The effect of snacking on continuously monitored glucose concentrations in analogue insulin basal bolus treatment regimens

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DECLARATION

I, Dr. Lukas Johannes Moolman, hereby declare that the dissertation, which I hereby submit for the degree Master of Science in Clinical Epidemiology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at another university.

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Date

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Chapter 1. Background and literature review

1.1. Introduction

According to the International Diabetes Federation it is estimated that more than 371 000 000 people suffered from diabetes mellitus in 2012. During this same period, with the exclusion of the North African countries, an estimated 15 000 000 Africans were living with diabetes. Eighty one per cent of these diabetics were undiagnosed and this figure is expected to double over the next 20 years. This region has also been identified as the region with the highest mortality rate due to this metabolic disease.[1]
The situation in South Africa in 2012 was little different with an estimated diabetes prevalence of 7.04%. This prevalence corresponds to approximately 2 000 000 diabetics of which 1 500 000 were undiagnosed. During this same period 63 000 people died due to diabetes related causes.[1]

1.2 Diabetes mellitus

1.2.1. Definition of diabetes mellitus

Diabetes mellitus is a metabolic abnormality characterized by a chronic hyperglycemia as well as disorders of the carbohydrate, fat and protein metabolism. This disorder is the result of multiple etiologies and is characterized by the abnormal secretion and or action of insulin.[2]

1.2.2. Classification of diabetes mellitus

Diabetes mellitus can be classified according to the underlying etiology. Type 1 diabetes mellitus is the result of an absolute insulin deficiency caused by a cellular-mediated autoimmune destruction of the pancreatic β-cells. A progressive insulin secretory defect, on the background of insulin resistance, results in a relative insulin deficiency that is the hallmark of type 2 diabetes mellitus. The third etiological group is known as the other specific types of diabetes mellitus and includes genetic defects of β-cell function, genetic

defects of insulin action, diseases of the pancreas, genetic syndromes, drug effects and endocrinopathies. Gestational diabetes and overt diabetes of pregnancy is the fourth group and is responsible for an increased risk for complications during pregnancy.[3]

1.2.3. Diagnosis of diabetes

The diagnosis of diabetes mellitus is based on the demonstration of dysglycemia by means of either acute or chronic glycemic markers. Acute markers include fasting plasma glucose concentration, random plasma glucose concentration and 2-hour post glucose tolerance test concentration. The HbA_{1c} is a chronic marker indicative of longstanding dysglycemia and has the advantage of being less influenced by analytic variability.[4]

Criteria for the diagnosis of diabetes[4]

HbA1c ≥ 6.5%
Fasting plasma glucose ≥ 7.0mmol/L
OGGT 2-h plasma glucose ≥ 11.1mmol/L
In the presence of classic hyperglycemic symptoms or a hyperglycemic crisis, a random plasma glucose ≥ 11.1mmol/L

In the absence of symptoms of hyperglycemia or a hyperglycemic crisis, a marker indicative of dysglycemia should be repeated before the diagnosis of diabetes mellitus can be made.[4]

1.3. Complications of diabetes mellitus

1.3.1. Acute complications

Two of the most serious acute complications of diabetes mellitus are diabetic ketoacidosis and the hyperosmolar hyperglycemic state. The fundamental abnormality in both these acute complications is a reduction of the effect of insulin combined with an increase in counter regulatory hormones.[5]

1.3.2. Chronic complications

Patients with all forms of diabetes of sufficient duration are vulnerable to the long-term complications of this metabolic disorder. Chronic complications can be divided into vascular and nonvascular complications and the risk for these complications increases as a function of the duration of the hyperglycaemia.[6]

1.3.2.1. Microvascular complications

Microvascular complications are responsible for significant morbidity and premature mortality. The spectrum of these complications include retinopathy, nephropathy and neuropathy.[2, 5, 6] In both type 1 and type 2 diabetic patients, the risk for microvascular complications can be substantially reduced by intensive glycemic control with multiple insulin injections.[7, 8]

1.3.2.2. Macrovascular complications

After adjustment for other cardiovascular risk factors, diabetes accounts for 75-95% of the excess risk for coronary arterial disease.[2, 9] Due to this excess risk as well as all the other risk factors found in diabetic patients, stroke and myocardial infarction is responsible for the most deaths in type 1 and type 2 diabetics.[10]

Tight glycemic control in type 1 diabetes mellitus has been shown to substantially reduce the risk for macrovascular disease. The effect of glycemic control on the reduction of macrovascular disease in diabetes mellitus type 2 seems to be more modest and of more benefit if treatment is optimized before the onset of atherosclerotic heart disease.[11]

1.3.2.3. Non vascular complications

Other diabetic complications include diabetic foot disease, sexual dysfunction, gastrointestinal disease, skin disease, bone disorders, rheumatic disorders, increased risk of infections and psychological abnormalities.[2]

1.4. Therapeutic considerations

1.4.1. Medical nutrition therapy

The ultimate goal of medical nutrition therapy in diabetic patients is to achieve optimal glucose control, optimize the lipid profile and to prevent the onset and progression of complications. Dietary carbohydrates are the major determinant of the postprandial glucose excursions but unfortunately this food group is an important part of the diabetic diet and cannot be excluded from the daily diet since it is an important source of energy, fiber, minerals and vitamins. Postprandial glucose excursions are influence by both the type and quantity of the carbohydrates consumed. The quantity of carbohydrates consumed is the main determinant of the postprandial response but the type of carbohydrate also plays a role. The recommended daily allowance for carbohydrates is an average of one hundred and thirty grams per day. Making use of the glycemic index, the postprandial glucose excursions due to the different carbohydrates can be quantified. This index is the area under the curve, above the fasting value, for the following two hours after a constant amount of carbohydrate is ingested. This value is then divided by a reference food, like bread, to produce the glycemic index. The glycemic load of a meal is determined by multiplying the glycemic index of the different carbohydrates with the amounts consumed. Lower glycemic index foods tend to reduce the postprandial glucose response and improve glycemic control.[12]

In insulin dependent diabetic patients it is important to match the amount of pre-meal insulin to the amount and type of carbohydrate consumed. Different methods can be used to determine the amount of carbohydrate consumed and the methods include carbohydrate counting, the exchange system and an experience-based estimation. No research to date has demonstrated one method as superior to another and all of these methods can be used for the estimation of the amount of carbohydrates ingested.[12]

The position statement of the American Diabetes Association[12] is not clear on optimal meal frequency in diabetic patients but a study by Delahanty et al[13] showed an increase in HbA_{1c} in patients that consumed night time snacks more than three times per week.[12, 13]

1.4.2. Oral antidiabetic agents

The oral antidiabetic drugs are the first line drugs to be used in type 2 diabetic patients and consist of biguanides, sulphonylureas, meglitinides, thiazolidinediones, gliptins and α-glucosidase inhibitors.[2]

1.4.3. Insulin

1.4.3.1. Different types of insulins

Insulin is an anabolic hormone and in states of insulin deficiency a state of catabolism is induced. The first time a patient was injected with insulin was in 1922 and the first commercial insulin was extracted from porcine and bovine pancreas. These first insulins were effective but impure and were often complicated by immune-mediated side effects. Additional purification steps resulted in mono component insulin with fewer side effects in the 1970's. With the discovery of recombinant DNA technology, human insulin became widely available which virtually eliminated the immune induced side effects.[2]
The biologic action of this injected soluble insulin was 5 to 6 hours and attempts were made to increase the duration of action. With the addition of protamin, a highly basic protein, and zinc the duration of action increased to twenty-four hours. The erratic absorption of this development was later improved by the development of NPH insulin where the protamine and zinc were added in stoichiometric proportions. In 1951 lente insulin followed and was produced by adding zinc in excess in an acetate buffer to produce relatively insoluble crystals. The duration of action of lente insulin was determined by the modulation of the size of the crystals by changing the pH of the solution. These long acting human insulins are used as basal insulin but are often considered undesirable due to their profile of peaks.[2, 14]
Short acting human insulin needs to be injected half an hour to an hour before having a meal because it's onset of action is about twenty to thirty minutes. Due to variability in absorption and a prolonged duration of action, this insulin carries a high risk for the development of hypoglycemic events.[2, 14]

Analogue insulins were developed due to these unfavorable kinetic properties of the human insulins.

1.4.3.2. Short acting analogue insulin

Changing of amino acid positions of the insulin molecule produces the analogue short acting insulins. These changes promote rapid dissociation and the formation of stable monomers allowing for rapid absorption. Absorption of this short acting insulin is within ten to fifteen minutes of a subcutaneous injection with peak activity within thirty to ninety minutes. The duration of action is 4-6 hours. Due to the rapid onset of action, these insulins can be injected immediately prior to the meals.
Several studies examining postprandial hyperglycemia have found analogue insulin to be superior to regular human insulin in lowering these excursions.[2, 14, 15]

1.4.3.3. Long acting insulin analogues

Changes in the insulin molecule promote a longer duration of action. There are currently two of these long acting analogues commercially available. Glargine is produced by three different amino acid changes to the B chain of the insulin molecule. A micro precipitate is formed after subcutaneous injection resulting in a slow release of insulin molecules from the injection depot. Detemir insulin is produced by the addition of a fatty acid to the molecule leading to a prolonged duration of action as well as reversible albumin binding.[2, 14]
Studies comparing the analogue insulins to NPH insulin demonstrated similar glycemic control with the advantages of less weight gain and a reduced risk for nocturnal hypoglycemia with analogue insulins.[2, 14]

1.5. Insulin regimens

1.5.1. Insulin regimens for type 1 diabetes mellitus

Type 1 diabetic patients suffer from an absolute insulin deficiency and require basal as well as prandial insulins. These requirements can be met by 3 different insulin regimens.

With the first insulin regimen patients make use the so-called premix insulins, consisting of a mixture of short acting and intermediate acting insulin.[2, 3, 14] This premix insulin is then injected twice daily according to a rather rigid regimen. Snacks in between meals, leading to weight gain, might be required to prevent hypoglycemic events.[14]

Separating the basal and bolus insulins allows for a more physiologic insulin regimen. Long acting basal insulin, like NPH or analogue insulin, is injected to control the fasting and pre-prandial glucose concentrations. Post-prandial glucose excursions are addressed by the injection of pre-prandial short acting bolus insulin like human regular or analogue insulin. This regimen is a more physiologic regimen with more flexibility than the previous regimen.[2, 14]

A third option in the type 1 diabetic population is the delivery of a short acting analogue via a constant subcutaneous infusion. The device delivers constant basal insulin that can be adjusted to provide different dosages over a twenty-four hour period. Bolus insulin is given at meal times and this can be delivered according to different infusion patterns.

1.5.2. Insulin regimens in type 2 diabetes mellitus

Patients suffering from type 2 diabetes mellitus have varying degrees of insulin deficiency that is progressive over time. These patients will typically progress from oral drugs to the addition of either basal or bolus insulin followed by a twice daily pre-mix or basal bolus insulin regimen.[2,14,15] The addition of basal insulin is necessitated when glycemic control is suboptimal in spite of the oral antidiabetic drugs. NPH insulin as well as the long acting insulin analogues can be used as add on options.[2, 14, 16] Insulin analogues offer equal efficacy in terms of glycemic control but is associated with less hypoglycemia as compared to NPH insulin.[14]

An equally effective approach would be to add prandial insulin to the existing oral antidiabetic agents. When this approach is compared to basal insulin addition it has a higher incidence of weight gain and hypoglycemic events as well as being more complicated.[14]

Twice daily premix insulin addresses both the basal and bolus requirements and is more effective in achieving glycemic targets when compared to basal insulin addition. When glycemic control deteriorates after the initiation of basal insulin, this type of insulin regimen might achieve better results. The down side of this type of insulin is a higher incidence of weight gain and hypoglycemic events when compared to analogue insulin.[2, 14]

Separate basal and bolus replacement is more physiologic and offers the best possible glycemic control.[14]

1.6. Assessment of glycemic control in diabetes mellitus

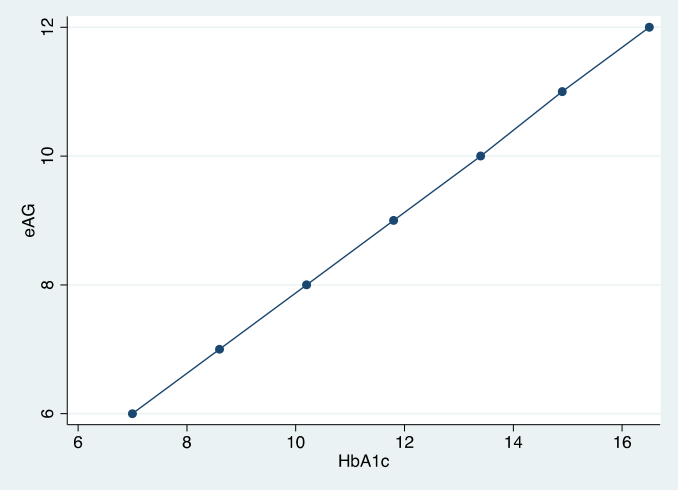
1.6.1. Self monitoring of blood glucose

Self-monitoring of blood glucose has led to a shift in glucose monitoring away from the physician to the patient. This shift in monitoring is important since it has been shown that more frequent self-monitoring is associated with clinically and statistically better control regardless of the type of diabetes mellitus or the therapy prescribed.[17] This self-test is conducted by collecting a microliter or less of blood by means of a finger prick. A test strip impregnated with glucose oxidase, glucose dehydrogenase or hexokinase then analyzes the blood sample. Quantification of the reaction where glucose is converted into gluconic acid and hydrogen peroxidase provides an indication of the blood glucose concentration. Results from these glucometers are not as accurate as laboratory methods but it is accurate enough for home monitoring.[18]

1.6.2. HbA_{1c}

The most widely used clinical test is measurement of glycated hemoglobin (also called HbA_{1c}). Making use of continuous subcutaneous glucose monitoring, it has been shown that there exists a direct relationship between HbA_{1c} and mean glycaemia.[19] This is a weighted relationship giving an estimation of the mean blood glucose during the previous hundred and twenty days.[18] Half of the value of the HbA_{1c} is determined during the month preceding the measurement, forty percent is contributed by the period ranging from day thirty to sixty and 10% by the period ranging from day sixty to hundred and twenty.[20, 21] Due to existence of this relationship, HbA_{1c} levels can be expressed as estimated average blood glucose for most patients with diabetes mellitus.[21]

Translating HbA_{1c} into estimated average glucose (eAG)[3]



A non-linear relationship exists between mean HbA_{1c} and the risks for macro vascular events, micro vascular events and death. There is evidence of the existence of thresholds below which no change in risk is achieved. The threshold for micro vascular events and death is 6.5% and for macro vascular events 7.0%. Glucose control above these threshold values is associated with an increase in risk for complications. A 1% increase in the HbA_{1c} is associated with a thirty eight percent higher risk for macro vascular events, a 40% higher risk for micro vascular events and a 38% higher risk to die.[22]

1.6.3. Continuous subcutaneous blood glucose monitoring

The interstitial blood glucose concentration can be measured by indwelling continuous blood glucose monitors.[23] Two forms of this continuous glucose monitoring exist. Personal continuous glucose monitoring is done with a device owned by the patient and glucose values are continuously visible.[24] The other form of monitoring is known as professional continuous glucose monitoring and the equipment is owned by the health care professional. With this form of monitoring the patient remains unaware of the glucose readings until they are downloaded and analyzed by the health care professional.[24] Unbiased results are the big advantage of this approach and it is used to evaluate the influence of diet, medications and other factors on diabetes control.[25]

A recent study has found that a good correlation (r=0.84) exists between average glucose, measured by continuous interstitial glucose monitoring, and HbA_{1c}. Due to the existence of this relationship, A_{1c} levels can be expressed as estimated average glucose for patients with type 1 and type 2 diabetes mellitus. An HbA1c value of 7% represents an average glucose concentration of 8.6 mmol/L (6.8-10.3) and an increase in the average glucose of 3.2 mmol/L above this value is equivalent to an HbA_{1c} of 9%. In the HbA_{1c} range from 7-9%, an increment of 1% would be more or less equivalent to an average plasma glucose concentration of 1.6 mmol/L and a change of 1.5% in the HbA_{1c} would be more or less equivalent to a change in an average blood glucose concentration of 2.4 mmol/L.[21]

Chapter 2. Rationale for the study and aims and objectives

2.1 Motivation for the study

The development of, especially microvascular, diabetic complications depend on long-term glycemic control as reflected by the HbA_{1c}.[2] The main determinant of the HbA_{1c} is the average blood glucose over the preceding three months.[23] The most physiologic way to control the blood glucose in insulin dependent diabetic patients is by making use of an analogue basal bolus regimen.[14] This regimen consists of basal insulin to suppress hepatic glucose output and mealtime bolus insulin. Due to the relative peak less basal profile and the predictable bolus profile, a basal bolus regimen with analogue insulin is associated with less hypoglycemic events as compared to human insulin.[2] Theoretically, due to the kinetic profile of analogue insulin, omitting snacks between meals should not be associated with hypoglycemic events and should actually be associated with an improvement in the average blood glucose. This improved control would be the result of a reduction in glucose excursions between meals and in addition to this it has been shown that more than three nighttime snacks per week lead to an increase in the HbA_{1c} values.[13]

2.2. Research question

2.2.1. Primary question

Can glucose control be improved if snacks are omitted between meals when treated with an analogue basal bolus insulin regimen?

2.2.2. Secondary question

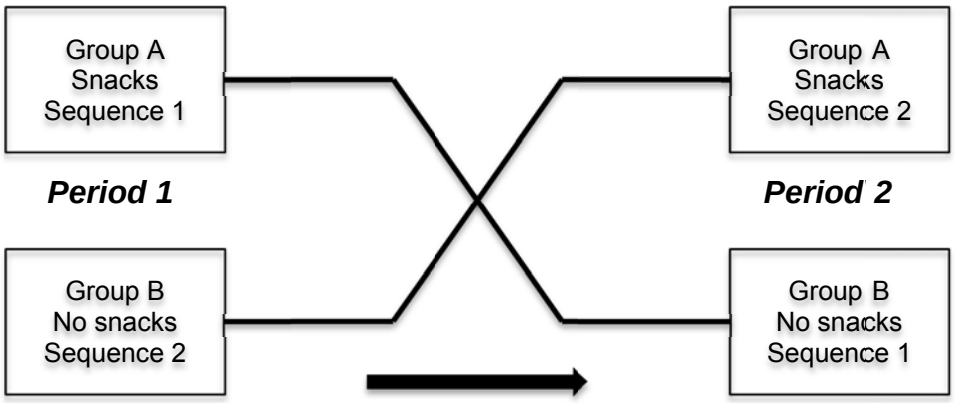
Are there differences in the number of hypoglycemic events, expressed as the hypoglycemic incidence rate, between those patients taking snacks and those not taking snacks.

Chapter 3. Methods

3.1. Study design

This was a clinical trial designed as a crossover study of the AB/BA design.

3.1.1 Schematic presentation



3.2. Setting

The study was conducted in a private internal medicine practice located at the Unitas Private Hospital in Centurion.

3.3. Patient/Research object selection

Existing diabetic patients treated with an analogue basal bolus insulin regimen were invited to participate in this study. The general health status and the presence of diabetic complications were not taken into account.

3.3.1. Inclusion criteria

- Patients had to be older than eighteen years of age

- Both type 1 diabetic patients and insulin dependent type 2 diabetic patients were considered
- Patients had to be treated according to an analogue basal bolus insulin regimen
- Patients had to have basal insulin requirements of equal to or more than twenty units per day
- Patients were required to make use of at least 5 units of bolus insulin per meal
- Informed consent should have been given by the patient

3.3.2. Exclusion criteria

- Patients that suffered from an acute febrile illness
- Patients that were travelling and in the process prevented from following their respective diabetic diets or from following up.
- Patients that were non-compliant in both their dietary and pharmacologic treatment prior to the trial
- Lack of informed consent

3.4. Ethics, informed consent and trial number

The study protocol, number 27/2012, was approved on 01/03/2012 by the research ethics committee of the faculty of Health Sciences of the University of Pretoria.

Patients could only be enrolled in the study after informed consent was obtained.

3.5. Randomization method

Patients enrolled in the study were allocated to the different study groups in an alternating fashion. The process involved the allocation of a patient to one

group with the following patient allocated to the other group. This process was repeated until all subjects were enrolled into the study.

3.6. Intervention

There were two groups of patients receiving analogue insulin according to the basal bolus regimen. The one group started the study by having snacks (A) during the first period followed by omission of snacks (B) during the second period. This was sequence number 1 of the study. Sequence number 2 started of with patients avoiding snacks (B) during the first period followed by the same patients having snacks (A) during the second period. The intervention in this study was the omitting of snacks between meals.
A dietician developed a nutritional plan consisting of three meals. This diet provided an average energy value of six thousand five hundred kilojoules and consisted of more or less fourteen to twenty-one grams of protein and more or less forty-five to fifty grams of carbohydrates per meal. During the snacking period, the patients were allowed to consume low glycemic index snacks that were equivalent to fifteen to twenty grams of carbohydrates. The carbohydrate content of the three meals were also reduced during the snacking phase with the result that the same total daily amount of carbohydrates were consumed during the snacking and non-snacking phases. The patients were allowed to choose one of the following snacks during the snacking phase:

	1	slice of whole-wheat, brown or rye bread
or	3	provitas
or	½	large whole-wheat or a brown bread roll
or	½	slice of brown bread and ½ cup of soup
or	1	whole-wheat scone, muffin, whole-wheat rusk or low GI rusk
or	1	cup of popcorn
or	1	cup of soup

During the non-snacking phase these snacks were then reincorporated into the 3 meals.

3.7. Outcome measure

3.7.1. Interstitial glucose measurement

By making use of an iPro continuous monitoring device, the interstitial glucose concentration was recorded for a total period of 14 days with a changeover at day seven. At the changeover the iPro device connected at day one was removed and a second device connected to the patient. The interstitial glucose concentrations during each of the two periods were thus measured for a period of seven days. Subcutaneous glucose sensors were inserted into the fat layer just under the skin with the aid of an insertion device. The insertion of these sensors was restricted to the abdominal area since the accuracy of the device is based on insertion in this specific area. After completing this step an iPro recorder, which will be recording the glucose levels during the next 7 days, was connected to the sensor. An occlusive dressing was then applied to keep the sensor and recorder in place.
Patients were required keep a log of their meals and they also had to test their blood glucose concentrations at least 4 times during the day. The blood glucose concentration readings were necessary for calibration of the iPro data.
After each seven-day period, the iPro sensor and recorder were removed and the recorder connected to a computer via an iPro dock. The data from the recorder was then extracted and uploaded to the Medtronic website. Once the data was uploaded, the blood glucose measurements had to be added to the patient's data for calibration purposes.
After this whole process various reports could be printed from this website and the one that was used in this study was the daily overlay report. This report provided us with the average interstitial glucose readings as well as the number of hypoglycemic events that occurred during the monitoring period.

3.7.2. Primary outcome

Average interstitial glucose concentration measured over the middle three-days of each seven-day monitoring period.

3.7.3. Secondary outcome

The secondary outcome was the number of hypoglycemic events that occurred during the middle three days of each monitoring period.
A hypoglycemic event was defined as a blood glucose concentration below 3.5 mmol/L. These events were expressed as a hypoglycemic incidence rate for each treatment group.

3.8. Sample size

In a study comparing analogue insulin to NPH and human insulin, the day to day within person variation in plasma glucose for an analogue basal bolus insulin regimen, was found to have a standard deviation of 2.88 mmol/L.[15]
Our study aimed to detect a difference of 2.4 mmol/L between the two groups. This difference is more or less equivalent to a HbA1c change of 1.5% (HbA_{1c} ≤ 9%).
The power to detect this difference was set at 0.8 and a two-sided significance level of α = 0.05 was chosen.

Formula for the calculation of sample size for crossover trials [26]

$$n = \frac{(Z\alpha/2 + Z\beta)^2 2\sigma^2}{\Delta^2} + ½Z^2_{\alpha/2}$$

$= (1.96 + 0.84)^2\ 2\sigma^2 / 5.76 + (1.96)^2 / 2$

$= 7.84 \times 16.58 / 5.76 + 1.92$

$= 22.6 + 1.92$

$= 24.5$

≈25 patients

Twenty five patients were required as total sample size.

3.9. Statistical analysis

3.9.1. Data entry

The data from the iPro transducers were uploaded to the Medtronic website and daily overlay reports for the two treatment periods were then in turn downloaded. The average interstitial glucose concentrations for the two treatment periods were then obtained from these reports and exported to STATA12.[27]

3.9.1.1. Primary outcome

The mean interstitial glucose concentration over a three-day period for both the snacking and non-snacking periods were analyzed and compared by employing the *pkcross* command in STATA. This command makes use of ANOVA models for the analysis of crossover study data.
Because the interstitial glucose concentration data for both the evaluation periods were skew, we log transformed the data in an attempt to improve the distributions towards normality. The log transformed mean interstitial glucose concentration for period two followed a normal distribution but that of period one was still skewed to the right. Although this is a parametric test, ANOVA is a robust analysis with regards to the distribution of the data.[28] In order to get around the influences of data that is not normally distributed, it is suggested that at least 25 participants need to be included for every condition that is being analysed.[28] Due to this characteristic of the ANOVA test we went ahead and utilized this statistical test for the analysis of our data. Before employing the pkcross command the data had to be reshaped by making use the *pkshape* command that was followed by testing of the data for equal variances. Employing the pkequiv command after the reshaping of the data did this.
The results of the ANOVA statistical test were afterwards confirmed by employing a paired t-test. Although the untransformed data for both the snacking and the non-snacking groups were skewed, the log-transformed data was normally distributed and this parametric test could be used

The problem with AB/BA designs is that there is not enough model degrees of freedom available to estimate treatment, period and carry-over effects effectively.[29] To address this problem, Senn[29, 30] in his paper and book on crossover trials, has recommended a sufficient wash out period between the two intervention periods in order to prevent possible carry over effects. In this study we only analyzed the readings during the three days in the middle of the seven-day monitoring period. This design allowed for a wash out period of four days between the two intervention periods (two days from the first period followed by two days from the following period) and we were of the opinion that the study design was sufficient to avoid significant carry over effect. To evaluate the adequacy of our washout period we did a test for carryover effect.

3.9.1.2. Secondary outcome

The number of hypoglycemic events, expressed as the hypoglycemic incidence rate, in both the groups were compared. The data was representative of paired count data and the McNemar test for matched pair data was employed to test for a statistically significant difference between the two groups.

3.9.1.3. Intention to treat analysis

Eleven of the patients in this study ended up with no uploaded data due to a technical problem with the iPro equipment. These patients, where all the data was lost, were included in an intention to treat analysis. Unfortunately no perfect solution exists in dealing with this complex problem.[30] We decided that the problem should be handled by assuming that there was no change between the two periods (period1 - period2 = 0).
Data analysis for this study was conducted in two phases. The first phase of the analysis was done with the successfully downloaded per protocol data and the intention to treat data was analyzed during the second phase. This

approach was followed in order to compare the impact of the missing data on the outcome of the per protocol analysis of the study.

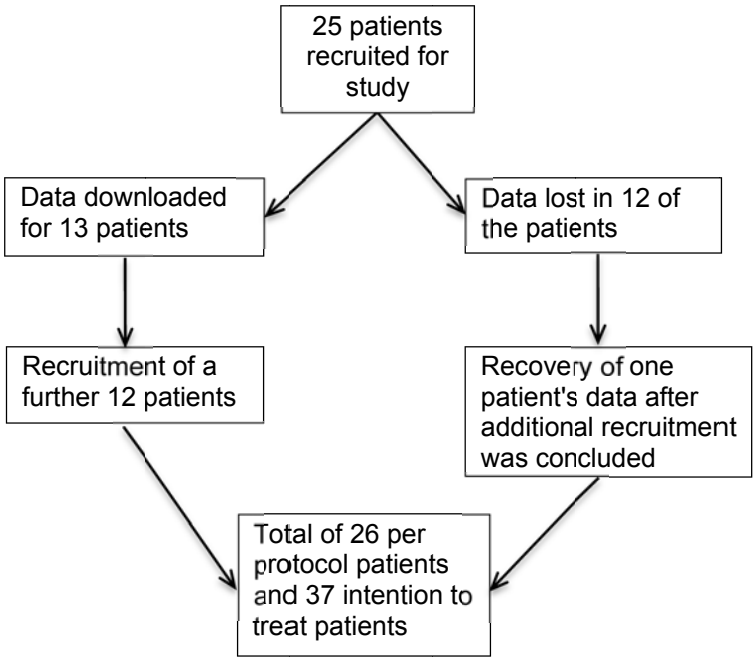
Chapter 4. Results

4.1. Descriptive statistics

4.1.1. Recruitment

Thirty-seven patients, meeting the inclusion and exclusion criteria, were eventually included in the study. During the trial, technical problems with the uploading of the data from the iPro transducers lead to a loss of the data of 12 of the patients. Therefore recruitment continued until the required target of 25 patients with uploaded data was reached. After achieving the target, a further one patient's data was recovered. A total of 26 patient's data were thus downloaded and analyzed successfully.

4.1.1.1. Flow diagram



4.1.2. Patient Demographics, clinical characteristics, glucose concentration data and hypoglycemic events

	Per Protocol	Intention to treat
Sample size	26	37
Average age	47.1	46.7
Sex		
Male	16	23
Female	10	14
BMI	32.5	32.9
Type diabetes mellitus		
Type 1	5 (19%)	8 (22%)
Type 2	21 (81%)	29 (78%)
Insulin dosage		
Long acting(U)	30.1	31.9
Short acting(U)	21.7	22.2
Mean glucose concentration		
Snack(mmol/L)	8.52	8.4
No snack(mmol/L)	7.89	7.95
Difference(mmol/L)	0.63	0.45
Hypoglycemic episodes		
No snack	13	13
Snack	18	18

This table compared the demographics, clinical characteristics, glucose concentrations and hypoglycemic events between the per-protocol and intention-to-treat patient groups. It is evident from this table that these groups were very similar in all regards except for the difference in the mean interstitial glucose concentration between groups. Addition of the patients with lost data to the intention to treat group lead to a reduction in the difference of the average glucose concentration between the snacking and non-snacking groups.

4.1.3. Description of the data

4.1.3.1. Variables

In order to have the data ordered and captured the following variables were created:

Dataset 1 for the analysis of glucose control. Both per protocol and intention to treat datasets were created.

- id: The identification number of the patient
- seq: The AB/BA sequence
    - Sequence 1 is snacking followed by non snacking (AB)
    - Sequence 2 is non snacking followed by snacking (BA)
- period1: The mean glucose for the middle three days of the first period
- period2: The mean glucose for the middle three days of the second period
- snack: The mean glucose for the middle three days of the snacking period
- nosnack: The mean glucose for the middle three days of the the non-snacking period

Dataset 2 for the analysis of the hypoglycemic incidence. Datasets were created for both the per protocol and intention to treat groups.

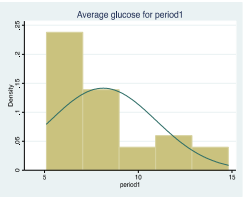
- id: The id of the patient
- snack: The number of hypoglycemic events for the middle three days of the snacking period (A)
- nosnack: The number of hypoglycemic events for the middle three days of the non-snacking period (B)

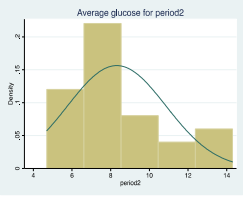
4.1.3.2. Distribution of the average glucose concentration data

4.1.3.2.1. Per protocol data

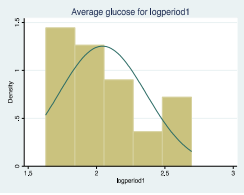
4.1.3.2.1.1. Mean interstitial glucose concentration for analysis with ANOVA

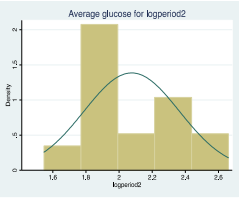
Untransformed data





Log-transformed data

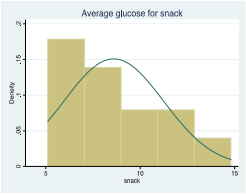


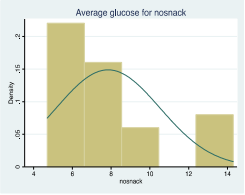


It is evident from the above graphs that the untransformed data was not normally distributed and that log transformation of this data lead to an improvement in the normality of the distribution of this data. The distribution for logperiod2 was normal but that of logperiod1, although improved, was still skewed.

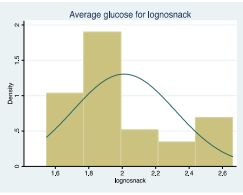
4.1.3.2.1.2. Mean interstitial glucose concentration for t-test analysis

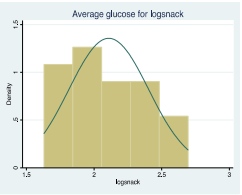
Untransformed data





Log-transformed data



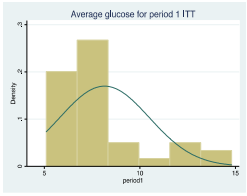


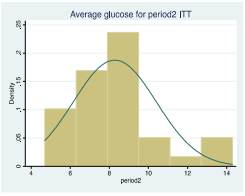
The untransformed data was not normally distributed and log transformation improved the distribution to such an extent that the transformed data was normally distributed.

4.1.3.2.2. Intention to treat data

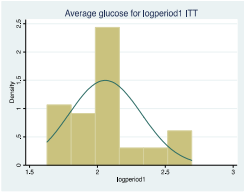
4.1.3.2.2.1. Mean interstitial glucose concentration for analysis with ANOVA

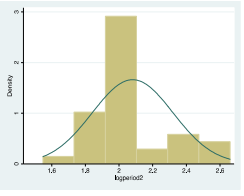
Untransformed data





Log-transformed data

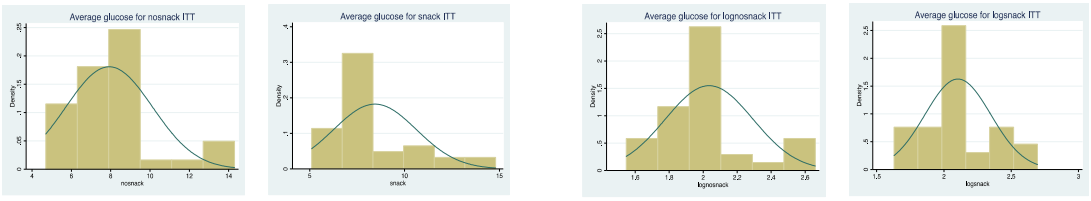




It is evident from the above graphs that the untransformed data was not normally distributed and that log transformation of this data lead to an improvement in the normality of the distribution of this data

4.1.3.2.2.2. Mean interstitial glucose according for t-test analysis

Untransformed data | Log-transformed data



The untransformed data was not normally distributed and log transformation improved the distribution to such an extent that the transformed data was normally distributed.

4.1.3.3. Hypoglycemic events

Hypoglycemic events are an example of paired count data.

4.1.3.3.1. Hypoglycemic events for per protocol analysis

- nosnack group: 0.5/3 day period
- snack group: 0.69/3 day period

4.1.3.3.2. Hypoglycemic events for intention to treat analysis

- nosnack group: 0.35/3 day period
- snack group: 0.48/3 day period

4.2. Analytic statistics

ANOVA					
Per protocol analysis					
	F	Prob>F	Regression coef.	Anti-log	95%CI
Treatment effect	4.99	0.035	-0.074	0.84	0.72-0.99
Sequence effect	0.39	0.54			
Period effect	0.42	0.52			
Carryover effect	0.71	0.40			
Intention to treat analysis					
	F	Prob>F	Regression coef.	Anti-log	95%CI
Treatment effect	4.76	0.035	-0.052	0.89	0.79-0.99
Sequence effect	0.39	0.53			
Period effect	0.39	0.54			
Carryover effect	0.72	0.40			

Paired t-test		
Per protocol analysis		
Log diff	Anti-log	95% CI
-0.076	0.84	0.71-0.98
Intention to treat analysis		
Log diff	Anti-log	95% CI
-0.053	0.89	0.79-0.99

McNemar test for paired count hypoglycemic data		
Per protocol analysis		
Chi2	df	Prob>chi2
6.33	7	0.50
Intention to treat analysis		
Chi2	df	Prob>chi2
6.33	7	0.50

A between group difference to the magnitude of 0.84mmol/L was demonstrated during the per-protocol analysis. The mean interstitial glucose concentration was lower in the non-snacking group as opposed to the snacking group

The p-values for the sequence-, period- and carryover effects were all in excess of 0.05 and we failed to reject the null hypothesis of no difference. No sequence effect, period effect or carryover effect was thus demonstrated for the per-protocol data

The intention to treat analysis also demonstrated a statistically significant difference of 0.89mmol/L between the two treatment groups. As with the-per - protocol analysis, the mean interstitial glucose concentration was lower during the non-snacking period of the study.

No period-, sequence- effect or carryover effects were demonstrated during the intention-to-treat analysis.

The occurrence of hypoglycemic events did not differ between the two treatment groups.

Chapter 5. Discussion and recommendations

5.1. Discussion

A difference in the mean interstitial glucose concentration between the snacking and non-snacking groups was demonstrated during the ANOVA evaluation of the log-transformed per-protocol data. This difference was 0.84mmol/L and the mean interstitial glucose concentration was lower in the non-snacking group as compared to the snacking group. The result of this ANOVA analysis was confirmed by a paired t-test analysis that was also conducted with log-transformed data.

A difference between the groups was demonstrated with the evaluation of the intention-to-treat data. The mean interstitial glucose concentration was 0.89mmol/L lower in the no-snack group as compared to the snacking group. Confirmation of this result, as with the per-protocol data, was done by analysis of the log-transformed intention-to-treat data with a paired t-test. This increase in the difference of the mean interstitial glucose concentration between the per-protocol and intention-to-treat data was not expected. The inclusion of the additional patients, with no difference in mean interstitial glucose concentration between the two periods, was expected to dilute the magnitude of the difference between the two treatment groups. This unexpected result was probably due to the fact that the addition of data with no difference between groups did not lead to a significant change in inter and intragroup variance.

Analysis of both the per-protocol and intention-to-treat data sets for evidence of sequence, period and carryover effect failed to demonstrate the presence of these effects.

This study confirms that the avoidance of snacks, in diabetic patients treated with an analogue basal bolus regimen, is associated with improved glycemic control. The magnitude of the reduction in mean interstitial glucose concentration in the per-protocol group will lead to an improvement of at least 0.5% in the HbA_{1c}. The importance of this can be appreciated when the UKPDS 35 results are reviewed.[31] This study was conducted to determine the association between hyperglycemic exposure and the development of both

microvascular and macrovascular complications. In this landmark study it was found that a one percent decrease in HbA_{1c} lead to a 14% reduction in fatal and non-fatal myocardial infarctions, a 37% decrease in microvascular endpoints, a 43% reduction in amputation or death from peripheral vascular disease, a 12% reduction in fatal and non-fatal stroke, a 19% decrease in cataract extraction and a 16% decrease in heart failure.[31]

The only other article found in the literature that examined the effect of snacking on glucose control was conducted in Finland in 1998. This article examined the effect of a 50% reduction in the carbohydrate content of snacks in patients treated with NPH basal and analogue bolus insulin. The participants in this study were treated according to the basal bolus insulin regimen. In patients compliant with the nutritional advice, of reducing the carbohydrate content of snacks by 50%, the authors demonstrated a 0.25% reduction in the $HbA_{1c.}$[32]

By avoiding snacks between meals, patients were expected to experience more hypoglycemic events. The number of hypoglycemic readings, defined as a glucose concentration of below 3,5 mmol/L, that occurred in each of the two periods were analyzed by the McNemar test. This test failed to demonstrate a statistically significant difference between the two groups and we could therefore concur that the avoidance of snacks was not associated with an increase in hypoglycemic events.

During this trial patients were allowed to consume low glycemic snacks. This is probably the reason for the relatively small difference in glucose concentrations between the two treatment groups. It is possible that the consumption of high glycemic index foods would lead to a larger difference between snacking and non-snacking groups. It would therefore be recommend that if snacks must be taken that it is chosen from the low glycemic index group of carbohydrates.

It can thus be concluded that by avoiding snacks between meals when treated with an analogue basal bolus insulin regimen, an improvement in glucose control without an increase in hypoglycemic events, could be achieved. Patients in clinical practice find it difficult to consume regular snacks in a working environment. The lack of a difference of hypoglycemic events between the two groups provided support for a simpler nutritional plan

consisting of only three meals. A nutritional plan that excludes snacks will make it easier to treat this group of patients.
From this discussion it is evident that avoidance of snacks, a simple and cheap intervention, can lead to improved blood glucose control as well as a reduction in the incidence of diabetic complications.

5.2. Limitations of the study

This study was conducted in a private internal medicine practice and is not representative of the general population. The results obtained can therefore not be extrapolated to the rest of the diabetic population.
Due to technical problems a vast amount of data was lost and the intention to treat analysis had a dilution effect on the magnitude of the difference.

5.3. Conclusion

This study has demonstrated that patients, treated at a private internal medicine practice in Centurion, benefitted from an intervention consisting of the avoidance of snacking between meals when they were treated with an analogue basal bolus insulin regimen.

5.4. Recommendations

Given the results of this study, it would be appropriate to conduct a study that is representative of the general diabetic population. This will enable doctors to have specific guidelines regarding snacking in diabetic patients treated according to an analogue basal bolus regimen.

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