also evidence that dietary fat and increased free fatty acids can impair insulin sensitivity and elevate glucose production from the liver (Wolpert *et al.*, 2013). Both situations increase the need for insulin.

Carbohydrates are the main macronutrient contributing to glycaemic elevation. However, as shown by the studies listed in Table 2.1, it is not only carbohydrates that affect glycaemia. In the absence of glucose, fat and protein are converted into glucose via glucose-producing metabolic pathways such as gluconeogenesis (Lee *et al.*, 2004) and can therefore also influence insulin requirements.

Fat influences post-prandial glycaemia in more ways than one. Firstly, fat delays gastric emptying of food matter, which delays digestion of macronutrients, including carbohydrates, and thus lengthens the total digestion time and duration of elevated

glycaemic blood glucose levels resulting from carbohydrate ingestion (Lee *et al.*, 2004). Secondly, digested fat turns into free fatty acids (FFA), and circulating FFA have been proposed to impair insulin sensitivity, which also results in prolonged hyperglycaemia (Wolpert *et al.*, 2013).

A study performed by Lee *et al.* (2004) involved a high-fat meal and a dual-wave insulin bolus with additional insulin calculated for both fat and protein, and showed that the post-prandial blood glucose curve was significantly lower compared to the same meal with a normal insulin bolus and based on carbohydrate counting only. The dual wave consisted of 65.9% of the bolus delivered immediately and an additional 34.1% delivered over 5.2 hours on average. This shows a much higher insulin requirement and much longer digestion time and corresponding glycaemic excursion duration than expected.

Proteins are digested to amino acids, which can also affect glucose levels in the blood via gluconeogenesis. This is a metabolic process in the liver through which substrates other than glucose are converted into glucose (Neu *et al.*, 2015).

In the next section, the available literature investigating the use of different CSII bolus settings for protein-rich and/or fat-rich meals is discussed and the need for better guidelines is highlighted.

2.7 Summary of current literature

Table 2.1 summarises recent studies in which different types of insulin boluses were used in an attempt to improve post-prandial hyperglycaemia in subjects with type 1 diabetes following high-protein and/or high-fat meals. Comparability between studies is impaired by the varied study designs and the duration for which post-prandial glycaemic curves were monitored. Olinder *et al.* (2009), for example, measured the post-prandial glycaemic curve for three hours only using different types of meal boluses, and concluded that there are no significant differences in the glycaemic excursions following high-fat and low-fat meals. On the other hand, Smart *et al.* (2013) measured post-prandial glycaemia up to five hours post-meal and found a marked increase in glycaemia between three and five hours post-meal. Neu *et al.* (2015) measured

glycaemic values up to 12 hours post-prandially and found the most significant difference in glycaemic values between fatty, protein-rich meals and carbohydrate-only meals to be six hours post-meal. Similar results were found by Lee *et al.* (2004), where significantly higher glucose levels were recorded at five hours post-meal when a normal insulin bolus was given for a high-fat meal.

Another factor complicating the comparison of results between studies using prolonged boluses is the lack of information on how the square and dual-wave boluses were decided on (Chase *et al.*, 2002; Olinder *et al.*, 2009). Other studies, including the study by Wolpert *et al.* (2013), used post-meal basal adjustments to allow for post-prandial hyperglycaemia corrections while Kordonouri *et al.* (2012) used CFP counting to determine the prolonged boluses. The study by Lee *et al.* (2004) used carbohydrate counting with an insulin-to-carbohydrate ratio, and used 50% of this ratio for both fat and protein to develop insulin-to-fat and insulin-to-protein ratios. There is no explanation or rationale as to why half of the carbohydrate ratio was used to quantify the hypothesised insulin requirements of fat and protein. In the study by Wolpert *et al.* (2013) the authors stated that an alternative method is needed to predict boluses for high-fat meals. In the study by Kordonouri *et al.* (2012), although the post-prandial hyperglycaemia was reduced, there was also a significant increase in hypoglycaemia after the meal, indicating that their method is not safe to use for all patients. As previously mentioned, the method proposed by Kordonouri *et al.* (2012) is even more complicated than simple carbohydrate counting, which makes it less appealing for patients, especially the paediatric population. All these studies concluded that high-fat and/or high-protein meals increase post-prandial glycaemia and that carbohydrate counting with standard or normal boluses is not sufficient to prevent this increase.

A study by Pańkowska *et al.* (2009) indicated that the regular use of dual-wave boluses for meals containing fat and protein in addition to carbohydrates reduced HbA1c levels. Yet, a study by Jankovec *et al.* (2008) showed that less than 50% of patients on pump therapy actually made use of other boluses than the standard bolus. Authors of other studies in this area of diabetes care have noted that the methods used to determine prolonged type bolusing should be as easy to perform as those currently used to

determine the normal or standard bolus otherwise patients will not use these methods (Olinder *et al.*, 2009). In other words, deciding which prolonged bolus to use and how to set it should not take much more effort than carbohydrate counting and delivering a normal bolus.

Most of the studies done on insulin requirements for fat and protein conclude that there is a need to give additional insulin for fat-rich and protein-rich meals, although only three of them attempted a mathematical measure to quantify this additional need for insulin (Kordonouri *et al.*, 2012; Lee *et al.*, 2004; Wolpert *et al.*, 2013). The small sample sizes and the different age ranges used in these studies make it impossible to formulate a method that patients can use to quantify insulin for fat and protein meal content. The study by Neu *et al.* (2015), for example, concluded that teenagers aged 16 years should set a dual-wave bolus for at least six hours for fatty, protein-rich meals as a significant glucose peak was seen at six hours, lasting up to 12 hours for some of them. The sample size of 15 patients is, however, not enough to draw firm conclusions. Also, where glucose patterns were followed without delivering additional insulin to correct hyperglycaemic values in the hours following the meal, no information is available on the possible age-specific additional insulin requirements for fat and protein.

Table 2.1: Types of bolus and food studies in type 1 diabetes

Author and year	Study design	Number of subjects	Ages (yrs)	Description of meal(s) and insulin boluses	Post-prandial BG profile	Outcomes measured	Results
<i>Chase et al., 2002</i>	Randomised, cross-over trial using CC. Total insulin kept the same for all 4 boluses, no additional insulin given. Note: Lispro insulin used; all other studies used soluble insulin; also allowed a pre-prandial level up to 11.1 mmol/L for meal initiation.	n=9	14-28	The same high-kilojoule meal (pizza and tiramisu): carbohydrates (53%), protein (11%) and fat (36%). Meals were consumed 4 times, once a week for 4 weeks. A different insulin bolus was administered every time: a single bolus, 2 separate half-boluses 90 minutes apart, an entire bolus as a square wave over 2 hours, and a dual wave with 70% as a bolus and 30% in the form of a square wave over 2 hours.	Blood glucose measured at -60, -30, 0, and every 30min thereafter for 6 hours post-prandial.	AUC, glucose excursion, time to peak excursion	Dual wave resulted in lowest glucose excursions at 90min and 120min compared to baseline glucose. Distributing insulin delivery over 2 hours (dual and square wave boluses) resulted in a significantly lower glucose reading 4 hours after the meal compared to a standard or split up bolus.
<i>Lee et al., 2004</i> <i>*Only study not including children</i>	Cross-over, repeated measures study. Insulin delivered was calculated by incorporating all macronutrients; for carbohydrates the normal insulin-carbohydrate ratio was used, and 50% of the insulin-carbohydrate ratio was used for both fat and protein.	n=10	47.9±12.5	In this study, 3 meals were given over 3 nights. Meal 1 was the control meal given with a normal bolus. Meal 2 was a high-fat meal given with a normal bolus. Meal 3 was a similar high-fat meal given with a dual-wave insulin bolus. The dual-wave insulin bolus consisted of 65.9% of the bolus delivered immediately and an additional 34.1% delivered over 5.2 hours on average.	Fasted for 16 hours post-meal, wore CGMS for this duration.	Glycaemic excursions, mean average hourly sensor values.	Three hours post-meal the glucose excursions were similar. Significant higher glucose levels were seen at 5 hours post-prandial in meal 2, the high-fat meal with a normal bolus, compared to the other meals. CGMS identified prolonged post-prandial glucose elevations, especially for the high-fat meals.

Author and year	Study design	Number of subjects	Ages (yrs)	Description of meal(s) and insulin boluses	Post-prandial BG profile	Outcomes measured	Findings
<i>Lodefalk et al., 2008</i>	Randomised, cross-over trial. CC used. No additional insulin given.	n=7	16.4 ± 0.7	Two meals with the same CHO and protein but different fat content. Meal 1 consisted of 320kcal and 2g fat. Meal 2 consisted of 640kcal and 38g fat. All subjects received 7 units insulin pre-meal.	Capillary blood glucose tests done every 30mins for 4 hours post-meal	Rate of digestion, post-prandial glucose levels	Rate of digestion effects post-prandial glycaemia. An initial delayed glycaemic response in the first 2 hours was seen post-meal for high-fat meal.
<i>O'Connell et al., 2008</i>	Open cross-over study. CC was used and correction boluses were not allowed post-meal.	n=20	8-18	Four different meals with different food items, close to equal calories, fat and carbohydrate (57g-60g) content but with different protein and GI values. Insulin bolus given either as a full, standard bolus or as a dual wave of 50%:50% over 2 hours.	3 hour post-prandial glycaemia assured with CGM	AUC, peak of glucose excursion, and time to peak excursion	High-GI meals had a significant upward post-prandial glucose excursion as well as a greater AUC for both types of boluses. For low-GI meals, the dual-wave bolus significantly decreased the post-prandial AUC by up to 47% compared to standard bolus.
<i>Olinder et al., 2009</i>	Teenage girls pasta study. Insulin only given for carbohydrates, correction boluses not allowed. Pre-meal glucose allowed to be up to 12 mmol/L.	n=15 with diabetes, 10 control or non-diabetic	13-20	Two pasta meals with different fat content was consumed 3 times. Same dose of insulin given in three different bolus types; 1) standard, 2) dual wave of 60% standard, and 3) 40% over 1 hour, square wave over 1 hour	CGM was used to monitor glucose 3 hours post-meal	Peak glucose excursion and AUC	No significant differences in post-prandial glucose profiles were seen between the four different bolus types in the diabetes group.

Author and year	Study design	Number of subjects	Ages (yrs)	Description of meal(s) and insulin boluses	Post-prandial BG profile	Outcomes measured	Findings
<i>Kordo-nouri et al., 2012</i>	Randomised controlled trial, the same meal given was 4 times, 1 – normal bolus CC, 2 – normal bolus CFP counting, 3 – dual bolus CC, 4 – dual bolus CFP counting. Performed in inpatient conditions. Basal rates not changed during test meals. Sensor alarm for upper glucose values was disabled. Additional insulin given pre-meal by calculating fat and protein.	n=42	6-21, mean age 12.3	Lunch meal of salami pizza, 50% CHO, 34% fat, 16% protein. Meal energy added up to 33% of daily energy needs, adjusted for age. Dual wave was 70%:30% delivered over 3 hours. For 1 FPU, 4 hours. For 2 FPU, 5 hours. For 3 FPU, 6 hours. For 4+ FPU, 6 hours.	Post-prandial measured with CGMs. Not allowed to eat anything else for 6 hours post-meal. Capillary tests done pre-meal; also 2 and 6 hours post-meal.	AUC over 6 hours, average glucose values were measured and compared	CFP counting had significantly lower AUC and AV for normal and dual bolus ($p<0.001$). More post-prandial hypoglycaemic events were found with CFP counting ($p<0.001$). Shows protein and fat also requires insulin.
<i>Smart et al., 2013</i>	Four-by-four randomised crossover trial done over 2 paediatric centres. Carbohydrate counting used. No additional insulin given.	n=33 total; 27 on CSII, 6 on MDI	8-17 mean age 12.2	Breakfast meals with same carbohydrates (20.5g) but different fat and protein contents were given: LFLP, LFHP, HFLP, HFHP. LF=4g fat, HF=35g fat, LP=5g protein, HP=40g protein. Individual standard or normal bolus given for each meal. Subjects ≤ 45 kg received 75% of all macronutrients.	Post-prandial glycaemia assured by using iPro 2 CGM for 5 hours post-meal.	Mean glucose excursions, time of glucose excursion	An increase in glucose excursions is seen for meals high in fat and/or protein from 3 to 5 hours post-meal. High-protein meals had a protective effect on hypoglycaemia.

Author and year	Study design	Number of subjects	Ages (yrs)	Description of meal(s) and insulin boluses	Post-prandial BG profile	Outcomes measured	Findings
<i>Wolpert et al., 2013</i>	Crossover study. Carbohydrate counting was used. Additional insulin added by adjusting basal insulin rate post-prandially.	n=7	55 ± 12	Two dinners were given, with identical protein and carbohydrate content but one meal was high in fat, 60g fat (HF), and one low in fat, 10g fat (LF). Total energy per day and per meal was based on individual requirement calculated by means of the Harris Benedict equation. Closed loop system was followed at night, with a standard meal bolus given 15min prior and adjusted basal rates post-meal based on CGM readings. Breakfast and lunch for 2 study days were also controlled. Protein and carbohydrate quantities in each meal were identical for each subject. Mainly saturated fat was used.	18 hour post-prandial period followed with CGM.	Glycaemia post-prandial AUC, insulin requirement per meal.	HF dinner requires more insulin than LF, $p < 0.01$, 12.6 ± 1.9 units vs. 9.0 ± 1.3 units. Despite extra insulin HF meal resulted in more hyperglycaemia post-prandially, $p < 0.0001$. Inter-individual differences were seen in the amount of insulin needed for fat. Supports fact that dietary fat increases glucose levels, and theory of free fatty acids' ability to impair insulin sensitivity, thus fatty meal could contribute to an insulin-resistant state post-prandially. The study concludes that there is a need for 'alternative insulin dosing algorithms' to account for high-fat meals. Authors report a need for studies in younger age groups.

Author and year	Study design	Number of subjects	Ages (yrs)	Description of meal(s) and insulin boluses	Post-prandial BG profile	Outcomes measured	Findings
<i>Neu et al., 2015</i>	Mono-centre, non-blinded, randomised, crossover trial. Hospital-based environment. Used only carbohydrate counting and gave a normal bolus insulin only for carbohydrates. Used home-based foods, not take-away foods as most other studies do.	n=15 total. 13female 2 male. CSII and MDI patients	16.8 ± 2.9	Two dinner meals on two consecutive days eaten, one meal a standard meal (SM) and the second meal a fat- and protein-rich meal (FPRM), both meals were equal in carbohydrates.	Glucose measured continuously overnight for 12hrs with CGM.	AUC, time to maximum AUC, difference of glucose levels 12 hours post-prandial.	Maximum glucose excursion in fat-protein rich meal seen at 6 hours post meal, significant differences between the two meals at 4 – 12 hours post-meal. Significant difference in the glucose levels between the two meals was seen the following morning. Study concludes that after 12 hours fat-protein rich meals still cause glucose excursions, and that dietary counselling should incorporate fat and protein when determining insulin as a need for additional insulin for fat-protein rich meals is clearly indicated.

*BG – blood glucose, CC – carbohydrate counting, CFP – carbohydrate, fat and protein counting, FPU – fat protein unit, AUC – area under the curve, HF – high fat, HP – high protein, LF – low fat, LFLP – low fat, low protein, LFHP – low fat, high protein, HFHP – high fat, high protein, MDI – multiple daily injections, CSII – continuous subcutaneous insulin infusion, CGM – continuous glucose monitoring, GI – glycaemic index, SM – standard meal, FPRM – fat-protein rich meal

2.8 Conclusion

Tight glycaemic control, both fasting and post-prandial, has been shown to reduce the risk of diabetes-related microvascular and macrovascular complications such as nephropathy, neuropathy and retinopathy, cardiovascular diseases and all-cause mortality (Lee *et al.*, 2004). However, once complications have manifested, even tight glycaemic control has a limited protective effect. Thus, it is crucial to prevent hyperglycaemia, including post-prandial hyperglycaemia, from as early as possible following diagnosis (Fullerton *et al.*, 2014).

For many type 1 diabetes patients, post-prandial glucose rises are one of the major challenges in diabetes care as these rises contribute greatly to glucose variability and overall glycaemic control (Chase *et al.*, 2002; Heptulla *et al.*, 2008). Some studies have indicated that even in the presence of a near normal HbA1c, late complications can still develop as a result of poor post-prandial glucose control (Danne *et al.*, 2006).

It is therefore imperative to adjust nutritional therapy and to accurately calculate the insulin need of each individual patient to improve the post-prandial hyperglycaemia that many patients experience. In fact, the American Diabetes Association recently recommended that patients with type 1 diabetes who have mastered advanced carbohydrate counting should be educated on the glycaemic influences of fat and protein meal content (Bell *et al.*, 2015). However, no specific guidelines have been developed as to what this education should entail. Prolonged boluses such as the square or extended, or dual- or multi-wave bolus features of CSII treatment have been developed by insulin pump companies in an attempt to address this issue specifically. The current and most widely used method of calculating insulin need, namely carbohydrate counting, has been shown to be inefficient in energy-dense, high-protein, high-fat meals. However, other methods used to better determine how to set these boluses by taking the protein and fat content of meals into consideration, such as CFP counting developed by Pańkowska *et al.* (2012), are novel research tools and suitable probably for only some patients due to the complexity of the methods. Hence, these methods may be impractical to implement in the day-to-day living of patients with type 1 diabetes and even more so in the paediatric population. Also, CFP counting has resulted in significantly more episodes of post-prandial hypoglycaemic events (Kordonouri *et al.*, 2012). Although the value of these methods should not be dismissed, determining the post-prandial blood glucose profiles in children with type 1 diabetes of different age groups could help to provide insight into how to better determine an

optimum dual-wave or extended bolus that is specific to the age of the child. This is of particular importance as only a few studies have investigated this issue in children with type 1 diabetes.

Children with type 1 diabetes are exposed to possible hyperglycaemia for many more years compared to those diagnosed in adulthood and have a greater chance of developing complications. Conducting this study to try and establish the true insulin requirement in children with type 1 diabetes for meals with different macronutrient content may help to guide future practices in avoiding post-prandial hyperglycaemia and to limit the exposure to hyperglycaemia and thus reduce the risk of both macro and microvascular complications later in life.

In 2014, ISPAD made the following level A evidence-based recommendation in terms of insulin treatment: “In all age groups, as close to physiological insulin replacement as possible and optimal glycaemic control must be the aim” (Danne *et al.*, 2014).

Optimal use of insulin pump therapy, including the accurate use of prolonged bolus insulin delivery, based on accurate data of true insulin needs for fat and protein content of meals, can help to achieve this goal by lowering post-prandial glycaemia.

The aim of this study was therefore to determine the post-prandial glucose profile of children with type 1 diabetes for meals with known, constant carbohydrate content but different fat and protein contents, using CGM or sensor technology and correction bolus insulin, in an attempt to develop simple guidelines for determining the insulin requirement of fat and protein and the optimal type of bolus for mixed meals.

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CHAPTER 3 – PROTEIN AND FAT MEAL CONTENT INCREASE INSULIN REQUIREMENT IN CHILDREN WITH TYPE 1 DIABETES – ROLE OF DURATION OF DIABETES

Authors: M. van der Hoogt, J.C. van Dyk, R.C. Dolman, M. Pieters

This chapter includes

- 3.1** Authors' instructions of the journal, *Pediatric Diabetes* (Impact factor: 3.4);
- 3.2** Proof of submission for publication; and
- 3.3** The article titled: "Protein and fat meal content increase insulin requirement in children with type 1 diabetes – role of duration of diabetes".

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3.3 Article submitted to *Pediatric Diabetes*

PROTEIN AND FAT MEAL CONTENT INCREASE INSULIN REQUIREMENT IN CHILDREN WITH TYPE 1 DIABETES – ROLE OF DURATION OF DIABETES

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3.3.1 Abstract

Background and objective: Hyperglycaemia remains a challenge in type 1 diabetes since current regimes used to determine meal insulin requirements prove to be ineffective. This is particularly problematic for meals containing high amounts of protein and fat. We aimed to determine the post-prandial glycaemic response and total insulin need for mixed meals, using sensor-augmented insulin pumps in children with type 1 diabetes.

Methods: Twenty two children with type 1 diabetes, aged 4 to 17 years on insulin pump therapy completed this home-based, cross-over, randomised controlled trial. Two meals with identical carbohydrate content – one with low fat and protein (LFLP) and one with high fat and protein (HFHP) contents – were consumed using normal insulin boluses. Blood glucose monitoring was done for 10 hours post-meal, with correction bolus insulin given two-hourly if required.

Results: The HFHP meal required significantly more total insulin (3.48 vs. 2.7 units) as a result of increased post-meal correction insulin requirement (1.2 vs. 0.15 units) spread over a longer duration (6 vs. 3 hours). The HFHP meals significantly increased the time spent above target glucose level. Duration of diabetes and total daily insulin use significantly influenced the post-prandial blood glucose response to the two meals.

Conclusion: When consuming carbohydrate-based mixed meals, children with type 1 diabetes on insulin pump therapy, required significantly more insulin over a longer period of time than the insulin requirement calculated using current regimes. This additional amount required is influenced by the duration of diabetes and total daily insulin use.

Keywords

carbohydrate, protein and fat, type 1 diabetes, glucose, insulin infusion systems

3.3.2. Introduction

For many people with type 1 diabetes, post-prandial hyperglycaemia remains one of the major challenges in diabetes care and contributes greatly to glucose variability and overall glycaemic control (1). Even in the presence of a near normal glycosylated haemoglobin (HbA1c), diabetes complications can still develop (1). Therapy includes lifetime management of exogenous insulin delivery either by injection or by subcutaneous insulin infusion, also known as insulin pump therapy, dietary and exercise management, as well as blood glucose monitoring with finger pricks and, for some, continuous glucose monitoring (CGM) systems (2).

Current practices for determining meal-time bolus insulin whether on multiple daily injections (MDI) or continuous subcutaneous insulin infusion (CSII) (insulin pump therapy) involve carbohydrate counting, usually advanced (Level 3) carbohydrate counting where individualised insulin-to-carbohydrate ratios are used (2). This and other methods of carbohydrate counting assume that only carbohydrates affect the post-prandial glucose rise in children with type 1 diabetes. However, many studies have indicated that factors such as the type of carbohydrate, the glycaemic index of the meal, and the fat, fibre and protein content of the meal play an important role in delaying post-prandial hyperglycaemia, and these factors should be considered when trying to optimise post-prandial glucose levels (3-5).

When using CSII, most pumps offer three modes to deliver bolus or meal-time insulin: the normal or standard bolus, the dual-wave or multi-wave bolus, and the square-wave or extended bolus. The use of only normal or standard boluses and carbohydrate counting alone, where all bolus insulin is delivered immediately and the dose is determined by the current blood glucose reading and carbohydrate content of the meal, can be ineffective in optimising post-prandial blood glucose levels for mixed meals as fat and protein have shown independent effects on

post-prandial hyperglycaemia (3-5). Consequently, Pańkowska *et al.* (6) developed a method of quantifying meal insulin based on all macronutrients of the meal (carbohydrate, fat and protein (CFP) counting). However, CFP counting may pose a risk for increased post-prandial hypoglycaemic events in the early hours post meal (3,6) and may be challenging to implement in the paediatric diabetes population. In 2014, the *Guide for nutritional management in children and adolescents with diabetes*, developed by the International Society for Paediatric and Adolescent Diabetes (ISPAD), indicated that randomised controlled trials, aimed at developing methods to better manage post-prandial hyperglycaemia after fat and protein rich meals, are needed (2).

The aim of this study was to determine the post-prandial glycaemic response and total insulin need for mixed meals with known, constant carbohydrate content but different fat and protein contents, using insulin pump therapy and CGM in children with type 1 diabetes.

3.3.3. Subjects

In total, 32 children with type 1 diabetes aged 4 to 17 years were recruited. The inclusion criteria were: use of sensor-augmented pump therapy for longer than one month; HbA1c \leq 9.6% (81 mmol/mol) for the last three months; World Health Organization BMI / age z-score of -1 to below 3, thus not including wasted or obese individuals; and total daily insulin use of \geq 0.5 u/kg to avoid inclusion of participants in the remission phase of diabetes.

The exclusion criteria were: smoking; coeliac disease; cystic fibrosis; concurrent conditions that can be associated with delayed gastric emptying or altered digestion; and the use of any medication or supplements that could influence gastric emptying, digestion or glucose levels,

such as glucocorticoids or oral anti-diabetic drugs. Participants had to be free of any acute illnesses at the start of the study.

The study was conducted in accordance with the Helsinki Declaration. Ethical approval was obtained from the Health Research Ethics Council of North-West University, South Africa (NWU-00042-15-S1).

3.3.4. Materials and methods

3.3.4.1 Study design

A home-based, cross-over, randomised controlled trial was performed. For all participants, optimal basal insulin rates, carbohydrate ratios and sensitivity factors were revised and adjusted by a paediatric endocrinologist before enrolment. Participants were randomised to treatment. The two meals were consumed at dinner time (18:00) under parental supervision, at least a day apart and within a month of one another, to ensure that factors which could potentially change HbA1c values such as illness, radical changes in diet or activity, or stress did not interfere with the results. Participants maintained their normal, habitual activity levels during the two study days. Upon enrolment, participants received detailed study instructions, a cooler bag with their two individual study meals, three Enlite sensors (Medtronic, Inc., MN, USA), a blood glucose meter (Bayer Contour 2.4 Next Link 2.4 blood glucose meter; Bayer Indianapolis, IN, USA) and 30 strips for the meter.

Sensor-compatible insulin pumps used in the study included the 554 Veo, 754 Veo, 722 Paradigm and 640G from Medtronic (Medtronic, Inc., MN, USA). For CGM, Medtronic Enlite Sensors were used with two different transmitters, the Gaurdian Link and Gaurdian Connect, as

two different pump models were used. Study meals could only be taken on days 2 to 5 of the sensor lifespan as a sensor is least accurate on day 1 and day 6 (7). All participants used rapid-acting insulin Novorapid (Novo Nordisk, Copenhagen, Denmark) in their pumps.

3.3.4.2 Test meals

Each participant received two different meals with the same carbohydrate content. One meal was high in fat and protein (HFHP) and the other low in fat and protein (LFLP). Meals consisted of smoked, skinless and boneless chicken breast, pre-prepared plain, white long-grain rice, ready prepared chicken gravy, and olive oil. The meals only required pre-heating in the microwave; no cooking was allowed. The fat and protein content was manipulated by the portion sizes of the chicken breast and the amount of gravy and olive oil. The rice was a low glycaemic index (GI) food.

The macronutrient content of the meals was calculated as follows: the total daily energy requirement for each participant was individually calculated using an age, weight and gender specific World Health Organization energy expenditure recommendation (8). The total carbohydrate per day was then calculated at 50% of total energy, since 50%-55% is recommended for children with type 1 diabetes (6). Of the total daily carbohydrates, 25% was allocated to each study meal. The amount of carbohydrates for both meals was kept constant in order for the LFLP meal to be used as the control for the HFHP meal. The fat and protein content per meal, calculated as percentage energy, were as follows: LFLP meal carbohydrates 60%, fat 25%, and protein 15%; HFHP meal carbohydrates 40%, fat 35% and protein 25%.

3.3.4.3 Meal consumption procedures and capillary blood glucose testing

A pre-prandial blood glucose level of 4 to 11 mmol/L was required before the study meal could be taken. If the level was not in the specified range, the participant was allowed to give a correction bolus and then have the study meal 30 to 60 minutes later, if the capillary test then fell within the recommended range. The blood glucose level and indicated carbohydrate content of the meal were entered into the bolus wizard feature of the pump and a normal insulin bolus was then delivered 10 minutes prior to eating each meal. To limit variability of gastro intestinal clearance affected by fluid intake, participants were not allowed to have more than two to three glasses of water 30 minutes pre-meal to two hours post-meal. Meals had to be consumed within 20 minutes. Consuming the study meal was not permitted on an evening where the participant experienced a severe hypoglycaemic event during that day. Participants were allowed a breakfast and a low fat, light lunch meal of their choice but were not allowed to have any food two hours prior to the study. The entire process was repeated for the second meal on a different night.

After consumption of the study meal, in addition to CGM, capillary blood testing was performed by a parent at 30 minutes post-meal and then every two hours after the start of the meal for 10 hours. Each blood glucose value was entered into the pump and a correction bolus (calculated by the pump) was delivered when required (also at 2-hour intervals). All hypoglycaemic events and carbohydrate treatments were entered into the pump. In the case of a blood glucose value dropping below 4 mmol/L, the study was terminated as additional food had to be given, but the time of the hypoglycaemic events was still recorded and used for data analysis.

In the case of a child not finishing a meal, not wearing the sensor for the required amount of time or not adhering to the study protocol, the meal missed was repeated and given on another day. If this resulted in an altered meal order, it was recorded accordingly.

3.3.4 Insulin infusion

Before initiating the study, sensor and pump infusion sites were checked by a paediatric endocrinologist for, among others, swelling, infection and lipodystrophy, and changed if necessary. Participants were encouraged to consume study meals on days where infusion sets were changed and not to wear infusion sets longer than three days to limit the risk of poor insulin infusion.

3.3.4.5 Outcome measures

Pump downloads to obtain the study data were done using Medtronic Carelink Software Professional version (Medtronic, Inc., MN, USA). Main outcomes measured for the two meals were: peak sensor glucose value post-meal i.e. maximum post-meal glucose excursion above 6 mmol/L; time to peak sensor glucose excursion i.e. the time it took, following consumption of the meal, to reach the maximum post-meal glucose excursion; time of first and largest correction bolus - times at which first and largest correction bolus insulin were required; total correction insulin - the total of additional (correction) insulin required post-meal; total meal insulin - the total amount of insulin needed for the meal, this includes meal bolus and correction boluses; additional insulin required - correction bolus as a percentage of total bolus insulin; area under the sensor glucose response curve (AUC) (≥ 8 mmol/L), using the trapezoidal method; and, finally, duration of elevated post-prandial glucose - total time of elevated post-prandial glucose spent above 6 mmol/L.

The following participant characteristics were investigated as potential co-variables related to the outcome measures: gender; age; weight (measured on a precision health scale with participants wearing only light clothing and no shoes); height (using a wall-mounted stadiometer with the head in the Frankfort Horizontal Plane); duration of diabetes; HbA1c (Siemens DCA Vantage System from Siemens Medical Solutions, PA, USA); total daily insulin; active insulin time setting; carbohydrate-to-insulin ratio; insulin sensitivity factor; and meal energy, carbohydrate, fat and protein content.

3.3.4.6 Statistical analysis

The computer software package IBM® SPSS® Statistics version 23 (Statistical Package for Social Sciences, IBM, New York, USA) was used. Significance was set at $p < 0.05$. Normally distributed data is reported as mean \pm standard deviation (SD) and non-parametric data as median (25th; 75th percentiles). The order of treatment effect was tested for using repeated measures analysis of variance (ANOVA). Since no order of treatment effect was observed for any of the outcome variables, the data of the two treatment periods was combined. Paired t-tests for normally distributed data, and the Wilcoxon Matched Pairs test for non-parametric data were used to compare the LFLP and HFHP meals. Repeated measures ANOVA was used to perform sensitivity analysis to test for the possible influence of the use of two different CGM transmitters. In order to determine the influence of inherent patient characteristics unrelated to the test meals on the outcome variables, univariate mixed models were performed and data reported as β (95% confidence intervals (CI)). Characteristics found to be significantly related to the outcome variables were tested for interaction with the test meals by creating interaction terms, using continuous variables, in separate mixed models. Bivariate models were used when adjusting for age, and for determining the combined effect of fat and protein.

3.3.5 Results

Of the 32 participants recruited, 22 successfully completed the study. Dropouts were the result of poor adherence to the study protocol (n=10). There was no difference in baseline characteristics between the participants who completed and those who did not complete the study (data not shown). Descriptive data for the 22 participants who completed the study is provided in Table 1. The mean age of the participants was 10.4 ± 4 years. Nine were female, and the median duration of diabetes was 3.5 (1.5; 8.0) years. Sixteen participants had a BMI/age z-score indicating normal weight ($z = -1$ to 1), five were at risk of becoming overweight ($z = 1-2$) and one was overweight ($z = 2-3$) (data not shown).

Insulin requirement and glucose response curve data for the two test meals are reported in Table 3.2. The HFHP meal required significantly more insulin than the LFLP meal, namely eight times more post-meal correction insulin (1.2 vs. 0.15 units), and 1.3 times (30%) more total meal insulin (3.48 vs. 2.7 units). The LFLP meal resulted in significantly more hypoglycaemic events compared to the HFHP meal (7 vs. 1). Although the HFHP meal did not cause a significantly higher peak sensor glucose value ($p=0.14$), the time to reach peak sensor glucose value was borderline significantly longer ($p=0.056$). However, the HFHP did result in a longer duration of elevated post-prandial glucose (364 vs. 185 min) and a significantly larger AUC (198 vs. 46.3). There was no difference in the time of first or largest correction insulin dose between the two meals. Sensitivity analysis for the use of two different CGM transmitters revealed that it did not have any effect on the outcome variables in response to the two test meals.

The characteristics inherent to the study population but unrelated to the test meals that impacted the outcome variables were also investigated, and the significant relationships

reported in Tables 1 and 2 of the Online Supplement (Tables 3.3 and 3.4). Additional adjustment for age did not significantly alter the results. Characteristics that showed significant association were tested for interaction with the test meals in subsequent analysis. Online Supplement Table 1 additionally shows that both protein and fat meal content influence total meal and correction insulin requirements. Total meal insulin increased by 0.12 units for every 1g increase in protein. This relates to one unit additional correction insulin for every 8g protein in a mixed meal already containing carbohydrates. For fat, total meal insulin increased by 0.24 units for every 1g increase, translating to one unit additional correction insulin for every 4g fat in a mixed meal already containing carbohydrates. This 2:1 ratio was confirmed in a bivariate model determining the effect of fat and protein combined and is in agreement with the observed effect on AUC, with 1g fat also having double the effect 1g of protein does (Online supplement Table 2).

There was a significant interaction between the test meals and duration of diabetes in terms of peak sensor glucose values ($p=0.014$). The difference in peak sensor glucose values between the two test meals was larger in individuals who have had diabetes for longer (Online Supplement, Figure 1). Similarly, interactions were observed between the test meals and duration of diabetes ($p<0.0001$), total daily insulin use (u/kg) ($p=0.003$) and HbA1c ($p=0.003$) in terms of AUC. The difference in AUC between the two test meals was larger in individuals who have had diabetes for longer (Online Supplement Figure 2), those with a higher total daily insulin use (Online Supplement Figure 3) as well as individuals with higher HbA1c (Online Supplement Figure 4). These differences remained after additional adjustment for age.

3.3.6. Discussion

Although there is emerging evidence that protein and fat influence the insulin requirement of children with type 1 diabetes (3, 4, 6, 10), the recommended method of calculating prandial insulin is still based on meal carbohydrate content only. This is due to inconsistent findings in the literature and increased post-prandial hypoglycaemia in children when following methods to increase meal insulin based on fat and protein content (3,6). This study emphasises the urgent need to revisit the calculation of insulin requirement as well as the manner in which it should be delivered when using insulin pump therapy in children with type 1 diabetes. Our data indicate that when comparing two meals with the same carbohydrate content but different fat and protein contents, the HFHP meal, representing a typical mixed-meal dinner and not a take-out meal as in most other studies, required on average eight times more post-meal correction insulin than the LFLP. This additional insulin was determined by allowing two-hourly post-prandial correction insulin boluses for 10 hours post-meal, which is a major difference in study design compared to other studies in the field. The addition of protein and fat to the meal did not result in significantly higher absolute blood glucose levels, but it did result in a significantly larger AUC and longer time spent above target. In addition, the duration of diabetes and the total daily insulin use significantly influenced the post-prandial blood glucose response to the two meals, with individuals having diabetes for longer and those with a higher total daily insulin use showing the largest differences between the two meals.

The addition of fat and protein to a CHO-containing meal significantly extended the duration of post-prandial hyperglycaemia from up to three hours for the LFLP meal to up to 8.5 hours for the HFHP meal. This is in agreement with Neu *et al.* (9) who found elevated blood glucose levels in adolescents for up to 12 hours following a HFHP meal (without allowing post-meal correction insulin) and three hours for a standard meal. This may suggest that even for LFLP mixed meals,

extended insulin delivery, to over three hours, can be considered, with individual ranges varying from one to five hours. For HFHP mixed meals, boluses may have to be set as long as six hours, with ranges from four to 8.5 hours. However, advising a patient to spread an extended bolus from anything between three and 8.5 hours is not very practical. It calls for the identification of factors (see below) contributing to this large inter-individual variation in order to accurately advise a patient on extended bolus duration and the amount of insulin required.

Eight hypoglycaemic events were recorded in this study: seven after the LFLP meal, five of which occurred during the first two post-prandial hours (data not shown). Similarly, in the study by Neu *et al.* (9), 60% of adolescents had hypoglycaemia after a standard meal with no hypoglycaemic events occurring after a HFHP meal. This emphasises the limitations of carbohydrate counting in children with type 1 diabetes and shows that in some instances it may overestimate the amount of insulin required. The fact that almost all of the hypoglycaemic events occurred in the LFLP meal supports findings from another study that has shown protein to be protective of hypoglycaemia (5). Participants who experienced hypoglycaemia with their LFLP meal still required additional correction insulin for their HFHP meal, highlighting the fact that protein and fat meal content significantly increases insulin requirement. Our study showed an average increased insulin need of 31% for HFHP meals in children with a 2:1 ratio for fat and protein. A closed-loop study by Wolpert *et al.* (4), in adults, showed an average increase of 42% for high-fat meals compared with low-fat meals, also with marked inter-individual differences.

In an attempt to identify factors contributing to this large inter-individual variation in glycaemic response and consequent insulin requirements, we investigated participant characteristics such as age, gender, weight, body mass, glycaemic control, duration of diabetes and markers of insulin sensitivity such as total daily insulin use. Our data showed that duration of diabetes and

total daily insulin use influenced the insulin requirement and blood glucose response to the two meals, also after adjustment for the different ages of the study participants. Individuals with a longer duration of diabetes showed a larger difference in peak sensor glucose value between the two test meals. Similarly, individuals who have had diabetes for longer, those with higher total daily insulin use and higher HbA1c, demonstrated a larger difference in AUC between the two meals. The above suggests that this phenomenon might be explained in terms of insulin resistance that develops with longer duration of type 1 diabetes, which may in turn influence insulin requirement and glucose response to different meal compositions. It is likely not a mere consequence of metabolic differences across the age range.

Insulin resistance is commonly associated with type 2 diabetes, but in recent years studies have shown it to be present in type 1 diabetes as well (10-12). Insulin resistance in type 1 diabetes is not thought to be associated with current glycaemic control (10) or with BMI, fat percentage, plasma lipids, visceral fat or physical activity level (10). Some of the main proposed mechanisms for insulin resistance in type 1 diabetes are inhibited insulin signalling, caused by chronic hyperglycaemia, an increase in plasma free fatty acids (FFA) and amino acids, as well as inflammatory processes (11). Insulin resistance may further be attenuated by chronic iatrogenic hyperinsulinaemia in people with type 1 diabetes (11), which could explain why longer duration of diabetes, and thus longer exposure to iatrogenic insulin, can cause insulin resistance, at any age. There is also evidence that dietary fat and increased FFA can impair insulin sensitivity and elevate glucose production from the liver (13,14). Excessive amino acid and lipid availability interfere with insulin signalling (11); this interference can therefore explain a state of reduced insulin sensitivity after HFHP meals. In the event of HFHP mixed meals, there is additional substrate for gluconeogenesis, in the form of FFA and amino acids, which may

explain why these types of meals have such an effect on the post-prandial glycaemic curve and which adds to the increase in total additional insulin requirement (11).

The large inter-individual variation in glycaemic response and concurrent insulin requirements observed begs the question whether one method of calculating insulin for meals containing different macronutrients can be used for all patients. The observed influence of duration of diabetes and the potential effects of insulin resistance may explain why current methods quantifying insulin requirement considering meal content and current blood glucose level only, such as CHO counting, may not be working adequately for all patients.

A limitation of this study is that it was performed with a rather small sample size due to the difficulty of recruiting and retaining patients in private care in addition to poor protocol adherence by the paediatric study population. The sample size was however in line with (5,15,16) or larger than (9,17,18) other studies published in this field. In addition, insulin resistance *per se* was not measured. As is done in practice, we used a lower sensitivity factor and higher insulin use per kilogram body weight as proxy markers for indicating insulin resistance. Furthermore, variables such as time of first and largest correction bolus were interpreted based on two-hourly blood glucose testing, due to the study protocol and not necessarily at the time when the highest post-prandial glycaemia occurred. Two-hourly finger prick testing and correction bolusing were chosen as for most patients the active insulin setting would not allow more frequent correction boluses. Lastly, this study did not distinguish between elevated post-prandial glucose due to food or to the effect of growth hormone and cortisol peaks in the early morning hours (14) (glycaemic patterns were followed until 04:00 in the morning). To our knowledge, this was the first study where post-prandial correction dosages were used to determine total meal insulin. Hence, our results should be confirmed in larger study populations before definite conclusions can be drawn.

Future studies should investigate if the time of day influences meal insulin requirements by testing the same meals at different times of the day. For HFHP mixed meals, the feasibility of the pre-meal addition of one unit insulin for every 4g fat and one unit for every 8g protein, in combination with the usual CHO ratio and CHO counting method, administered in prolonged bolus form, should be investigated. Acknowledging the large inter-individual differences in glycaemic response to meals; this method may also not be suitable for all children with type 1 diabetes but may address the need of specifically patients who have post-prandial hyperglycaemia following these type of meals. The contribution of duration of diabetes, and the concomitant development of insulin resistance, to insulin requirements should also be further elucidated. In addition, future research should explore the use of extended boluses which can be spread over a mean of six hours, ranging from four hours to 8.5 hours, for HFHP mixed dinner meals. As this duration is likely to be dependent on more factors, not investigated in this study, the identification of these additional factors should receive priority.

This study highlights the additional insulin needed for typical mixed meals in children with type 1 diabetes and shows that all macronutrients require insulin, with the addition of each 1g of fat requiring double the amount of correction insulin compared to each 1g of protein. For the first time, duration of diabetes (regardless of age) is shown to be strongly associated with post-prandial hyperglycaemia, likely due to the development of insulin resistance. CHO counting alone fails to prevent post-prandial hyperglycaemia, especially in HFHP mixed meals, and in future, bolus wizard set-ups might require more input than target ranges, carbohydrate ratios and sensitivity factors. Duration of diabetes, fat and protein ratios and maybe even time of day might all form part of essential inputs to prevent post-prandial hyperglycaemia.

3.3.7 Acknowledgements

We would like to give special thanks to all patients, and their parents, for participating in this study and to Dr Marike Cockeran for statistical advice.

3.3.8 Conflict of interest

This work was supported by a Medtronic grant providing funding for study materials.

3.3.9 Authors' contributions

M. vd H. conceptualised and executed the study, performed the statistical analysis and wrote the paper. J. v D. conceptualised the study, oversaw medical aspects and treatment of participants, and critically reviewed the final manuscript. R. D. conceptualised the study and critically read the final manuscript. M. P. conceptualised the study, performed statistical analysis and co-wrote the manuscript.

3.3.10 References

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Table 3.1: Characteristics of the study population (n=22)

Variable	Mean \pm SD / median (25 th ; 75 th percentile)
Participant characteristics	
Male n (%)	13 (59)
Age (years)	10.4 \pm 4.00
Weight (kg)	39.0 \pm 17.0
Height (m)	1.41 \pm 0.25
Duration of diabetes (years)	3.5 (1.5 ; 8.0)
HbA1c (%)	8.23 \pm 0.82
(mmol/mol)	66 \pm 18
Total daily insulin (u/kg)	0.75 \pm 0.18
Active insulin or insulin on board setting time (h)	3.32 \pm 0.72
CHO ratio for study meal (g CHO / insulin unit)	8.34 (9.68 ; 22.0)
Insulin sensitivity factor for night (mmol/L glucose / insulin unit)	5.50 \pm 3.57
Test meals characteristics	
Meal carbohydrates (g)	40.2 \pm 9.08
Meal energy (kcal) HFHP	396 \pm 91.0
Meal protein (g) HFHP	26.6 \pm 6.72
Meal fat (g) HFHP	15.3 \pm 4.03
Meal energy (kcal) LFLP	273 \pm 63.2
Meal protein (g) LFLP	10.6 \pm 3.37
Meal fat (g) LFLP	7.72 \pm 2.25

SD – standard deviation; BMI – body mass index; HbA1c –glycosylated haemoglobin; CHO – carbohydrate; HFHP – high fat, high protein meal; LFLP – low fat, low protein meal

Table 3.2: Insulin dosage and glucose response curve comparisons between test meals

Dependent variable	LFLP	HFHP	P
Total correction insulin (units)	0.15 (0 ; 0.53)	1.2 (0.48 ; 2.32)	<0.0001
Total meal insulin (units) ^a	2.70 (1.68 ; 5.80)	3.48 (2.43 ; 7.81)	<0.0001
Additional insulin required (%) ^b	11.3 ± 5.36	31.1 ± 16.3	<0.0001
Time of 1 st correction bolus (min post-meal)	352 ± 204	352 ± 170	0.81
Time of largest correction bolus (min post-meal)	399 ± 192	420 ± 160	0.7
Basal suspend duration (min)	86.1 ± 79.9	53.3 ± 53.9	0.052
Duration of elevated post-prandial glucose (min) ^d	185 ± 124	364 ± 142	<0.0001
Peak sensor glucose value post-meal (mmol/L)	9.22 ± 2.09	10.3 ± 2.77	0.14
Time to peak sensor glucose value (min post-meal)	233 ± 204	342 ± 178	0.056
Sensor glucose peak excursion ^c	3.42 ± 1.94	4.29 ± 2.77	0.18
AUC (above 8 mmol/L)	46.3 (0 ; 211)	198 (11.7 ; 505)	0.02
Occurrence of hypoglycaemic events n (%)	7 (32)	1 (0.05)	0.02

^a food bolus and correction boluses; ^b correction bolus expressed as % of initial food bolus;

^c (difference between peak sensor glucose value and target of 6 mmol/L); AUC – area under the sensor glucose curve, ^d total time of elevated post-prandial glucose spent above 6 mmol/L. Data reported as mean ± SD or median (25th ; 75th percentile) depending on normality.

3.3.11 Online supporting information

Table 1: Effects of participant and meal characteristics on insulin dosage

Table 2: Effects of participant and meal characteristics on glucose response curve

Figure 1: Interaction between duration of diabetes and peak sensor glucose value

Figure 2: Interaction between duration of diabetes and area under the curve

Figure 3: Interaction between insulin use in units per kg and area under the curve

Figure 4: Interaction between HbA1c and area under the curve

Table 3.3: Effects of participant and meal characteristics on insulin dosage

Independent predictor *	Total correction insulin (units)		Total meal insulin (units) ^a	
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
Participant characteristics				
Gender	-0.95 (-3.91; 2.02)	0.51	-0.66 (-5.43 ; 4.13)	0.78
Age (months)	0.03 (-0.00 ; 0.06)	0.08	0.07 (0.03 ; 0.11)	0.003
Weight (kg)	0.08 (-0.00 ; 0.16)	0.58	0.20 (0.95 ; 0.31)	0.001
Height (cm)	0.05 (0.01 ; 0.09)	0.02	0.12 (0.66 ; 0.18)	<0.0001
Duration of diabetes (years)	0.43 (0.21 ; 0.64)	<0.0001	0.74 (0.43 ; 1.06)	<0.0001
HbA1c (%)	1.06 (-0.73 ; 2.85)	0.23	1.38 (-1.15 ; 4.28)	0.331
Insulin : carbohydrate ratio	-0.17 (-0.33 ; -0.00)	0.05	-4.25 (-0.63 ; -0.21)	<0.0001
Insulin sensitivity factor ^c	-0.36 (-0.75 ; 0.03)	0.07	-0.86 (-1.40 ; -3.12)	0.004
Total daily insulin (units/kg)	11.5 (5.07 ; 17.9)	0.001	21.3 (12.4 ; 30.2)	<0.0001
Test meal characteristics				
Meal energy (kcal)	0.02 (0.01 ; 0.02)	0.002	0.03 (0.02 ; 0.04)	<0.0001
Meal protein (g)	0.11 (0.05 ; 0.17)	0.001	0.12 (0.06 ; 0.19)	0.001
Meal fat (g)	0.21 (0.10 ; 0.32)	0.001	0.24 (0.11 ; 0.36)	0.001
Meal carbohydrate (g)	0.16 (0.01 ; 0.30)	0.043	0.37 (0.17 ; 0.57)	0.001

* Independent predictors were entered as single predictors in the mixed models ^a food bolus and correction boluses; ^b correction boluses expressed as a % of initial food bolus

^c mmol/l glucose / insulin unit

Table 3.4: Effects of participant and meal characteristics on the glucose response curve

Independent predictor *	Peak post-meal sensor glucose		AUC (above 8mmol/l)	
	value (mmol/l)			
	β (95% CI)	<i>P</i>	β (95% CI)	<i>P</i>
Participant characteristics				
Gender	-0.23 (-2.29 ; 1.82)	0.82	-33.2 (-346 ; 280)	0.83
Age (months)	0.02 (-0.00 ; 0.04)	0.08	2.34 (-0.71 ; 5.47)	0.12
Weight (kg)	0.04 (-0.02 ; 0.10)	0.17	6.21 (-2.60 ; 15.0)	0.16
Height (cm)	0.02 (-0.01 ; 0.06)	0.13	3.21 (-1.69 ; 8.12)	0.19
Duration of diabetes (years)	0.32 (0.17 ; 0.48)	<0.0001	53.9 (30.1 ; 77.8)	<0.001
HbA1c (%)	1.13 (-0.01 ; 2.26)	0.05	197 (26.5 ; 367)	0.03
Insulin : carbohydrate ratio	-0.11(-0.22 ; 0.00)	0.051	-16.0 (-33.3 ; 1.39)	0.07
Insulin sensitivity factor ^a	-0.17 (-0.45 ; 0.11)	0.22	-33.7 (-74.9 ; 7.40)	0.10
Total daily insulin in units/kg	7.29 (2.84 ; 11.7)	0.003	1288 (656 ; 1920)	<0.001
Test meal characteristics				
Meal energy (kcal)	0.01(0.00 ; 0.15)	0.012	1.62 (0.53 ; 2.72)	0.005
Meal protein (g)	0.08 (0.01 ; 0.15)	0.02	16.5 (6.75 ; 26.3)	0.002
Meal fat (g)	0.15 (0.03 ; 0.27)	0.019	30.8 (13.2 ; 48.4)	0.001
Meal carbohydrate (g)	0.08 (-0.03 ; 0.18)	0.16	12.6 (-3.70 ; 29.0)	0.122

^a mmol/l glucose / insulin units; AUC – area under the sensor glucose curve

* Independent predictors were entered as single predictors in the mixed model

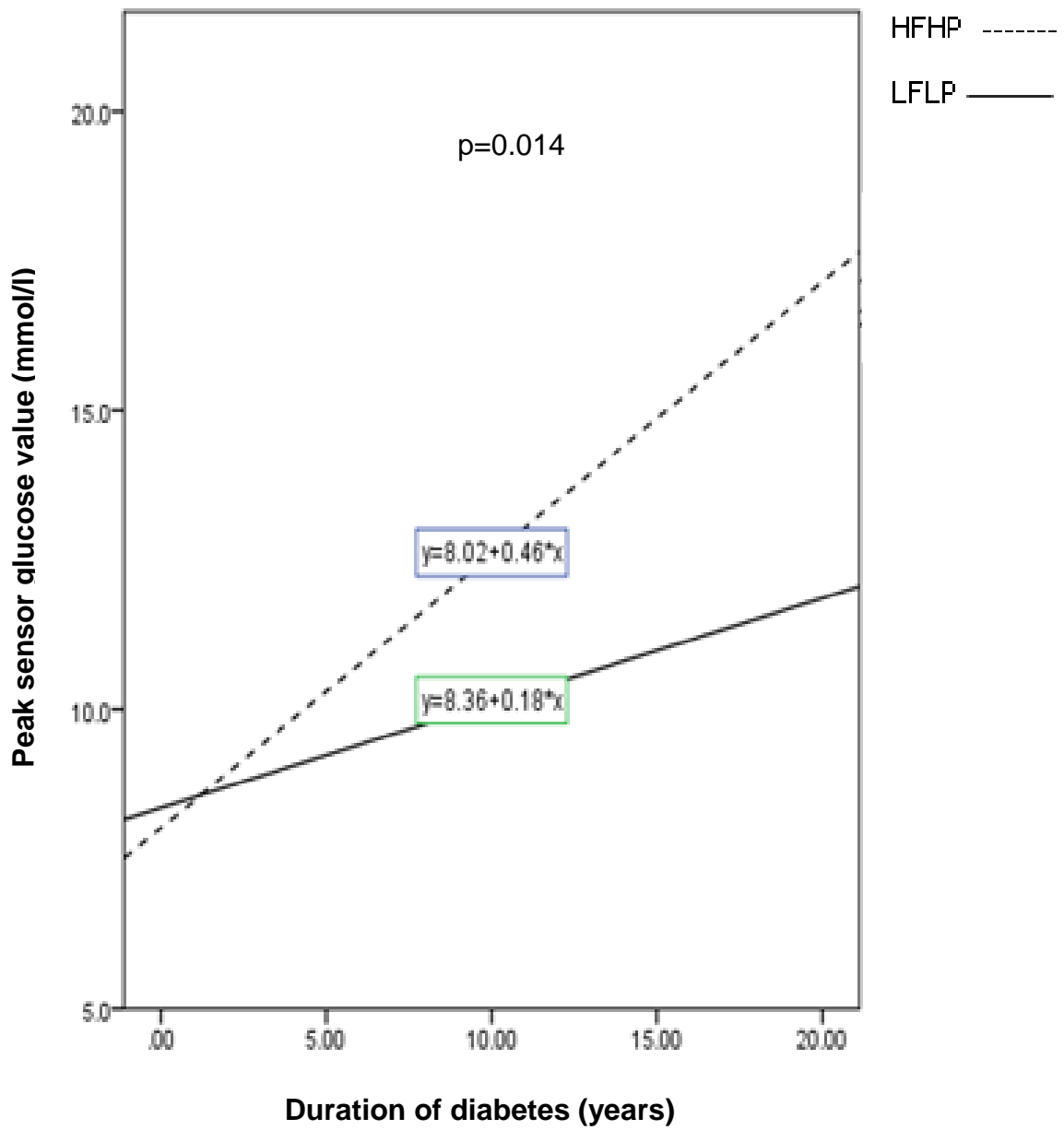


Figure 3.1: Interaction between duration of diabetes and peak sensor glucose value

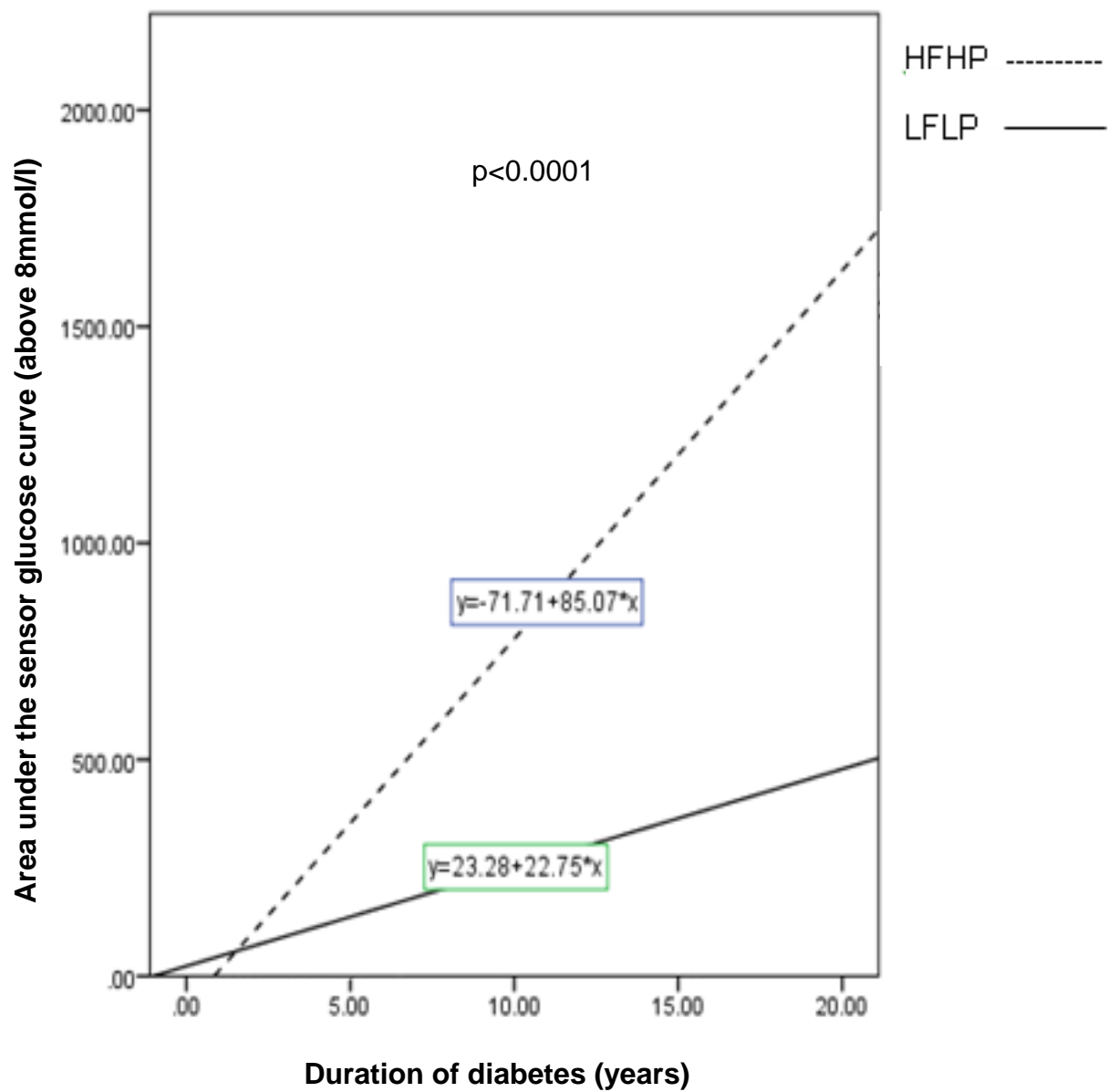


Figure 3.2: Interaction between duration of diabetes and area under the sensor glucose curve

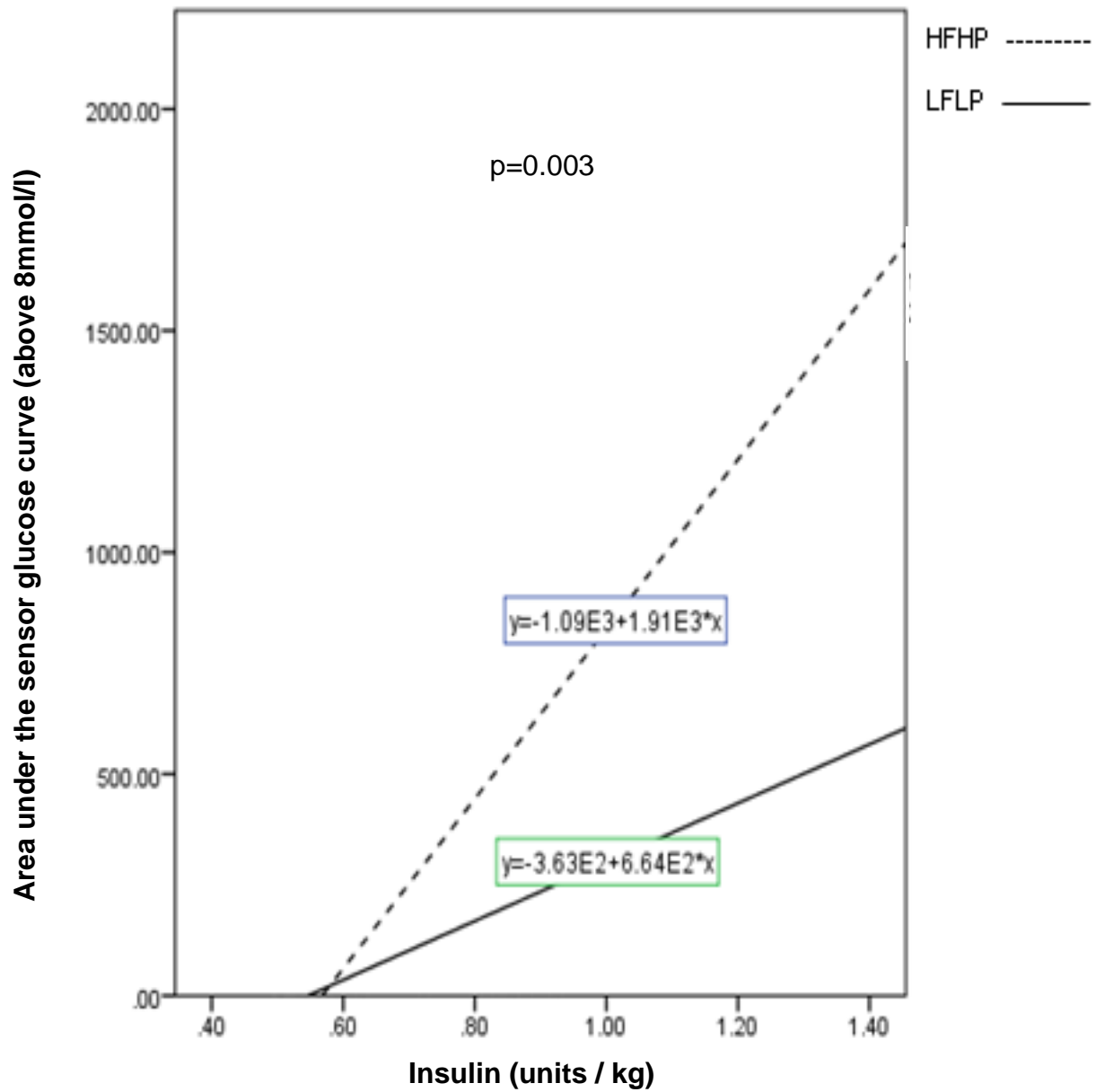


Figure 3.3: Interaction between insulin use in units per kg and area under the sensor glucose curve

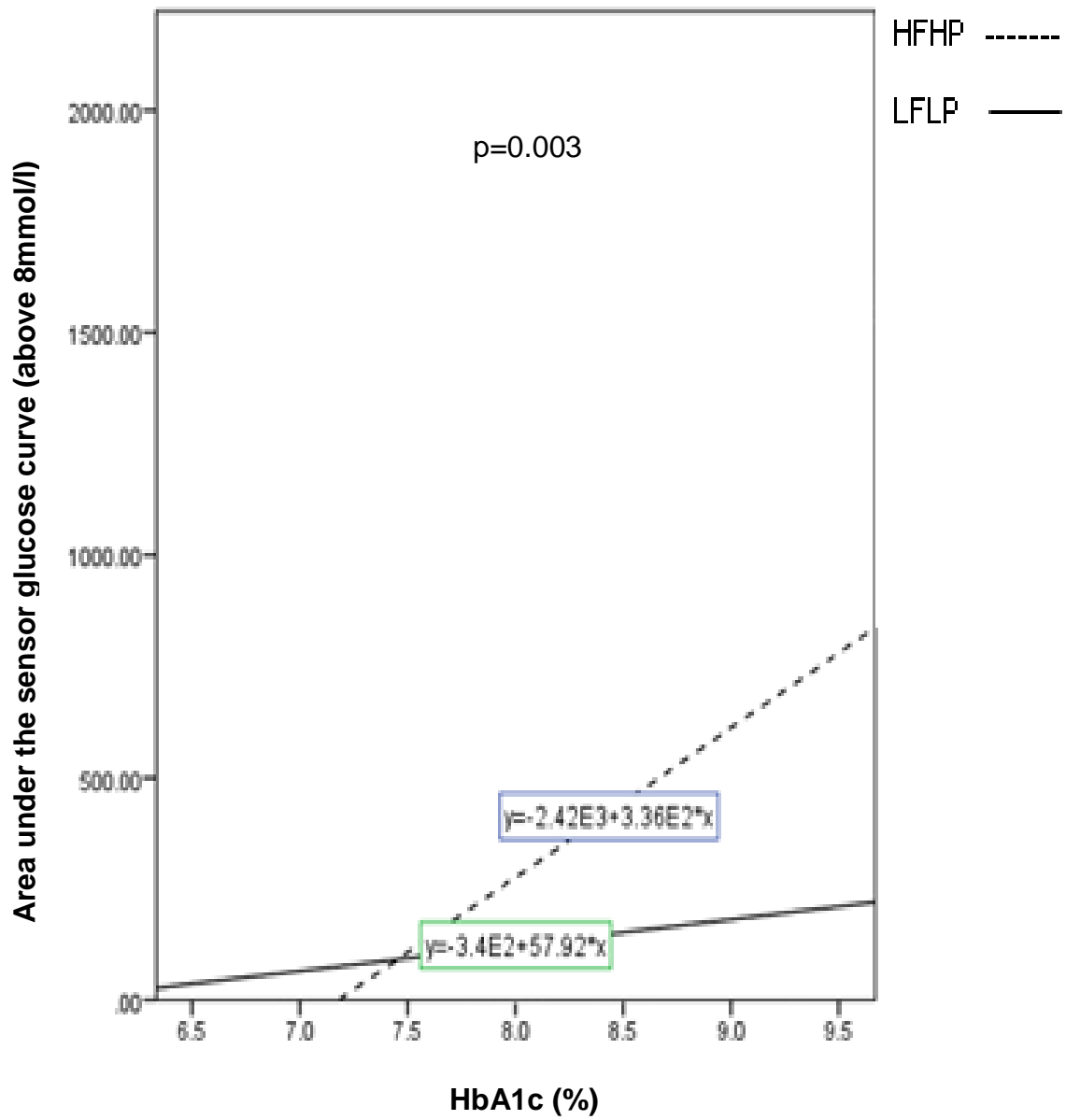


Figure 3.4: Interaction between HbA1c and area under the sensor glucose curve

ADDENDA

ADDENDUM A: Information leaflet and parental permission form



APPROVED

HREC stamp

PARTICIPANT INFORMATION LEAFLET AND PARENTAL PERMISSION FOR PARENTS AND LEGAL GAURDIANS OF PARTICIPANTS

TITLE OF THE RESEARCH PROJECT: The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes.

REFERENCE NUMBERS:

PRINCIPAL INVESTIGATOR: Prof. Marlien Pieters

ADDRESS: Little Company of Mary Hospital, Groenkloof, Pretoria

CONTACT NUMBER: 012 460 9700 / 072 369 4865

Your child / grandchild is being invited to take part in a research project that forms part of the Master's degree of Maryke van der Hoogt, one of the researchers. Please take some time to read the information presented here, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied and that you clearly understand what this research entails and how you could be involved. Also, you and your child's / grandchild's participation is **entirely voluntary** and you are free to decline to participate. If you say no, this will not affect you or your child / grandchild negatively in any way whatsoever. Your and your child's / grandchild's level of care or relationship with your diabetes care team will not be influenced in any way by your

decision to allow or not allowing your child / grandchild to participate in this study. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee of the Faculty of Health Sciences of North-West University (NWU)** and will be conducted according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the Health Research Ethics Committee members or relevant authorities to inspect the research records.

What is this research study all about?

This study will be conducted in the outpatient setting at Dr van Dyk's consulting rooms at Little Company of Mary Hospital in Groenkloof, Pretoria, and at your home, and will involve two intervention meals for your child / grandchild to eat. The post-prandial blood glucose curve following the two intervention meals will then be monitored using finger prick testing and a blood glucose sensor. We will provide you with two meals that have the same carbohydrate content, but different fat and protein content, for your child / grandchild to eat at dinner time at home on two different weeknights. During the study your child / grandchild will be asked to wear an Enlite continuous glucose monitoring sensor, and after the two dinner meals we will ask you as parent / legal guardian / grandparent to assist in doing two-hourly finger prick tests. The study will be conducted by experienced health researchers trained in the field of type 1 diabetes in children.

The aim of this study is to determine the true post-prandial glycaemic curve and total insulin need for high-fat, high-protein meals in children with type 1 diabetes.

The objectives of this study are:

- To determine the effect of adding fat and protein to carbohydrates in the form of a high-fat, high-protein mixed meal on the post-prandial glucose profile of children with type 1 diabetes.
- To use the post-prandial glucose profiles collected in this study to determine the prandial insulin requirement of children with type 1 diabetes for a high-fat, high-

protein meal based on the amount and timing of insulin given as bolus corrections that were needed in the hours post-prandially.

- To identify participant characteristics that may influence the post-prandial glucose response to a high-fat, high-protein meal.

Why have you and your child / grandchild been invited to participate?

You have been invited to participate because your child / grandchild has type 1 diabetes, and already uses an insulin pump and continuous glucose monitoring sensor as part of his/her diabetes care.

Your child / grandchild has also complied with the following inclusion criteria: is between 4 – 17 years and 11 months old, has a current HbA1C \leq 9.6%, has been on insulin pump and continuous glucose monitoring sensor therapy for more than a month and is not in the remission phase of diabetes.

Your child / grandchild will be excluded if: He or she does not fit the inclusion criteria, is smoking, has coeliac disease or has any condition that could be associated with delayed gastric emptying such as gastroparesis, is using any medication or supplement that could influence gastric emptying, or which can influence digestion or glucose levels, such as glucocorticoids or any oral anti-diabetic drugs such as Metformin for 7 consecutive days in the 3 months prior to the study. Any child who falls ill during the study will be excluded from data analysis. If your child / grandchild recovers and would like to still participate in the study we would gladly allow that if the study is still in its intervention phase by the time of your child's / grandchild's recovery.

What will your responsibilities be?

You as a parent / legal guardian / grandparent will be requested to accompany your child / grandchild to his/her routine doctor's visits to ensure his/her pump settings are at an optimal level and that you are comfortable with using the sensor and know which alarms to disarm and which not. You will also be asked to ensure your child / grandchild eats the two study meals and not take any additional fluid or foods for the two dinners indicated. You will also be asked to do sensor calibration by doing a finger prick on your child / grandchild before, after 30 minutes of eating, and every 2 hours for 10 hours after

the dinner meal for both evenings. This means a finger prick must be done at 17:50 just before the meal, the meal must be eaten at 18:00, the second finger prick must be done at 18:30 and then at 20:00, 22:00, 00:00, 02:00 and the last one at 04:00. If your child / grandchild is older and able to do some of the finger pricks him/herself that is allowed. Please just check up on them to ensure that no tests are missed in the hours after the two meals. Please note it is only for two meals and thus two evenings of doing these additional finger prick tests. You will be asked to inform the research team as soon as the two study days have been completed and to allow us to download the information on the pump as is usually done during your child's / grandchilds' clinical visits.

Your child / grandchild will be expected to: Read through or listen to the researchers explain the study procedure, eat the two meals and adhere to the protocol in terms of additional fluid and foods for the two dinners, wear the pump and sensor continuously for the study duration and allow you to do the indicated finger pricks during the two study nights. The two study nights do not have to be two consecutive nights or even two nights in the same week in order to limit the effect of night-time blood glucose testing on your sleep. We request that the two study nights just be completed within two weeks of one another.

What will the responsibilities of the researcher be?

The researcher will explain the study protocol clearly and also be telephonically available for assistance or enquiry before, during and after the study. The researcher will provide the study meals, additional blood glucose strips and the blood glucose meter, the three Enlite sensors and also the gift voucher once the two study days have been completed and the data collected from your child's insulin pump.

On your request, after data collection, a third party, not the researcher will make your child's / grandchild's individual data available. The anonymous, combined results of the study will be presented to any parent / legal guardian / grandparent who is interested at a support group meeting once the study has been completed for all required participants and the data has been analysed.

Will you benefit from taking part in this research?

The direct benefits for you and your child / grandchild as a participant will be to see the true effect of different dinner meals on your child's / grandchild's post-prandial glucose levels.

The indirect benefit will be that data collected in this study will hopefully give enough insight to formulate easy methods of determining how to use a dual and square wave on your child's / grandchild's insulin pump for future fatty and protein rich meals to ultimately have less high glucose values after these meals and improve his/her overall diabetes care. The data collected will be used to better educate other families with type 1 diabetes on insulin pump therapy as well.

Are there risks involved in your taking part in this research?

There are only minimal risk and inconveniences for you or your child / grandchild to participate in this study. The inconveniences in this study are that you as the parent / legal guardian / grandparent will have to do the 2-hourly finger pricks for 10 hours after the meal; 6 finger pricks after the meal, during the two nights, will affect your sleep for the two nights. The benefits of the data collected from this study outweigh the inconveniences and risks.

What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

Should you or your child / grandchild have the need for further discussions during or after the two study days and glucose monitoring have been completed, an opportunity will be arranged for you to discuss your discomforts with the researcher or other members of the research team. This will also be documented in order to improve planning of future studies in the field. You and your child / grandchild have the right to discontinue the study at any point if you feel the need to do so.

Who will have access to the data?

Anonymity will be maintained as the data collected by your child's / grandchild's blood glucose profiles will not be linked to his/her name, only to gender and age. Upon enrolling in the study, your child / grandchild will receive a number and all data analysis will be done using the numbers, not your child's / grandchild's particulars. A third party will allocate numbers to each child. The researchers will not know which pump downloads belong to which child; only the age and gender of the child will be known to the researchers. On request of the child or his/her parents / legal guardian / grandparents, personalised feedback will be given through the third party. Confidentiality will be ensured by the way the data will be captured and by deleting the digital recordings and downloads once the data has been transcribed. Only the researchers will have access to the data. Data will be kept safe and secure by protecting all electronic downloads with a password. As soon as data has been transcribed it will be deleted from the recorders. Data will be stored for five years.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study but the study meals, 30 blood glucose strips, a blood glucose meter and 3 Enlite sensors will be provided free of charge. If both study days were successfully completed, your child's / grandchild's pump downloaded for the data, your child / grandchild will receive a gift of appreciation in the form of a R200 gift voucher. Different vouchers will be given for the different age groups. This will be given on the day you bring your child's / grandchild's pump for the research team to download the data.

Who will fund this study?

The companies Medtronic and Bayer will be approached to fund the expenses related to this study such as the study meals, printing costs, gift vouchers, blood glucose meters and blood glucose strips.

Is there anything else that you should know or do?

- You can contact Maryke van der Hoogt on 012 460 2700 or 072 369 4865 if you have any further queries or encounter any problems.
- You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl on 018 299 2094 or at carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.
- You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

The findings of the research will be shared with you by Maryke van der Hoogt at one of the usual pump support evening groups and via an email to those who are not able to attend the evening groups.

Declaration by parent / legal guardian / grandparent of participant

By signing below, I, agree to take part in and allow my child to take part in a research study entitled “The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes”.

I declare that:

- I have read this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions to both the person obtaining consent, as well as the researcher and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*)20...

.....
Signature of person obtaining consent

.....
Signature of witness

Declaration by researcher

I, *Maryke van der Hoogt*, declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of researcher

.....
Signature of witness

ADDENDUM B: Child verbal assent form for children aged 4 years – 6 years and 11 months



PARTICIPANT INFORMATION LEAFLET AND CHILD VERBAL ASSENT FORM FOR PARTICIPANTS AGED 4 years to 6 years and 11 months (48 months – 83 months)

TITLE OF THE RESEARCH PROJECT: The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes.

REFERENCE NUMBERS:

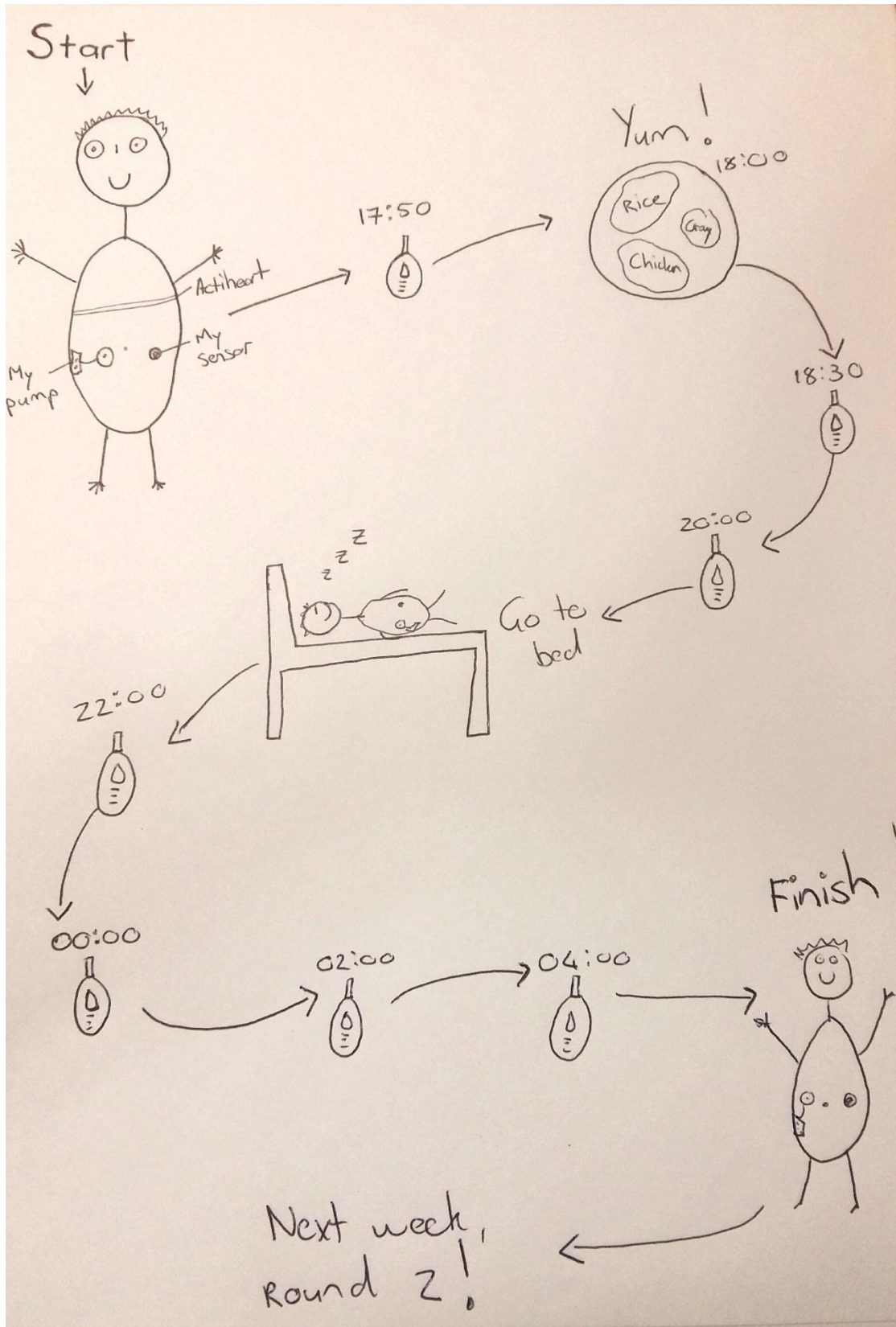
PRINCIPAL INVESTIGATOR: Prof. Marlien Pieters

ADDRESS: Little Company of Mary Hospital, Groenkloof, Pretoria

CONTACT NUMBER: 012 460 9700 / 072 369 4865

We are going to do a study with kids who have type 1 diabetes, like you, and who are also wearing pumps and sensors. The reason why we want to do this study is to see if we are giving you, and other children with type 1 diabetes, enough insulin when you eat foods like meat, chicken, pasta, fish, pizza and so on. You only have to be part of this study if you want to, no one will force you to do it. If you do feel like joining in, this is how it's going to work:

What is going to happen?



What is true and what is not true: (Read out loud to child)

True	Not true
I am going to wear my own pump and sensor the whole time, like usual.	I will take off my pump and sensor.
I am going to get rice, chicken and gravy to eat for 2 nights. It will be in 2 different weeks.	They are going to make me eat Brussels sprouts and snails for 2 nights.
There will be more finger pricks on the 2 nights, but mom or dad will do them when I sleep.	I have to wake up to do more finger pricks on the 2 nights.
I have to finish my food on the 2 nights.	I can leave food behind or eat other food on the 2 nights.
It is my choice if I want to do this, if I don't want to do this I don't have to.	Someone will be mad at me if I say no to doing this.
I will do this at my own home with mom or dad.	I have to come and sleep in hospital for this study.

Do you have any questions?

(Write questions or concerns of child as well as response)

Participant's agreement to enrol in study (tick the box that applies):

Yes

No

Declaration by person obtaining consent

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*)20...

.....
Signature of person obtaining consent

.....
Signature of witness

Declaration by researcher

I, *Maryke van der Hoogt*, declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.

- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....

Signature of researcher

.....

Signature of witness

ADDENDUM C: Child written assent form, children aged 7 years – 13 years and 11 months



PARTICIPANT INFORMATION LEAFLET AND CHILD WRITTEN ASSENT FORM FOR PARTICIPANTS AGED 7 YEARS to 13 YEARS and 11 MONTHS (84 months – 167 months)

TITLE OF THE RESEARCH PROJECT: The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes.

REFERENCE NUMBERS:

PRINCIPAL INVESTIGATOR: Prof. Marlien Pieters

ADDRESS: Little Company of Mary Hospital, Groenkloof, Pretoria

CONTACT NUMBER: 012 460 9700 / 072 369 4865

Introduction

I am going to give you information and invite you to be part of a study. You can choose whether or not you want to be part of this study. This study forms part of my Master's degree, meaning I am also learning while you take part in this study. We have talked to your parent(s)/guardian about this study and they know that we are also asking you for your agreement. If you are going to take part in this study, your parent(s)/guardian also have to agree. But if you do not wish to take part in the study, you do not have to, even if your parents have agreed.

You may discuss anything in this form with your parents or friends or anyone else you feel comfortable talking to. You can decide whether to participate or not after you have talked it over. You do not have to decide immediately.

There may be some words you don't understand or things that you want me to explain more about because you are interested or worried. Please ask me to stop at any time and I will take time to explain.

Why am I doing this research?

As you know, you have type 1 diabetes mellitus, which is a sickness that makes the amount of sugar in your blood too high because there is not enough insulin in your body. To treat your diabetes you use your insulin pump and your sensor to give your body the insulin it needs for food, exercise and other things such as learning and playing. You and your parents are also doing 'carb (carbohydrate) counting' to ensure your insulin pump works out the correct amount of insulin you need for a meal. As you may have seen, sometimes after eating a meal that has a lot of meat, fish or chicken, or maybe a lot of fat, your blood glucose stays high a while after eating that meal, even if you did the carbohydrate counting correctly. It is not your fault that your blood sugar stays high after some meals. What we want to try and do with this study is to work out how much extra insulin children need for fatty, meaty meals so that you and other children with diabetes can still eat these meals in future but hopefully not have the high blood sugar numbers afterwards.

Do I have to do this?

You don't have to participate in this study if you don't want to. It's up to you. If you decide not to participate in this study, it's okay and nothing changes. I will still be your dietician; everything stays the same as before. Even if you say "Yes" now, you can change your mind later and it will still be okay.

What is this research study all about and what is going to happen to me?

You will come to see Dr van Dyk or me during a usual visit. At this visit, and after your doctor confirms that you are able to participate in the study, and only after you and your parent(s)/guardians have read, understood and signed the patient information and parental permission document form, I will ask you, and your parents, if you would like to be part of this study. I will also collect other information from your medical file like your growth chart.

Dr van Dyk will measure in your blood the level of a molecule that tells you how high your blood sugar has been on average over the last 2 to 3 months. This is called the HbA1c test. The measurement will be done by us in this office. A blood drop will be taken from your finger using your own, usual pricker (lancet) and put into a specific device which will give your doctor your blood results in a few minutes; the procedure is the same as for your daily blood glucose tests when you use your blood glucose meter. Results will be then reported in your file.

For the rest of the study I am going to ask you to eat two meals with the same, already known 'carbs' (carbohydrates), so you don't have to do the 'carb counting' yourself. The two meals will be eaten on two different school nights at home; you are not coming to hospital to eat the meals. The meals will consist of rice, a smoked chicken breast and some chicken gravy. On one night the chicken breast and gravy will be a little more than the other night. I am going to ask you to insert a new sensor the days before eating the meals and a new infusion set for your pump on the days of eating the meals. Please wear the sensor for at least 10 hours after the meal, like you would do normally. Please try not to disconnect the sensor or the pump in the night of the study meal. I am also going to ask your mom or dad or guardian to do finger prick tests and put the number into your pump, every two hours after the meal; that will be five tests for each night. You don't even have to wake up for this. Please allow them to do these extra tests just for the two nights of the study.

Will it hurt?

For the HbA1c test the finger prick might hurt for just a second. It might get a little red and might turn bluish. This should go away in a day or so. This will not hurt more than the normal fingerpick that you do to measure your sugar in your blood. The insertion of the sensor, your pump's infusion set and the capillary tests or finger pricks will also be exactly the same as what you are used to. Nothing else will be done to you.

Why are they doing this study?

As you know, sometimes when you eat fatty meals or a lot of meaty foods your blood glucose goes a bit high after the meal and you might struggle to get it down. We are trying to see how much insulin children with diabetes really need for fatty meals or meals with a lot of protein foods like meat and chicken. This is important as it can help us to think of ways to avoid high blood glucose numbers after these meals, and that will mean improving your diabetes care.

Do I get anything for being in this study?

Yes, if you complete the two study days and wear your insulin pump and your sensor correctly, you will get a R200 gift voucher from us to say thank you. Also, the data we collect from this study could possibly help us to work out ways to give insulin for fat and protein and thus help yourself and other children with type 1 diabetes to have fewer high blood glucose readings after eating fatty or protein rich meals. In the long run, if we can manage to have fewer high blood glucose readings after meals, it would help to lessen the chances of developing complications of diabetes later in life.

All information collected about you for this study will be kept confidential (will not be told to anyone not involved in the study) and your name will not be used in study reports. When the study is finished I will write a report about what was learned. The report will not name you or say that you were in the study.

If you sign your name on this paper you agree (and give assent) to be in the study. You will be given a copy of this document to keep after you have signed it. Your

parent(s)/legal guardian will also sign this document in addition to the Patient Information and Informed Consent document that they will sign to give consent on behalf of you to participate in this study.

Declaration by participant

By signing below, I, agree to take part in a research study called “The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes”.

Make sure that you agree with the following things before signing your name on the next page:

- I have read this form or someone explained it to me and I understand it.
- I have had a chance to ask questions to both the person giving me this form, as well as the researcher (Maryke van der Hoogt) and all my questions have been answered clearly.
- I understand that taking part in this study is **voluntary, meaning it’s my own choice**, and I have not been told against my will to take part.
- I may choose to leave the study at any time and this will not affect me in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.
- If I agree to this study it means that;
 - for two nights I will have the dinner given to me by the researcher and not eat other foods that night,
 - I will allow my parents to do the extra finger pricks on these two nights,
 - I will allow the researchers to download my insulin pump when I am done with the two study days

- I will come with my parent / legal guardian to have my pump downloaded and receive my gift voucher if I completed the two study days.

Do not sign below if you do not agree with all 6 dots above. If you do agree and you want to sign and take part in this study, you may sign your name.

Signed at (*place*) on (*date*) 20...

.....
Signature of participant

Declaration by person obtaining consent

I (*name*) declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of person obtaining consent

.....
Signature of witness

Declaration by researcher

I, *Maryke van der Hoogt*, declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of researcher

.....
Signature of witness

ADDENDUM D: Adolescent consent form for children aged 14 years to 17 years and 11 months



PARTICIPANT INFORMATION LEAFLET AND ADOLESCENT CONSENT FOR PARTICIPANTS AGED 14 years – 17 years and 11 months (168-215 months)

TITLE OF THE RESEARCH PROJECT: The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes.

REFERENCE NUMBERS:

PRINCIPAL INVESTIGATOR: Prof. M Pieters

ADDRESS: Little Company of Mary Hospital, Groenkloof, Pretoria

CONTACT NUMBER: 012 460 9700 / 072 369 4865

You are being invited to take part in a research project that forms part of the Master's degree of Maryke van der Hoogt. Please take some time to read this information, which will explain the details of this project. Please ask the researcher any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied and that you clearly understand what every part of this research study entails and how you could be involved. Also, your participation is **entirely voluntary** and you are free to refuse to participate. If you say no, this will not affect you negatively in any way whatsoever. Your level of care or relationship with your diabetes care team

will not be influenced in any way by your decision to participate or not to participate in this study. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the **Health Research Ethics Committee of the Faculty of Health Sciences of North-West University** and will be carried out according to the ethical guidelines and principles of the international Declaration of Helsinki and the ethical guidelines of the National Health Research Ethics Council. It might be necessary for the Health Research Ethics Committee members or relevant authorities to inspect the research records.

What is this research study all about?

This study will be conducted in the outpatient setting at Dr van Dyk's consulting rooms at Little Company of Mary Hospital in Groenkloof, Pretoria, and at your home, and will involve two meals for you to eat. The meals will consist of rice, smoked chicken breast and gravy. The carbohydrates of both meals will be the same, but the fat and protein content will be different. The post-meal blood glucose pattern or curve following the two study meals will then be monitored. We will provide you with two meals and ask you to eat each meal at dinner time at home on a weeknight. During the study you will be asked to wear an Enlite continuous glucose monitoring sensor, and after the two dinner meals we will ask you or your parent / guardian to assist in doing two-hourly finger prick tests five times after each meal.

The study will be conducted by experienced health researchers trained in the field of type 1 diabetes in children.

The aim of this study is to determine the true post meal glycaemic curve and total insulin need for high-fat, high-protein meals in children with type 1 diabetes. The objectives of this study are:

- To determine the effect of adding fat and protein to carbohydrates in the form of a high-fat, high-protein mixed meal on the post-prandial glucose profile of children with type 1 diabetes.

- To use the post-prandial glucose profiles collected in this study to determine the prandial insulin requirement of children with type 1 diabetes for a high-fat, high-protein meal based on the amount and timing of insulin given as bolus corrections that were needed in the hours post-prandially.
- To identify participant characteristics that may influence the post-prandial glucose response to a high-fat, high-protein meal.

This data will be valuable as it could help us to determine ways to avoid getting high blood glucose numbers after fatty, high-protein meals. If we do get answers from this research, other children with diabetes could benefit from this study as well.

Why have you been invited to participate?

You have been invited to participate because you have type 1 diabetes and already use an insulin pump and continuous glucose monitoring sensor as part of your diabetes care.

You have also complied with the following inclusion criteria: are between 4 and 17 years and 11 months old, your most recent HbA1C is $\leq 9.6\%$ and you have been on insulin pump and continuous glucose monitoring sensor therapy for more than a month.

You will be excluded if: You do not fit the inclusion criteria, are smoking, have coeliac disease or have any condition that could be associated with delayed gastric emptying such as gastroparesis, is using any medication or supplement that could influence gastric emptying, or which can influence digestion or glucose levels, such as glucocorticoids for 7 consecutive days in the 3 months prior to the study or any oral anti-diabetic drugs at any time from being diagnosed. If you fall ill during the study you will be excluded. If you recover and still want to participate in the study we will gladly allow it. Please be honest about the exclusion criteria. If you are smoking or using un-prescribed drugs or medications please do not participate in the study.

What will your responsibilities be?

You will be requested to attend your usual doctor's visits to ensure your pump settings are at an optimal level and that you are comfortable with using the sensor and know

which alarms to disarm and which not. You will also be asked to eat the two study meals and not take any additional fluid or foods above that which we allow for the two dinners indicated. You will also be asked to do sensor calibration by doing a finger prick before, after 30 minutes of eating, and every 2 hours for 10 hours after the dinner meal for both evenings. The first finger prick should be done at 17:50, the meal eaten at 18:00, and the remaining finger pricks must be done at 18:30, 20:00, 22:00, 00:00, 02:00 and 04:00. You can ask your parents to do the last 2 or 3 finger pricks of that night when you are asleep. Please note it is only for two meals and thus two evenings of doing these additional finger prick tests. You don't have to do both nights in one week. We will ask that you try to complete both nights in a two-week period at least. You will be asked to inform the research team as soon as the two study days have been completed and allow us to download the information on the pump as is usually done in your clinical visits.

The two study nights do not have to be two consecutive nights or even in the same week in order to limit the effect of night-time blood glucose testing on your sleep. We request that the two study nights just be completed within two weeks of one another.

Will you benefit from taking part in this research?

The direct benefits to you as a participant will be to see the true effect of a different dinner meals on you post (after) meal glucose levels.

The indirect benefit will be that data collected in this study will hopefully give enough insight to formulate easy methods of determining how to use a dual and square wave bolus on your insulin pump for future fatty and protein-rich meals to ultimately have less high glucose values after these meals and improve your overall diabetes care. The data collected will be used to better educate other families with type 1 diabetes on insulin pump therapy as well.

Are there risks involved in your taking part in this research?

There are only minimal risk and inconveniences for you to participate in this study. The inconveniences in this study are that you and/or your parents will have to do the two-hourly finger pricks for 10 hours after the meal, thus five finger pricks, during the two

nights, this will affect your or their sleep for the two nights. The benefits of participating in this study outweigh the inconveniences or risks.

What will happen in the unlikely event of some form of discomfort occurring as a direct result of your taking part in this research study?

Should you feel that you want to talk to anyone during or after the two study days and glucose monitoring have been completed, an opportunity will be arranged for you to discuss your discomforts with the researcher or other members of the research team. This will also be documented in order to improve planning of future studies in the field. You have the right to discontinue the study at any point if you feel the need to do so.

Who will have access to the data?

Anonymity will be maintained as the data collected via your blood glucose profiles will not be linked to your name. You will receive a study number, and the researchers will not know which number belongs to which child. A third party will allocate numbers to each child. The researchers will not know which pump downloads belong to which child, just the age and gender of the child will be known. Confidentiality will be ensured by the way the data will be captured and by deleting the digital recordings and downloads once the data has been transcribed. Only the researchers will have access to the data. Data will be kept safe and secure by protecting all electronic downloads with a password. As soon as data has been transcribed it will be deleted from the recorders. Data will be stored for five years. Data in this term does not mean the pump downloads itself.

Will you be paid to take part in this study and are there any costs involved?

No, you will not be paid to take part in the study but the study meals, 30 blood glucose strips, a blood glucose meter and three Enlite sensors will be provided free of charge. If both study days have successfully completed and if your pump has been downloaded for the data, you will receive a gift of appreciation in the form of a R200 gift voucher. Different vouchers will be given for the different age groups. This will be given on the day you bring your pump for the research team to download.

Who will fund this study?

The companies Medtronic and Bayer will be approached to fund the expenses related to this study such as the study meals, printing costs, gift vouchers, blood glucose meters and blood glucose strips.

Is there anything else that you should know or do?

- You can contact Maryke van der Hoogt on 012 460 2700 or 072 369 4865 if you have any further queries or encounter any problems.
- You can contact the Health Research Ethics Committee via Mrs Carolien van Zyl on 018 299 2094 or at carolien.vanzyl@nwu.ac.za if you have any concerns or complaints that have not been adequately addressed by the researcher.
- You will receive a copy of this information and consent form for your own records.

How will you know about the findings?

The findings of the research will be shared with you by Maryke van der Hoogt at one of the usual pump support evening groups and by an email to those who are not able to attend the evening groups.

Declaration by participant

By signing below, I, agree to take part in a research study entitled “The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes”.

I declare that:

- I have read this information and consent form and it is written in a language with which I am fluent and comfortable.

- I have had a chance to ask questions to both the person obtaining consent, as well as the researcher and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.
- I may be asked to leave the study before it has finished, if the researcher feels it is in my best interests, or if I do not follow the study plan, as agreed to.
- If I agree to this study it means that;
 - for two nights I will have the dinner given to me by the researcher and not eat other foods that night,
 - I will allow my parents to do the extra finger pricks on these two nights,
 - I will allow the researchers to download my insulin pump when I am done with the two study days
 - I will come with my parent / legal guardian to have my pump downloaded and receive my gift voucher when I have completed the two study days.

Signed at (*place*) on (*date*) 20....

.....
Signature of participant

.....
Signature of witness

Declaration by person obtaining consent

I (*name*) declare that:

- I explained the information in this document to

- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of person obtaining consent

.....
Signature of witness

Declaration by researcher

I, *Maryke van der Hoogt*, declare that:

- I explained the information in this document to
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.
- I did/did not use an interpreter.

Signed at (*place*) on (*date*) 20....

.....
Signature of researcher

.....
Signature of witness

ADDENDUM E: Hand-out to parents

Key notes for participating in the study “The effect of protein and fat meal content on the insulin requirement of children with type 1 diabetes”.

- Place a new Enlite sensor on Sunday, calibrate the sensor 3 to 4 times in the first 24 hours. On the study day calibrate **3** times, leave one calibration test for the night. Do not calibrate with *every* night-time test.
- **Do the study days any day from Monday to Thursday, on days 2 to 5 of the sensor. Not on day 1 which is Sunday or on day 6 which is Friday.**
- The study can't be done on a day that your child is sick or if s/he had a severe hypo on this day, severe meaning a third party had to assist with the hypo.
- The meals have to be eaten on a weeknight at dinner time, as close as possible to **18:00**.

The steps for blood glucose testing and bolussing for this night are as follows:

1. **Enter all blood glucose tests into the pump.**
2. Test around 17:00 to allow time for doing a correction bolus for a level above 11 *mmol/L*. Test at 17:50. If the test is between 3.9 and 11 *mmol/L*, proceed with the dinner meal. If the test is still above 11 *mmol/L*, do another correction bolus and proceed with the dinner meal after 30min to 1hour. Again do a blood glucose test before starting the meal. *(Remember in this case that all the post-meal tests will be done later than the indicated times.)*
3. Bolus 10min before starting to eat. Allow the normal correction bolus. Only enter the carbs as indicated on the meal packet. Only use a normal bolus, not a dual or square wave.
4. Try to finish the dinner meal in 20 minutes' time, no longer. Do not give any other food or calorie or caffeine containing drinks for the next 10 hours. Normal amounts of water are allowed. If you have to give food or calorie containing drinks, such as in the event of hypoglycaemia, the study day will have to be repeated and you need not continue with the night-time blood glucose testing on this night. Please inform me of this the next day.
5. Do the **first post-meal test 30 min** after starting the meal, that is at **18:30**. Allow a correction bolus if necessary, although most of the time the active insulin won't allow it.

6. **For the next 5 blood glucose tests** your child does not have to be awake or woken for any of the remaining blood glucose tests. Allow a correction bolus for any of these tests if necessary and any of these tests may be used for calibration if you want to and if it is a stable test. (Not >22.2 mmol/L, not <2 mmol/L, and no arrows on the sensor graph screen.) Remember 3 calibrations in total for the day.
7. Do the **second post-meal test 2 hours** after starting the meal, that is at **20:00**.
8. Do the **third post-meal test 4 hours** after starting the meal, that is at **22:00**.
9. Do the **fourth post-meal test 6 hours** after starting the meal, that is at **00:00 or midnight**.
10. Do the **fifth post-meal test 8 hours** after starting the meal, that is at **02:00**.
11. Do the **sixth and final post-meal test 10 hours** after starting the meal, that is at **04:00**.
12. Two study days, each with a different meal, must be done. They can be done in the same week or the first in one week and the second the following week. Consider who will be doing the night-time blood glucose checks and the subsequent sleep interruption. The easiest may be to do a Monday and Wednesday night, or a Tuesday and Thursday night both in the first week, as long as the exercise done on these days are similar.
13. If you choose to do it over two weeks, a new sensor must be placed on the second Sunday again and the process followed again as explained previously.
14. The two study days can be completed in school or holiday time, as long as the physical activity of the two days are similar and both days are still weekdays, but not Fridays.
15. Return to the clinic after the two weeks and two completed study days, have the pump downloaded and collect the token of appreciation for your child. This is not an appointment and will not be charged or claimed from your medical aid.

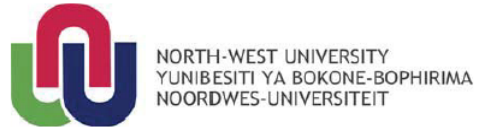
Contact me at any time if you are uncertain about any aspect of this study. Thank you very much for your personal trouble, and for allowing your child to participate in this study. Most of all, thank you for adding time and effort to the research done in type 1 diabetes in children.

Regards

Maryke vd Hoogt

072 369 4865 / 012 469 7900
marykevdh@gmail.com

ADDENDUM F: Ethical approval 2015



Private Bag X6001, Potchefstroom
South Africa 2520

Tel: (018) 299-4900
Faks: (018) 299-4910
Web: <http://www.nwu.ac.za>

Institutional Research Ethics Regulatory Committee

Tel +27 18 299 4849
Email Ethics@nwu.ac.za

ETHICS APPROVAL CERTIFICATE OF PROJECT

Based on approval by Health Research Ethics Committee, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your project as indicated below. This implies that the NWU-IRERC grants its permission that, provided the special conditions specified below are met and pending any other authorisation that may be necessary, the project may be initiated, using the ethics number below.

Project title: THE EFFECT OF PROTEIN AND FAT MEAL CONTENT ON THE INSULIN REQUIREMENT OF TYPE 1 DIABETIC CHILDREN															
Project Leader: Prof M Pieters															
Ethics number:	N	W	U	-	0	0	0	4	2	-	1	5	-	A	1
	<small>Institution</small>			<small>Project Number</small>					<small>Year</small>		<small>Status</small>				
<small>Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation</small>															
Approval date: 2015-07-22				Expiry date: 2016-07-31				Category				N/A			

Special conditions of the approval (if any): None

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The project leader (principle investigator) must report in the prescribed format to the NWU-IRERC:
 - annually (or as otherwise requested) on the progress of the project,
 - without any delay in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the NWU-IRERC. Would there be deviations from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the NWU-IRERC and new approval received before or on the expiry date.
- In the interest of ethical responsibility the NWU-IRERC retains the right to:
 - request access to any information or data at any time during the course or after completion of the project;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the project are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the NWU-IRERC or that information has been false or misrepresented,
 - the required annual report and reporting of adverse events was not done timely and accurately,
 - new institutional rules, national legislation or international conventions deem it necessary.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your project. Please do not hesitate to contact the IRERC for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis

Digitally signed by Linda du Plessis
DN: cn=Linda du Plessis, o=NWU,
ou=Vaal Triangle Campus,
email=linda.duplessis@nwu.ac.za,
c=ZA
Date: 2015.07.27 10:55:47 +02'00'

Prof Linda du Plessis

Chair NWU Institutional Research Ethics Regulatory Committee (IRERC)

ADDENDUM G: Diabetes Centre permission letter

Dr Jacobus van Dyk (Ing)

MB. Ch.B. M Med

Ing No: 2000/016503/21

Pr No: 3201856

(Pediatriese Endokrinoloog / Paediatric Endocrinologist)

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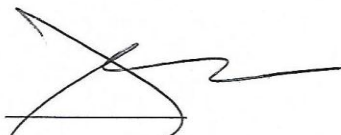
07 May 2015

Re: Permission for conducting the study with protocol title "The effect of protein and fat meal content on the insulin requirement of type 1 diabetic children"

I, Dr JC van Dyk, hereby give permission to Maryke van der Hoogt, MSc Dietetics student and researcher, to conduct the above named study at the outpatient paediatric diabetes centre of Little Company of Mary Hospital.

Recruitment will take place here at the diabetes centre, the intervention phases of the study will be conducted in the home environment of each participant, and data collection (electronic downloads of insulin pumps) will occur at the diabetes centre.

One hundred and fifteen patients who meet inclusion criteria will be recruited at the diabetes centre to allow 35 participants in each indicated age group.



Dr JC van Dyk

