University of Pretoria
Faculty of Health Sciences
School of Medicine

Capillary β – Hydroxybuterate testing in patients with diabetic ketoacidosis

Author: Eluned F Delport
Student nr: 93195738

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Supervisor: Prof P Rheeder
Co-supervisor: Prof DG van Zyl

Contact details:
Department of Internal Medicine
Endocrinology Unit
Steve Biko Academic Hospital

Tel: 012 354 1211
Cell: 082 924 3458
e-mail: eluned.delport@up.ac.za

I, Eluned Florence Delport, ID 7410140072080, hereby declare that this document, submitted for the partial fulfilment for the degree MSc (Clinical Epidemiology) is my original work (except where acknowledgements indicated otherwise). I further declare that the whole work, or any part of it, has not been submitted for another degree at this or other university.

Signed:

2013/10/31

E.F. Delport Date

ABSTRACT:

Introduction: Diabetic ketoacidosis (DKA) is a major life-threatening complication of both type 1 and type 2 diabetes. Insulin treatment for diabetic ketoacidosis is guided using the changes in blood glucose levels, blood gas analysis and urine ketone measurement. Evidence for the use of capillary β -Hydroxybuterate in the monitoring of therapy for diabetic ketoacidosis is not sufficient, and needs to be evaluated in adult patients.

The present study was undertaken to determine if quantitative measurement of β-Hydroxybuterate can simplify the management of diabetic ketoacidosis, and if the correction of hyperketonaemia could predict resolution of diabetic ketoacidosis.

Methods: A prospective, descriptive study, evaluating measurement of capillary β-Hydroxybuterate in patients with diabetic ketoacidosis was performed. The relationship between capillary β-Hydroxybuterate levels and values of pH was assessed during treatment and as a possible end point evaluation for intravenous insulin therapy.

Patients were recruited at two hospitals over a 24 month period. All patients were treated according to a standard DKA management protocol. Data was collected until resolution of DKA and all patients were followed up until discharge.

Results: 54 patients were included in the analysis. The mean age of included patients was 36.4 years (SD 10.32). The relationship between capillary ketones, (β -hydroxybuterate) and pH was explored using non- linear mixed models, fitted with restricted cubic splines. The results from this analysis suggest a complex, time dependant association between pH and measured β - Hydroxybuterate.

Assessing if β-Hydroxybuterate could predict normalisation of pH, was done with logistic regression (with subject-specific intercept and slope) including restricted cubic splines. From this model it is evident that the cut-off values for resolution, using capillary ketones, is definitely time dependent.

Conclusion: This study did not show to a clinically significant, applicable relationship between capillary β-Hydroxybuterate levels and values of pH during treatment of DKA. A single cut-off value for DKA resolution could not be determined, suggesting that β-Hydroxybuterate capillary measurement alone cannot be used as a possible end point evaluation for intravenous insulin therapy.

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Abbreviations

ADA	American Diabetes Association
AIC	Akaike information criterion
BHB	β - Hydroxybuterate
DKA	Diabetic Ketoacidosis
HCO_3	Bicarbonate
ISPAD	International Society for Paediatric and Adolescent Diabetes
POC	Point of Care
SD	Standard deviation
SEMDSA	Society for Endocrinology, Metabolism and Diabetes of South Africa

Chapter 1

Introduction and Literature Review

1. Introduction

Diabetic ketoacidosis is a major life-threatening complication of both type 1 and type 2 diabetes. Delay in the diagnosis and treatment of diabetic ketoacidosis (DKA) contributes to an increase in the morbidity and mortality associated with DKA. [20,21]

During DKA, ketones are synthesised as an alternative source of energy. [24,26,27] Acetoacetic acid is the initial ketone formed, which is reduced to β-hydroxybuterate (BHB) or decarboxylated to acetone. Formation of these ketone bodies leads to the development of a metabolic acidosis which often is severe. Until recently glucose, venous pH and bicarbonate were used to diagnose and monitor response to treatment in DKA.[43,45]

The current diagnostic criteria for diabetic ketoacidosis are non-specific, and limited due to the qualitative, rather than quantitative nature. The measurement of ketones in urine and in blood is done as adjuncts to both the diagnosis and as part of the monitoring of diabetic ketoacidosis. The criteria for resolution of DKA do not include ketone measurement, even though this is often used in clinical practice. Measurements of ketone bodies are indicated in all diabetic patients where the risk of ketotic decompensation exists.[46]

Currently the American Diabetes Association (ADA) recommends the use of blood ketone testing, based on the measurement of β- Hydroxybuterate, rather than urine ketone testing, for the diagnosis and monitoring of diabetic ketoacidosis. The scientific evidence supporting these recommendations is based on expert consensus or clinical experience (level E).[25,60,61]

Although specific measurement of β-Hydroxybuterate is available, further studies are needed to ascertain whether this test offers clinical advantage over existing management approaches (e.g. measurement of serum bicarbonate (s- HCO_3), pH and glucose or urine ketones). BHB measurement can be done at the bedside with a hand held meter, and has been shown to be reliable, correlating very well with laboratory values.

The purpose of this study is to evaluate if measurement of blood ketones, (using capillary BHB), correlates well with values of venous pH levels, and can therefore offer clinical advantage in the management of diabetic ketoacidosis. It also aims to assess if capillary BHB can be used to predict resolution of diabetic ketoacidosis, and therefore be used as an end-point for intravenous insulin therapy.

A blood gas provides information on acid-base balance, but is quite expensive. β-Hydroxybuterate level measurement with a finger-prick hand held device is quick and easy to use, offers rapid results and is less expensive to use than blood gas analysis.[42] If a close correlation of BHB values is confirmed, this could be used to simplify management of diabetic ketoacidosis and be used in a more cost-effective way to monitor treatment and resolution of diabetic ketoacidosis in adult patients. It could then be utilized as an alternative to blood gas analyses, especially in areas where blood gas analysis is not readily available.

2. LITERATURE OVERVIEW

DIABETES MELLITUS

i. Introduction:

Diabetes mellitus is a group of diseases characterized by hyperglycemia. The pathogenic processes leading to the development of diabetes range from destruction of the pancreatic β - cells, with an absolute or relative deficiency of insulin secretion, to abnormalities resulting in resistance to insulin action.[1] Deficient insulin action affects the metabolism of carbohydrates, fat and protein.

Most cases of diabetes can be classified into two groups; type 1 diabetes, where the cause is an absolute deficiency of insulin due to destruction of the β-cells in the pancreas, and type 2 diabetes, caused by a combination of insulin resistance and inadequate insulin secretion.

ii. Prevalence of diabetes:

The prevalence of diabetes is increasing worldwide.[2] The prevalence was estimated to be 4.0% in 1995 and to rise to 5.4% by the year 2025.[3] The global burden of diabetes is expected to rise from 135 million in 1995 to 300 million in 2025. Type 2 diabetes is the predominant form of diabetes in sub-Saharan Africa, accounting for 70 - 90% of cases. [4,5]

In South Africa, diabetes mellitus is a common disease, although there is limited data available on the prevalence. The IDF Atlas estimates that South Africa has about 2.6 million people suffering from diabetes.[3,6]

The incidence of childhood T1DM varies worldwide, with the highest reported incidences of T1DM occurring in Finland and Sardinia. [7] In sub-Saharan Africa, it is estimated that about 10% of diabetic cases are due to T1DM. [7] There is limited

information on the epidemiology of type 1 diabetes in South Africa, [8,9] and it appears to be less common than in Western countries. The low prevalence could however be due to under or misdiagnosis of these patients.

Findings of studies from South Africa suggests that type 1 diabetes mellitus patients in South Africa were older at time of diagnosis (median 22 years) when compared to European patients (median 12 years), and occurred slightly more in females than in males (F:M 1.55:1).[9,10,11]

The reported prevalence of Type 2 diabetes Mellitus in South Africa varies from 3% to 28.7%, as shown in table 1.1.[12] The prevalence differs due to different populations and different age groups in each study.

Table 1.1 The prevalence of Type 2 diabetes in different South African population groups[3]

Population	**Region (Number of participants)**	**Prevalence (%)**	**Age range (years)**
African[13]	Cape Town, urban (729)	8.0	30 +
African[14]	QwaQwa, rural (853) Mangaung, urban (758)	4.8 6.0	25+
African[15]	Durban, urban (479)	5.3	15+
Mixed [16]	Cape Town (200)	28.7	65+
Mixed [17]	Cape Town, peri-urban (974)	10.8	15-86
European [18]	Durban, urban (396)	3.0	15-69
Indian [19]	Durban, urban (2479)	13.0	15+

ACUTE DIABETIC KETOACIDOSIS:

Diabetic ketoacidosis (DKA) is a life threatening complication of both type 1 and type 2 diabetes mellitus, although it is more commonly seen in patients with type 1 diabetes.[20] DKA occurs due to an absolute or relative deficiency in insulin, leading to hyperglycaemia with alterations in fluid and electrolyte balance. DKA usually evolves rapidly, over a 24-hour period. Diabetic ketoacidosis is characterized by hyperglycaemia, hyperketonaemia and a metabolic acidosis.

i. Epidemiology:

DKA occurs most commonly in patients with type 1 diabetes, but may also occur in patients with type 2 diabetes mellitus. The annual incidence of DKA is between 4.6 to 8.0 per 1000 person years among patients with diabetes.[20] Diabetic ketoacidosis occurs more commonly in children and adolescents. Approximately 100 000 hospital admissions for DKA occur in the United States every year, with an average cost of $13 000 per patient per hospitalization. Thus, the annual expenditure for the care of patients who have DKA's may exceed $1 billion.[21]

Before the discovery of insulin in 1921, the mortality of DKA was 100%, but currently the mortality rate is less than 5% in experienced centers.[20] In a previously published Danish study, the mortality was reported to be 4%.[22] In Africa, however, the mortality rate is unacceptably high, with studies from Kenya, Tanzania and Ghana reporting rates of 26 to 29%.[23]

ii. Pathophysiology:

Diabetic ketoacidosis (DKA) is a complex disorder, accompanied by multiple metabolic derangements.[24] DKA results from grossly deficient insulin availability, combined with an increase in counter regulatory hormones such as glucagon, cortisol, catecholamines and growth hormone.[25] This type of hormonal imbalance enhances hepatic and renal gluconeogenesis, impairs glucose utilization in

peripheral tissues and increase glycogenolysis, resulting in severe hyperglycaemia. Further catabolism of muscle and fat is responsible for ketosis and acidosis.[26]

Glucagon plays an important role in initiating hyperglycaemia and ketogenesis.[27] In the setting of insulin deficiency, the action of glucagon on the liver and adipose tissue is unopposed. Increased glucose production from the liver and kidney and decreased cellular glucose uptake results in hyperglycaemia, leading to osmotic diuresis, dehydration and loss of sodium, potassium and other electrolytes.[28] This is often aggravated by vomiting.

A transition from carbohydrate metabolism to protein and fat catabolism takes place, with release of amino acids and free fatty acids into the circulation.[24,25,26] These provide substrates for gluconeogenesis in the liver. Long chain fatty acids derived from triglycerides in the adipose tissue are the main source for ketone body formation in the liver. Increased muscle proteolysis also adds amino acids to the hepatic substrate. Hepatic fatty acid oxidation and ketone body synthesis results in a metabolic acidosis, with marked ketonuria. Ketone bodies include acetone, β-hydroxybuterate (BHB), and acetoacetate. Increased secretion of catecholamines and cortisol can contribute to the increases in glucose and ketoacid production.[27]

Persistent hyperglycaemia causes an osmotic diuresis, leading to severe dehydration, electrolyte disturbances, renal function impairment and worsening of the existing metabolic acidosis. Furthermore, acidosis promotes vomiting, worsening dehydration and renal insufficiency, thus impairing renal compensation for the metabolic acidosis. Life threatening electrolyte disturbances may occur, of which hypokalaemia and hyperkalaemia are most common. The shift of free water from intracellularly to the extra cellular compartment may lead to hyponatreamia.[24]Associated cardiac dysrfhythmias, acute brain injury, pulmonary or cerebral oedema, and thrombotic complications may occur if DKA is not treated promptly.[29,30]

iii. Clinical features and diagnosis of diabetic ketoacidosis:

The most common precipitants for the development of DKA are infections [31-34]and the omission of insulin therapy.[34] Other precipitants include acute myocardial infarction,[31,35] cerebrovascular incidents,[35,36] acute pancreatitis,[37] severe stress or trauma,[31,38,39] alcohol intoxication[40] or drugs.[41]

The clinical picture of a diabetic ketoacidosis evolves quite rapidly, with symptoms that include polyuria, polydipsia and polyphagia, nausea, vomiting and dehydration. Abdominal pain may be present, especially in children.[25,28] Clinical features of dehydration may be present, and patients with severe metabolic acidosis usually have deep, rapid breathing (Kussmaul's breathing).[42] The state of consciousness is variable, ranging from normal awakeness to confusion and finally coma.

In 2003, the American Diabetic Association modified the diagnostic criteria of DKA, with the introduction of severity categories. Table 1.2 depicts typical findings in patients presenting with a diabetic ketoacidosis.[25, 43] These findings can be applied to classify the severity of the diabetic ketoacidosis.

Table 1.2 Diagnostic criteria for diabetic ketoacidosis

	Mild	**Moderate**	**Severe**
Plasma glucose (mmol/L)	>13.9	>13.9	>13.9
Arterial pH	7.25-7.30	7.00-7.24	<7.00
Serum bicarbonate (mmol/L)	15-18	10-14	<10
Urine ketones	Positive	Positive	Positive
Serum ketones	Positive	Positive	Positive
Anion gap	>10	>12	>12
Alteration in sensorium	Alert	Alert/ Drowsy	Stupor/ Coma

Adapted from ADA position statement[25,43]

iv. Treating diabetic ketoacidosis:

The management of a diabetic ketoacidosis should include intravenous fluid hydration, correction of hyperglycaemia with continuous intravenous insulin administration, and electrolyte replacement.[44] A summary of the management of DKA in adults is given in table 1.3.[45]

Table 1.3 Management of patients with diabetic ketoacidosis

	IV fluids	Insulin	Electrolytes	Other
Admission	0.9% NaCl: • 1 litre in the first hour • (15 - 20 ml/kg)	IV bolus: • Regular insulin 0.1-0.15 IU/kg then • Continuous infusion 0.1 U/kg/h • Prepare as 20 U in 200 ml 0.9% saline • For 80kg person: 8 U = 80 ml/h or 80 micro drops/min	**Bicarbonate:** If pH 6.9 - 7.0 • 50 mmol/l NaHCO3 in 200 ml 0.45% saline over 1 h pH < 6.9 • 100 mmol/l NaHCO3 in 400 ml 0.45% saline over 1h	Remember: • Urine MCS • Blood cultures if pyrexial • Chest X-ray • ECG • Subcut heparin • NG tube if comatose • ± U - catheter • O2 if PO2 < 80 mmHg • CVP if > 65 yrs, underlying cardiovascular disease or hypotensive
After 1 hour	Reassess: • Hydration status • s-Na^+ level Continue • 0.9% NaCl if s-Na^+ normal/ low, 250 - 500 ml/h (4-14ml/kg, depending on hydration status) Change to 0.45% NaCl if s-Na^+ is elevated Replace half fluid deficit in first 12 hrs (s-osmolality should not change > 0.3 mOsm/kg)	Increase insulin infusion if glucose does not decrease by 5 mmol/l/h	Repeat 2-hrly until pH > 7.0 **Potassium:** Always check potassium level before starting insulin therapy Replace according to K^+ concentration: • s-K+ >5.0 mmol/l: no K+ supplementation, but check 2 hourly • s-K^+ 3.0 - 5.0 mmol/l: add 20 mmol in each litre of IV fluid to keep serum K+ between 4 - 5 mmol/l • s-K+ < 3.0 mmol/l: add 40 mmol to initial fluid and withhold insulin till K > 3.0 mmol/l	Adequate treatment of underlying disease e.g. MI, infection Monitor and chart: • Glucose: hourly capillary glucose until ≤ 14 mmol/l, then 2-hourly • K+, pH, urine output and ketones, blood pressure and heart rate 4-hourly
Blood glucose < 14 mmol/L	Change to 5% dextrose or 5% dextrose in 0.45% NaCl solution	Decrease infusion by half Then adjust infusion rate 2-hrly based on s-glu level, as follows: • S-glu < 5.6 mmol/l: decrease by 10 ml/h, and give 25 ml of 50% dextrose IV • S-glu 5.6 - 8.9 mmol/l: decrease by 10 ml/h • S-glu 9 - 12.2 mmol/l: no change • S-glu 12.3 - 15.6 mmol/l: increase by 10 ml/h • S-glu> 15.6 mmol/l: increase by 10 ml/h and give bolus regular insulin of 8 IU IV When patient able to eat initiate a multidose insulin regimen. Continue IV insulin 1 - 2 h after SC begun	**Phosphate:** Replace if s-PO_4 < 0.33 mmol/l using: • oral phosphate solution (15 ml tds) • or IV Potassium Phosphate solution (14 mmol in 1L rehydration solution)	

Adapter from Rheeder and Oosthuizen, JEMDSA 2004; 9(1): 22-4 [12]

Severe acidosis is usually reversed by insulin and fluid replacement, but patients with severe acidaemia may benefit from bicarbonate replacement.[25,28] Specific treatment for precipitating factors such as a possible infection should be given if present. Frequent monitoring is needed to evaluate the patient's response to treatment and to assess for the occurrence of complications. Insulin therapy should be continued until diabetic ketoacidosis has resolved.

The criteria proposed by the American Diabetic Association (ADA) for resolution of DKA requires the achievement of a serum bicarbonate level of greater than 18 mmol/L, venous pH of greater than 7.30 and a glucose level <11 mmol/l.[46] The indicators of recovery used in most institutions are a pH greater than 7.3 and urine must be ketone free.[42]

Arterial blood sampling is an invasive and painful procedure, with a slight risk of vascular injuries, thrombosis or haemorrhage.[47] Venous blood sampling is easier, more rapid, and safer with less pain. Results of studies on patients with DKA indicated a good correlation between arterial and venous samples regarding pH values, suggesting that arterial blood sampling can be replaced by venous blood sampling to evaluate pH. [48,49,50] Hypotension may increase acidity of the venous sample, as well as increase the difference between venous and arterial blood gas pH values, though the amount of increase doesn't seem to be significant ($p > 0.05$)[50,51]

Evidence for the use of point-of-care β-Hydroxybuterate measurement, for the determination of DKA recovery, is accumulating. For monitoring capillary BHB, a near patient point - of - care device is available. It is a quantitative and enzymatic test, using the same equipment as for home capillary blood glucose determination, but with specific strips.[52] However, there is currently no evidence detailing a specific value of β-Hydroxybuterate which best agrees with DKA resolution. One previous study uses normalization of β-Hydroxybuterate (levels < 0.5 mmol/l) as an endpoint for intravenous insulin therapy, [54] whereas a second study suggests an end point of pH > 7,3, plus two consecutive β-Hydroxybuterate measurements < 1.0 mmol/l.[55]

KETONE BODIES:

Ketone bodies are produced during states of low carbohydrate availability or intake as a side product of lipolysis.[56] The main ketone bodies are acetoacetate, β-hydroxybuterate and acetone.[24,31] The liver produces acetoacetate during free fatty acid catabolism when glucose is not readily available. BHB is formed in the mitochondria of the hepatocytes, through the reduction of acetoacetate. Acetone is formed by the spontaneous decarboxylation of acetoacetate and is mainly excreted via the lungs, causing the sweet odour on the breath of patients with ketoacidosis.[57]

Ketone bodies are formed as a source of energy for the brain, which cannot use free fatty acids, and other organs. Ketone bodies are converted into energy in these organs, during the process of ketolysis, and then utilized to fuel intracellular metabolic activities. Diabetes is the most common cause of pathologically elevated ketone levels.[56]

i. Measurement of ketone bodies:

Measurement of ketone bodies is indicated in all diabetic patients where the risk of ketotic decompensation exists. Ketones in the urine or blood are usually measured with dipsticks based on the nitroprusside reaction, which only measures acetoacetate and acetone, but not β - Hydroxybuterate, which is the strongest and most prevalent ketone body in DKA.[57]

During diabetic ketoacidosis, the ratio of β - hydroxybuterate to acetoacetate increases from 1:1 to as much as 5:1, making β - hydroxybuterate the most prevalent ketone body, causing acidosis.[24,25] During therapy, BHB is converted to acetoacetate, which leads to the false impression that the ketosis is not resolving when measuring urine ketones.

Specific measurement of serum β- Hydroxybuterate can be used for diagnosis and monitoring of DKA.[26,54,55] Laboratory measurement of BHB is not practical in the management of diabetic ketoacidosis, as it takes too long and is not routinely available.[55] β- Hydroxybuterate measurement can be done by the bedside with a hand held meter, which has been shown to be reliable, with precision testing results varying by no more than 3.2% to 5.9%, and accuracy correlating very well with laboratory values (r = 0.959).[57,58] The presence of ketones can now be detected with finger prick testing, with the results available within thirty seconds.[52,53]Most investigators define normal BHB levels in a diabetic patient as less than 0.5 mmol/l, and the patient is at risk of a ketoacidosis if the level is above 3 mmol/l.[59]

Currently the American Diabetes Association (ADA) recommends the use of blood ketone testing, based on the measurement of β- Hydroxybuterate, rather than urine ketone testing, for the diagnosis and monitoring of diabetic ketoacidosis.[25] In the ADA position statement, it is also recommended that ketones should be tested during acute illness, stress or pregnancy, as well as with persistently elevated blood glucose levels (16.7 mmol/L) or when symptoms of ketoacidosis are present.[60] The scientific evidence supporting these recommendations is based on expert consensus or clinical experience (level E).[25,60,61]

The Joint British Diabetes Societies Inpatient Care Group for the Management of Diabetic Ketoacidosis in Adults has included near patient testing for BHB for the monitoring of treatment of DKA in their guidelines. The evidence for use was however drawn from accumulated professional knowledge and consensus agreement.[26]

The advantages of capillary β- Hydroxybuterate measurement are that it is easy to use, with rapid results[52,53] and it saves cost by eliminating laboratory measurements.[57,62,63] In user performance evaluation studies, lay user results were comparable to trained operator results on using ketone meters. Lay user results were comparable to trained operator results, and the first-time users found the test strip easy to use.[52] This may improve management of patients presenting with a diabetic ketoacidosis.

ii. Use of capillary β-Hydroxybuterate in diabetic ketoacidosis

Evidence is available showing an advantage for capillary ketone determination for detection of patients presenting with diabetic ketoacidosis, where a blood β-Hydroxybuterate value of 3 mmol/L or above predicts its existence with a positive likelihood ratio of 15.[55,59,64]

The use of β- Hydroxybuterate levels to monitor the treatment of diabetic ketoacidosis was evaluated in a protocol of insulin therapy adjustment to treat ketosis according to hydroxybuterate levels, compared to a protocol based on blood glucose levels.[54,55] Based on BHB levels, ketosis cleared approximately fourteen hours earlier than in the conventional regimen. This was, however, not correlated with pH, pCO2 or bicarbonate levels, the current measurements used to evaluate acidaemia.

The value of capillary β-Hydroxybuterate monitoring in the treatment of diabetic ketoacidosis in children was also evaluated.[65] This study showed that the changes in BHB levels correlated strongly with changes in pH, bicarbonate and pCO2. The study concluded that bedside monitoring could be a safe and less expensive alternative to blood gas monitoring in the treatment of DKA in children. The mean age of study participants was 12.1 years, with an interquartile range of 7.1 to 15.1 years.

In a second study [66] it was shown that there is an inverse relationship between serum total CO_2 and β-Hydroxybuterate levels (r = - 0.69). This study was done to assess the severity of DKA in adult patients.[65] These findings were also supported by two other studies, showing statistically significant, but slightly weaker correlations ($r=-0.41$, $p<0.05$[67] and $r=-0.56419$, $p < 0.05$).[68]

There is currently not sufficient evidence that changes in capillary β-Hydroxybuterate levels and changes in pH, pCO_2 and bicarbonate correlate in adult patients with diabetic ketoacidosis, or that it is useful in the monitoring of these patients.

In another study, the clinical application of capillary β-Hydroxybuterate testing in evaluating a new end-point for intravenous insulin therapy in the treatment of DKA was assessed.[54] This study, done in 25 children aged 1 to 14 years, showed that there is acceptable agreement between bedside capillary BHB levels and laboratory measurements. It also concludes that a new treatment end-point of two successive measurements of less than 1mmol/L plus a pH of >7.3 could be used.

Lastly, a study in children and adolescents with diabetic ketoacidosis, reported the use of an end-point for intravenous insulin therapy based on the normalization of blood ketone bodies. It also showed that quantitative determination of β-Hydroxybuterate levels could reduce patient time and costs in an intensive care unit.[69,70]

3. MOTIVATION AND AIM OF THIS STUDY

Insulin treatment, in patients presenting with diabetic ketoacidosis, is generally guided using the changes in blood glucose levels, blood gas analysis and urine ketone measurement. Monitoring of treatment of diabetic ketoacidosis therefore involves expensive laboratory blood work-up, including the blood gas evaluation. A blood gas provides information on acid-base balance, but is quite expensive and not always readily available in South African public hospitals.

β- Hydroxybuterate, the strongest and most prevalent ketone body leading to the ketoacidosis, is rarely measured. Ketones in the urine or blood are usually measured with dipsticks based on the nitroprusside reaction, which only measures acetoacetate and acetone, but not BHB. During therapy, BHB is converted to acetoacetate, which leads to the false impression that the ketosis is not resolving.

The American Diabetes Association recommends the use of blood ketone testing based on the measurement of β- Hydroxybuterate, rather than urine ketone testing, for the diagnosis and monitoring of diabetic ketoacidosis. Evidence for the use of this in the monitoring of therapy for diabetic ketoacidosis is not sufficient, and needs to be evaluated in adult patients.

Although specific measurement of β-Hydroxybuterate is available, further studies are needed to ascertain whether this test offers clinical advantage in adults over existing management approaches (e.g. measurement of serum CO_2, pH and glucose or urine ketones). The present study was undertaken to determine if quantitative measurement of β- Hydroxybuterate can simplify the management of diabetic ketoacidosis, and if the correction of hyperketonaemia, (using capillary BHB measurement) could predict resolution of diabetic ketoacidosis (pH > 7.3 and HCO_3 > 18 mmol/L). β-Hydroxybuterate measurement could then be used as end-point for intravenous insulin therapy in the management of diabetic ketoacidosis.

CHAPTER 2

HYPOTHESIS AND METHODS

1. SUMMARY OF STUDY METHODS

This was a prospective, descriptive study, evaluating measurement of capillary β -- Hydroxybuterate in patients with diabetic ketoacidosis. The aim was to evaluate the relationship between capillary β - Hydroxybuterate levels and values of pH and bicarbonate levels, during treatment of diabetic ketoacidosis.

The secondary aim was to assess if capillary β - Hydroxybuterate could predict resolution of diabetic ketoacidosis, and therefore be used as a possible end point evaluation for intravenous insulin therapy. It could therefore contribute to the monitoring and management of DKA.

Patients were recruited at two hospitals over a 24 month period. All patients were treated according to a standard DKA management protocol implemented at both hospitals. Data was collected until resolution of DKA (serum bicarbonate >18 mmol/L, venous pH >7.30 and glucose level <11 mmol/l). All patients were followed up until discharge.

2. HYPOTHESIS AND OBJECTIVES

OBJECTIVES:

1. Primary objective:

The purpose of this study was to evaluate the relationship between measured capillary ketone levels, using capillary β - Hydroxybuterate, with values of pH and bicarbonate levels, during treatment of diabetic ketoacidosis.

2. Secondary objective:

The secondary aim was to assess if capillary β - Hydroxybuterate values could predict resolution of acidosis (venous pH >7.30) and therefore be used as an end-point for intravenous insulin therapy, in the management of diabetic ketoacidosis.

HYPOTHESIS

1. Changes in capillary β - Hydroxybuterate levels correlate closely with changes in values of pH and bicarbonate levels, and therefore offer clinical advantage in the management of diabetic ketoacidosis.

2. Normalization of β - Hydroxybuterate values can be used to predict resolution of diabetic ketoacidosis, and could be used as an end-point for intravenous insulin therapy in diabetic ketoacidosis.

3. METHODS

3.1. STUDY DESIGN

This was a prospective, descriptive study, to evaluate if measurement of β - Hydroxybuterate levels adds value to the management of diabetic ketoacidosis and as a possible end point evaluation for intravenous insulin therapy.

3.2. SETTING

The study was done at two teaching hospitals, Steve Biko Academic Hospital and Kalafong Hospital, situated in the Gauteng Provence of South Africa. Patients who presented with an acute DKA at these hospitals were recruited for inclusion into the study. Most of these patients came from surrounding referral areas.
The study forms part of a larger study on DKA management by Prof. D.G. van Zyl. Patients selected for this larger study (who fulfilled the inclusion and exclusion criteria and where informed consent was obtained), were included in this study.

3.3 PATIENT SELECTION

3.3.1 Sample selection:

Patients with an acute diabetic ketoacidosis, who presented at either Steve Biko Academic hospital or Kalafong Hospital, and who fulfilled the inclusion and exclusion criteria, were recruited for inclusion into the study.

Subjects were given an information leaflet and had to give informed consent prior to enrolment into the study (Addendum 1).

3.3.2 Subject Inclusion criteria:

1. Previously known or newly diagnosed diabetes mellitus (Type 1 or type 2)
2. Older than 18 years of age
3. Venous pH at presentation between 6.9 and 7.2
4. Presence of urinary ketones on dipstick > 2+
5. Capillary blood glucose or venous blood glucose > 13 mmol/L
6. Verbal informed consent given by the patient or proxy consent by a relative if the patient was unable to give consent*

* A patient was considered unable to give consent if the Glasgow coma scale was less than 15/15 or if the patient was considered to be in a mental unsuitable condition to make an informed decision.

3.3.3 Subject exclusion criteria:

1. Patients not complying with the inclusion criteria
2. Acidosis due to other causes than DKA
3. Patients declining verbal consent
4. Patients unable to give consent, with no family member present to give proxy consent (See * above)
5. Need for cardiovascular supportive agents
6. Need for ventilatory support

3.4 MEASUREMENTS

Background clinical characteristics, diabetes type (Type 1, Type 2 or unknown) and DKA triggering factors were recorded for each patient.

Observations were done according to the determined study schedule and recorded onto a precompiled data sheet (see Addendum 2).

All blood samples were taken according to a predetermined study schedule (see Addendum 3).

Capillary glucose, capillary β - Hydroxybuterate, pH, pCO_2 and bicarbonate (HCO_3) levels were measured at baseline (time 0), and then after one hour. Further measurements were done according to the last pH value, as indicated by the schedule:

- Baseline (time 0)
- Time 0 + 1hour
- Then according to pH:
  - pH 6.9 – 7.2 repeat after 3 hours
  - pH 7.21 – 7.3 repeat after 2 hours
  - pH 7.31 – 7.33 repeat after 1 hours
  - pH 7.34 – 7.36 repeat after 30 min
  - pH > 7.36 stop measurement

Laboratory values for serum sodium, potassium, chloride, CO_2, urea and creatinine was done at baseline, after 1 hour, and again when pH > 7.36.

Serum calcium, magnesium phosphate, albumin and protein were done at baseline and again after pH > 7.36.

Urine ketone measurements were done at baseline, at pH level between 7.21 – 7.3 and when pH > 7.36.

Ketone measurement:

Capillary ketone levels (β - hydroxybuterate) were measured with a Medisense Optium glucose ketone meter (Abbott Laboratories). For ketone measurement, 5 micro litres of blood is needed per measurement.

The assay range is 0.0 to 6.0 mmol/l. Precision testing shows that results vary by no more than 3.2% to 5.9%. Accuracy correlates very well with laboratory values (r = 0.959).

Ketone bodies in the urine were assessed with a semi quantitative reagent strip, using Combur 9 urine dipsticks (Roche diagnostics).

pH, bicarbonate and pCO_2 measurement:

Venous blood pH, bicarbonate and pCO_2 measurements were done on a Copenhagen Radiometer ABL 700 blood gas analyser (Steve Biko Academic Hospital and Kalafong hospital) or Cobas B221 blood gas analyser (Steve Biko Academic Hospital).

The analyser measures pH, bicarbonate and pCO_2 levels and calculates temperature-adjusted values.

The analyser is calibrated automatically on a fixed 6 hourly schedule. Reagents are checked on a daily basis and replaced if necessary. Each measurement requires 85 micro litres of heparinised blood.

Glucose measurement:

Capillary glucose measurements were done using an Accu-check Active glucometer and testing strips (Roche Diagnostics). Only 1 micro litre of blood is needed per measurement.

The glucometer has an accuracy of r = 0.987. Precision, as indicated by the reported coefficient of variance, ranges from 2%, at 7.44 mmol/L, to 1% at 26.1 mmol/L glucose.

Urea, creatinine, electrolytes:

All blood tests were analysed at the local National Health Laboratory Service of each of the hospitals, using an automated Beckman-Coulter DXC

Determining resolution of diabetic ketoacidosis:

The criteria proposed by the American Diabetic Association for resolution of DKA were used.

This requires fulfilment of all three criteria, namely:

a. Serum bicarbonate >18 mmol/L
b. Venous pH >7.30
c. Glucose level <11 mmol/l

A standard management protocol was used at both hospitals. All patients were followed up until discharge.

4. DATA MANAGEMENT

Data capturing

Audit data was recorded on a precompiled data sheet (Addendum 2 and 3). The data was then captured on a Microsoft Access database. All the data was recorded and captured anonymously.

Data cleaning and editing was done in Excel, from where it was transferred to STATA 12 (Stata Statistical Software: Release 12, StataCorp 2011), SAS version 9.2 (SAS Statistical software analysis, SAS Institute Inc 2011) and R statistical software (R project for Statistical Computing, version R 3.0.0, R-LME4 package; R Foundation 2013) for statistical analysis.

Statistical analysis

Baseline descriptive statistics, for all subjects included in the study were evaluated using STATA 12. Quantitative variables were presented as the mean and standard deviation (SD).

The relationship between β-Hydroxybuterate levels and pH over time were explored using linear mixed models, fitted with a random intercept term and random slopes. This takes into account the within individual correlation, by allowing the intercept and slope to vary for each individual. This was done using the mixed procedure in SAS (proc mixed in SAS)

Two outliers were removed from the analysis, since they were unusual clinical cases that took much longer too respond compared to the other patients, for unknown reasons unrelated to this study.

The above mentioned models were then fitted with restricted cubic splines included, since the relationship between pH and β-Hydroxybuterate was non-linear.
The non-linear mixed model procedure in SAS was also used (nlmixed), with pH as the response variable and capillary ketones and time as the explanatory variables.

Further analysis was performed to investigate whether capillary β-Hydroxybuterate could predict normalization of pH. The relationship between pH and capillary ketones was evaluated, using R statistical software, LME 4 package. pH was analysed as a binary outcome, where pH = 0 if the pH </= 7.3 or pH = 1 if the pH > 7.3. For this analysis, a logistic regression model was used, again taking the random effects into account, by allowing the intercept and slope (time) to vary for each individual.

5. ETHICAL CONSIDERATIONS

The ethical principles of the Declaration of Helsinki were followed, and all patients gave written informed consent to participate in the study.

Ethics committee:

The protocol was approved by the Ethics committee of the Faculty of Human Health Sciences of the University of Pretoria.

Protocol number: s274/2007 (see Addendum 4)

Informed consent:

All eligible patients with diabetic ketoacidosis were approached for inclusion to the study done by Prof. D.G. van Zyl. Patients had to give informed consent prior to enrolment into the study. Informed consent to the bigger study included informed consent to participate in this study.

All information obtained in this study is regarded as strictly confidential. Reported data does not include any information which identifies the participants of the study.

CHAPTER 3

Results of Study

A total of 129 patients presented to the study hospitals during the study period, and were screened for possible inclusion to the study. Of these 129 patients, seven declined participation to the study and 65 patients did not fulfilled all the in or exclusion criteria. At the end of the study, 57 patients were enrolled with diabetic ketoacidosis. All the patients were over 18 years of age. DKA was diagnosed using the 2003 American Diabetes Association criteria.[25]

Five patients were enrolled with a pH between 7.2 and 7.29, the other 52 had a pH < 7.2. All other inclusion criteria were fulfilled in participating patients. Three patients were excluded from the data analysis, due to missing data.

Figure 3.1 represents the study process schematically

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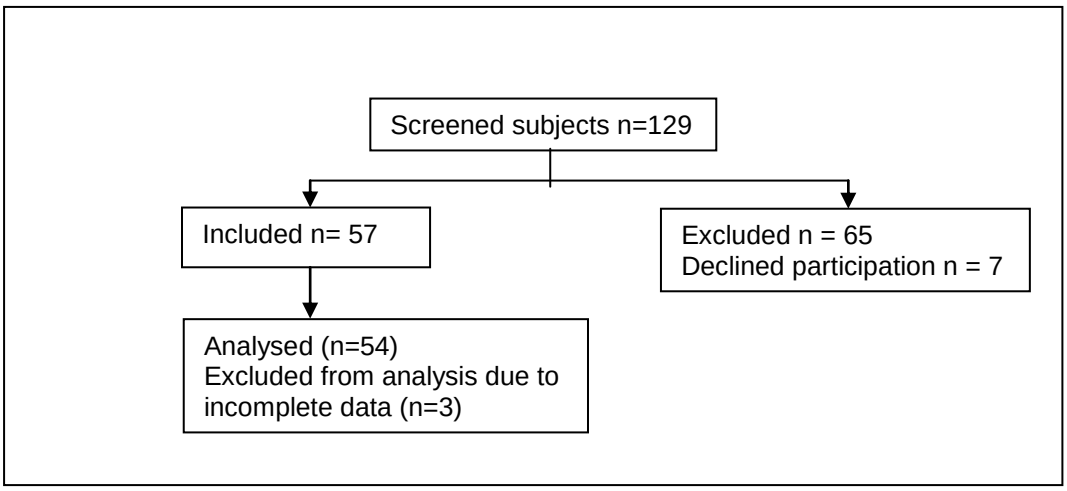

Figure 3.1: Schematic presentation of study process

All patients included received treatment according to the current diabetic ketoacidosis treatment regimen implemented at Steve Biko Academic Hospital and Kalafong hospital.[12] The specific treatment protocol is based on the intravenous administration of fast-acting insulin, with the purpose of reducing the hyperglycaemia.

During the study period, six hypoglycaemic events, with blood glucose < 3.5 mmol/l, were recorded. All the patients were followed up until resolution of the diabetic ketoacidosis. The mean in hospital duration for the patients was seven days. No deaths occurred during the study.

1. Baseline and descriptive results

Data from 54 of the enrolled patients were included in the final analysis. Of these 54 patients, 22 were enrolled at Steve Biko Academic Hospital and 32 at Kalafong Hospital.

Table 3.1 presents the baseline characteristics of the patients included in the study.

Table 3.1 Baseline characteristics of study population (numbers or mean (SD))

Variable	Study population n = 54
Gender (M/F)	31/23
Age (Years)	36.4 (10.32)
Newly diagnosed	22
Type of diabetes • Type 1 DM • Type 2 DM • Secondary diabetes • Uncertain	 27 4 1 22
Identifiable precipitant • Non-compliance • Infection • Other	30 14 11 5
Severity of DKA • Mild • Moderate • Severe	 5 39 10
Baseline results • Capillary glucose (mmol/l) • Capillary ketones • pH • HCO_3 (mmol/L)	 25.9 (8.28) 4.41 (1.38) 7.11 (0.09) 7.31 (3.53)

Mean values and standard deviations are presented unless otherwise stated

The mean age of the included patients was 36.41 years (SD 10.32). Thirty-one male patients (57.4%) and twenty-three female (42.6%) patients were enrolled into the study. Twenty two of these patients were newly diagnosed with diabetes.

Ten patients were classified as having a severe diabetic ketoacidosis, 39 had a moderate DKA and five patients had a mild DKA. In 30 of the patients an identifiable precipitant was present, of which non-compliance was the most common (14 patients), followed by an underlying infection (11 patients).

On admission, the average blood glucose was 25.9 (SD 8.28) mmol/l, the average capillary ketones was 4.41 (1.38) mmol/l and the average pH was 7.11 (0.09).

The median time to reach a venous pH of 7.32 for the 0.9% sodium chloride solution was 683 minutes (SD 694.40), with the longest time for an individual to normalize pH (pH > 7.3) was 3210 minutes.

2. Evaluating relationship between capillary ketones and pH

The relationship between capillary ketones, (β-Hydroxybuterate) and pH was explored using linear mixed models, fitted with a random intercept term and random slopes, and also with a quadratic time effect (time x time). This allows comparison of changes in response variables over time and also accommodates repeated measurements and possible within-individual correlation.

A new variable for time was created such that time = time/1000. Two outliers were removed from the analysis, since they were unusual clinical cases that took much longer too respond compared to the other patients, for unknown reasons unrelated to this study.

Capillary ketones, as the explanatory variable and pH as the outcome variable was used to model the relationship.

The following models were evaluated:

Model 1: No Random Effects added

Model 2: Random Intercept included

Model 3: Random Intercept and Slope (Time) included

Model 4: Random Intercept and Slopes (Time), including quadratic time effect (Time x Time)

Mixed models were used to take into account the correlation between repeated measurements belonging to the same individual. This model also takes into account the natural heterogeneity of individuals, allowing for different pH levels at initial presentation (random intercept), and also different response rates among individuals over time (random slope).

Testing model assumptions

Testing the need for the random intercept, random slopes and random quadratic time effect was done using a mixture of chi-square distributions as seen in table 3.2.

Table 3.2 Testing model assumptions (1)

Model	Effect	Log Likelihood
Model 1	No Random Effects	-933.0
Model 2	Random Intercept	-1146.1
Model 3	Random Intercept and Slope (Time)	-1271.00
Model 4	Random Intercept, Slopes (Time) and quadratic time effect (Time x Time)	-1344.00

A likelihood ratio test using a mixture of chi-square distributions was further used to test the need of the random quadratic time effect and random linear time effect as shown in table 3.3 (p-value < 0.01).

Table 3.3 Testing model assumptions (2)

Models	Effect	DF	p-value
Model 4 vs. Model 3	Random Intercept and Slopes (Time and Time*Time) versus Random Intercept and Slope (Time)	3 , 2 respectively	<0.01
Model 3 vs. Model 2	Random Intercept and Slope (Time) versus Random Intercept	2, 1 respectively	<0.01

Model results

Model 4 was shown to have the best fit, (see table 3.2 and table 3.3) with all terms being statistically significant (p<=0.05). This model, with a random intercept and slopes, including random quadratic time effect (time x time) was used. This indicates the variability of the pH as a function of time.

Using this model, the results are as follows:

Table 3.4 Model results

Effect	Estimate	Std Error	DF	t Value	Pr > |t|
Intercept	7.1554	0.019	53	384.54	<.01
Capillary ketones	-0.00835	0.01	269	-3.28	0.02
Time	0.4659	0.04	52	11.82	<.01
Time x Time	-0.2684	0.03	51	-9.18	<.01

Predicting pH

Using the results from the above model, pH could therefore be predicted with the following formula:

Predicted pH = 7.1554 + 0.4659 x (time) - 0.2684 x (time x time) - 0.00835 x capillary ketones

The above formula indicates the complex association between pH and of β-Hydroxybuterate during treatment of patients with diabetic ketoacidosis. It further characterizes the non- linear relationship, which changes over time.

Figure 3.2 graphically depicts the observed pH over time in the data set (a), and the predicted pH over time calculated using the derived formula (b).

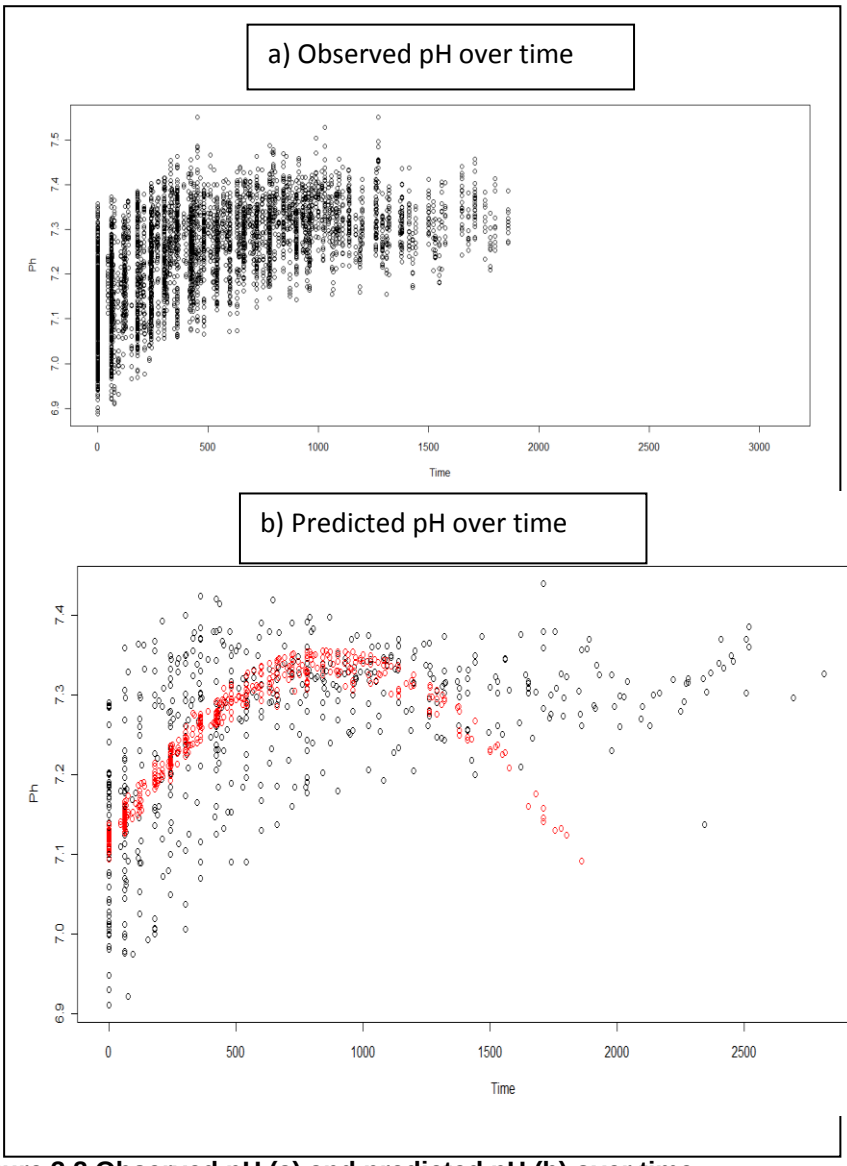

Figure 3.2 Observed pH (a) and predicted pH (b) over time

From the displayed graphs, it can be visualized that the derived formula does not accurately describe the relationship between pH and β – Hyroxybuterate over time. The graph suggests that a spline function would better define the relationship over time.

Restricted cubic splines:

Restricted cubic splines can be used to model complex non – linear relationships between variables over time.

A spline is a function of a continuous variable which allows the slope representing the variable to change at specified values (knots). The knots indicate where a change in slope will occur, splitting the timescale, to allow a linear relationship of the studied variables in the area between two knots.

Since the relationship between pH and capillary ketones was non-linear, a non-linear mixed model, with random intercept and random slopes effect was fitted including restricted cubic splines for the time variable, thus creating a new model, model 5.

pH was used as the response variable and capillary ketones as the explanatory variable.

Knots were introduced on the x-axis located at specific times. The number of knots (2, 3, 4 or 5) were tested by comparing the Akaike Information Criterion (AIC) of each model. The model containing three knots returned an AIC of -1233, and was therefore found to be the best. (Table 3.5)

Table 3.5 Comparing number of knots in models with cubic splines:

Number of knots	AIC	Log likelihood
2	-1222.4	
3	-1233	-1249
4	-1224	-1240
5	-1221	-1237

New variables time1 and time2 were created, indicating time periods in between knots where linear relationships between variables were observed.

Model 5: Linear mixed model, with random intercept and random slope (restricted cubic splines).

Using this model containing 3 knots, the parameter estimates were as follows: (See Table 3.6)

Table 3.6 Parameter Estimates

Effect	Estimate	Std Error	DF	t Value	Pr > |t|
Intercept	7.1599	0.019	52	399.51	<0.01
Capillary ketones	-0.00673	0.003	52	-2.64	0.01
Time1	0.3234	0.023	52	13.99	<0.01
Time2	-0.3362	0.052	52	-6.48	<0.01

From the table above we see that adding the additional terms improve the fit of the model, with all terms being statistically significant (p<=0.05).

Figure 3.3 shows the result of the fit we get from the model

Predicting pH

Using the results from the above model, pH could therefore be predicted with the following formula:

Predicted pH = 7.1599 - 0.00672 x (capillary ketones) + 0.3234 x (time1) - 0.3362 x (time2)

This formula which includes cubic spline variables, again suggests a complex, non-linear relationship between pH and of β- Hydroxybuterate (capillary ketones), during treatment of patients with diabetic ketoacidosis. It also indicates that this relationship changes over time.

Adjusted for time, this can be interpreted as follows:

> For every one unit increase in capillary ketones, pH decreases by 0.00672 (p-value=0.01)

Figure 3.3 displays the graphs, depicting observed and predicted pH over time, using the above formula from model 5 (including restricted cubic splines)

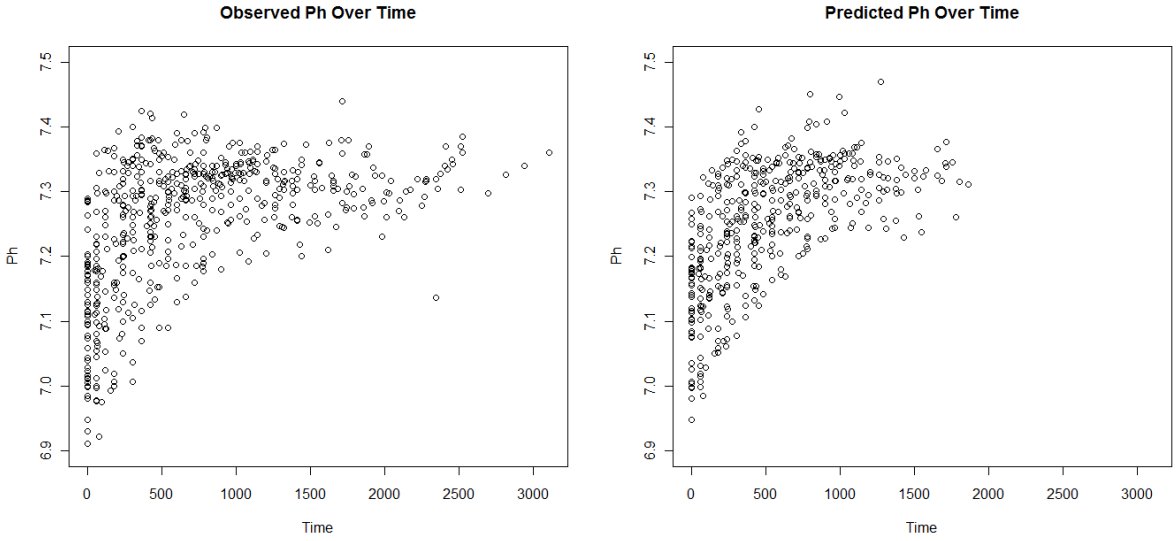

Figure 3.3 Observed pH over time and predicted pH over time

From these graphs, an improved prediction of pH can be seen when the formula derived from Model 5 is used.

Calculating pH using model 5

Using this model: pH = 7.1599 - 0.00672 x (capillary ketones) + 0.3234 x (time1) - 0.3362 x (time2)and using 4 randomly selected patients from the dataset, the pH value for each time point can be calculated, as shown in the tables 3.7 to 3.10.

Table 3.7: Prediction using Model 5 (PID=12)

Actual pH	Capillary ketones (mmol/L)	Time (minutes)	Calculated pH	95% CI
7.203	6.4	0	7.117	7.089 ; 7.145
7.293	2.0	248	7.225	7.200 ; 7.251
7.371	3.1	360	7.252	7.228 ; 7.276

Table 3.8: Prediction using Model 5 (PID=13)

Actual pH	Capillary ketones (mmol/L)	Time (Minutes)	Calculated pH	95% CI
7.263	7.3	0	7.111	7.081 ; 7.140
7.180	5.2	60	7.144	7.119 ; 7.170
7.300	5.4	120	7.162	7.137 ; 7.188
7.260	5.8	240	7.197	7.172 ; 7.223
7.211	4.8	360	7.240	7.215 ; 7.266
7.189	1.9	480	7.294	7.268 ; 7.319
7.230	5.8	780	7.337	7.300 ; 7.375
7.317	3.6	1050	7.398	7.355 ; 7.442
7.342	3.7	1140	7.410	7.362 ; 7.458
7.347	1.9	1200	7.430	7.382 ; 7.478
7.365	2.3	1260	7.434	7.382 ; 7.486

Table 3.9: Prediction using Model 5 (PID=17)

Actual pH	Capillary ketones (mmol/L)	Time (Minutes)	Calculated pH	95% CI
7.094	6.3	0	7.118	7.090 ; 7.145
7.104	6.6	112	7.152	7.124 ; 7.179
7.194	4.8	206	7.194	7.169 ; 7.218
7.165	5.3	346	7.233	7.207 ; 7.259
7.185	4.6	526	7.288	7.260 ; 7.315
7.218	3.7	706	7.336	7.306 ; 7.367
7.240	2.1	801	7.366	7.335 ; 7.398
7.253	3.2	942	7.384	7.346 ; 7.422
7.273	2.5	1062	7.407	7.365 ; 7.450
7.282	1.4	1191	7.432	7.385 ; 7.479
7.311	0.6	1311	7.451	7.398 ; 7.504
7.317	0.6	1371	7.458	7.401 ; 7.514
7.323	0.9	1521	7.469	7.403 ; 7.535

Table 3.10 Prediction using Model 5 (PID=35)

Actual pH	Capillary ketones (mmol/L)	Time (Minute)	Calculated pH	95% CI
7.149	4.3	0	7.131	7.104 ; 7.158
7.179	4.1	45	7.147	7.121 ; 7.173
7.270	3.3	253	7.218	7.194 ; 7.242
7.296	3.7	365	7.249	7.225 ; 7.273
7.257	3.6	465	7.278	7.253 ; 7.303
7.320	3.6	525	7.294	7.268 ; 7.320
7.350	3.2	585	7.312	7.284 ; 7.339
7.419	2.6	645	7.330	7.302 ; 7.358

3. Evaluating pH normalization and capillary ketones

The secondary aim of this study was to assess if capillary β-Hydroxybuterate could predict normalization of pH (resolution of diabetic ketoacidosis), and therefore be used as a possible end point evaluation for intravenous insulin therapy.

For this evaluation, pH was analyzed as a binary outcome, where pH = 0, if the pH </= 7.3 or pH = 1, if the pH > 7.3.

A logistic regression model was used, and again fitted with a random slope and random intercept. This takes taking account the within individual correlation, by allowing the intercept and slope (time) to vary for each individual. The models are all logistic regression models (with a subject-specific intercept and slope).

Four different models were fitted and evaluated.

Model 1: Including only capillary ketones as a covariate (assuming the relationship does not change over time).

Model 2: Including capillary ketones and time as covariates.

Model 3: Included capillary ketones and time as covariates, and also the interaction between capillary ketones and time.

Model 4: Restricted cubic splines were added to get a better understanding of the data and how the different models change the cut-off decision.

The scatterplots in figures 3.6 to 3.8 shows the observed capillary ketones over time.

- Green dots represent the capillary ketone measurements which correspond to an observed pH > 7.3
- Black dots correspond to an observed pH </= 7.3.

The lines in the plots indicate at which capillary ketone values, dependent on time, the probability of pH >7.3 is 0.5.

Model 1:

Including only capillary ketones as a covariate (assuming relationship does not change over time)

$$\frac{\exp(\hat{a}+\hat{b}*capketones)}{1+\exp(\hat{a}+\hat{b}*capketones)}=0.5$$

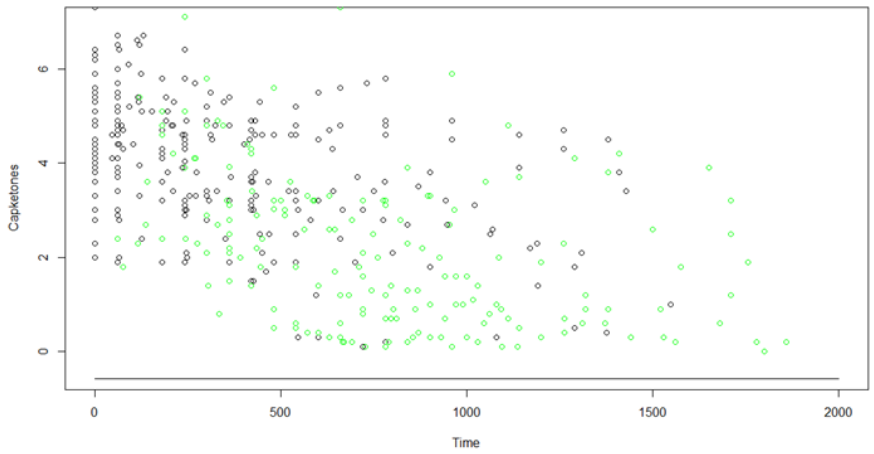

Figure 3.4 Model 1

Model 2:

Including capillary ketones and time as covariates

$$\frac{\exp(\hat{a}+\hat{b}*capketones+\hat{c}*time)}{1+\exp(\hat{a}+\hat{b}*capketones+\hat{c}*time)}=0.5$$

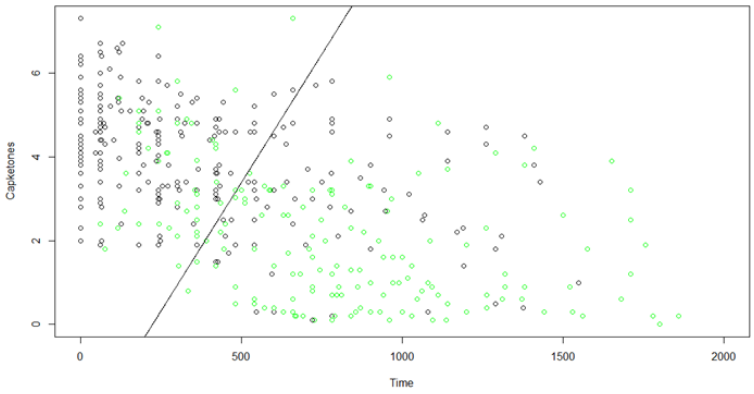

Figure 3.5 Model 2

Model 3

Including capillary ketones, time and interaction between capillary ketones and time. For Model 3, the parameter estimates failed to converge and this model was not explored further.

Model 4

Including capillary ketones and time as covariates, fitted with restricted cubic splines

$$\frac{\exp(\hat{a}+\hat{b}*capketones+\hat{c}_1*time_1+\hat{c}_2*time_2+\hat{c}_3*time_3)}{1+\exp(\hat{a}+\hat{b}*capketones+\hat{c}_1*time_1+\hat{c}_2*time_2+\hat{c}_3*time_3)}=0.5$$

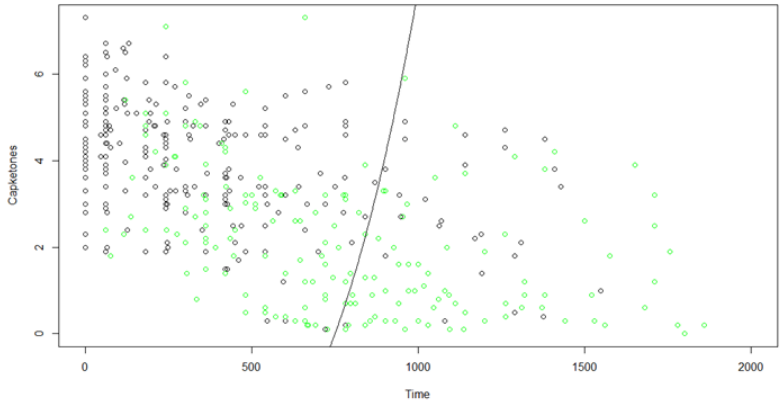

Figure 3.6 Model 4

Testing Model Assumptions

The need for different covariates was tested and the AIC of the different models and the model fits were compared using the likelihood ratio test. From this analysis it was found that Model 4 (AIC = 254.71) was the best model to predict pH normalisation.

Testing model assumptions

Table 3.7 Testing model assumptions

Model	Effect	AIC
Model 1	No Random Effects	334.97
Model 2	Random Intercept	279.20
Model 3	Random Intercept, Slopes (Time) and quadratic time effect (Time x Time)	Excluded from analysis
Model 4	Restricted cubic splines	254.71

Table 3.8 Likelihood ratio test comparing models

Comparison	Chi-squared	Df	p-value
Model 1 vs Model 2	55.77	1	2.94e-14
Model 2 vs Model 4	28.48	2	6.527e-07

This model was applied to obtain cut-off values for capillary ketones which correspond to a pH = 7.3. This was however dependent on time, and resulted in a curve over time, rather than a single cut-off value

From the graph of Model 4 (see figure 3.8), it is also evident that the cut-off values subjects as either recovered or not, using capillary ketones, is definitely time dependent.

The results from this analysis again confirm a complex, time dependant association between pH and measured β-Hydroxybuterate.

CHAPTER 4

Discussion

The background of the present study was to evaluate if using β - Hydroxybuterate offers clinical advantage over measurement of pH, as has been reported by other authors. Bedside capillary β - Hydroxybuterate monitoring has the potential to be a valuable method of guiding intravenous insulin therapy and evaluate normalisation of pH in patients with diabetic ketoacidosis. The measurement of capillary β - Hydroxybuterate has become popular in recent years, but is mainly used to predict the presence of ketoacidosis in a patient presenting with hyperglycaemia.

1. Results from this study

In the present study, using a hospitalised population of patients presenting with diabetic ketoacidosis, we have observed that there is no constant relationship between pH and of β- Hydroxybuterate (capillary ketones) over time. A complex, non-linear relationship was found and the expected decrease in β – Hydroxybuterate, accompanied by an increase in pH, could not be seen in the analysis.

Different models were evaluated to establish an association between pH and β – Hydroxybuterate, or if BHB could be used to predict pH values, when ketone body measurement was done using a POC device during the course of DKA treatment.

Firstly, based on restricted cubic spline modelling, this work suggests a non-linear relationship β-Hydroxybuterate and pH values. The results further indicated that this relationship changes over time, resulting in a complicated mathematical formula to derive pH.

Secondly, pH values was analysed as a binary outcome to evaluate resolution of DKA. A non-linear terms included model was again developed, using restricted cubic splines, to obtain cut-off values for capillary ketones which correspond to a pH = 7.3. A single cut-off value could not be determined and the analysis implicated that the cut-off values for resolution versus still ketoacidosis, using capillary ketones, is definitely time dependent.

Despite the fact that the capillary ketonaemia technique is easy to perform, its clinical use would be limited if complicated formulas has to be used to achieve reliable results.

2. Study results in relation to other studies

In contrast to the findings in this study, previously published studies indicated a correlation between β –hydroxybuterate and pH measurement, with a decrease in β-hydroxybuterate accompanied by a rise in pH. Evidence from these studies showed that capillary ketonaemia is directly related to the severity of acidosis.

A study of 68 children diagnosed with DKA found a moderate negative correlation between capillary β-Hydroxybuterate measurement and venous pH ($r=-0.63$; $p<0.01$). This correlation was still statistically significant when time dependant levels of pH were evaluated. [21]These findings were also supported in study evaluating 14 paediatric patients with DKA, showing a significant, but weak negative correlation between β – Hydroxybuterate and pH ($r=-0.41$, $p<0.05$)[23]

Also in concordance with these above mentioned studies, were the results is a Spanish study on thirty patients with type 1 diabetes, admitted for DKA. Even though the correlation of these parameters were statistically significant ($p < 0.01$) a strong correlation was yet again not observed ($r =-0.56419$). The correlation analysis was carried out based on the Pearson correlation coefficient. The impact of repeated measures with intra-individual correlation, was however not explored.[24]

The reason why the findings of this study did not show a linear correlation between measured β-Hydroxybuterate and pH values are not fully clarified.

One plausible reason may be that the pH level could be influenced by acid - base balance from other situations.

Fluid resuscitation with large volumes of sodium chloride may lead a hyperchloraemic acidosis, which will delay normalization of the pH. Acidosis may also be attributed to underlying sepsis or mild lactic acidosis if the patient is hypotensive.

A metabolic alkalosis, due to vomiting may give a false normal value. To minimize the problem created by underlying pathology, we excluded the patients from the in the study who had severe sepsis, needed cardiovascular supportive agents or ventilatory support or if other reasons for the acidosis where clinically evident.

A second possible reason for these study results could be explained by the statistical methods used to explore the relationship. Observations over time in one individual cannot be assumed to be independent, and the correlation between repeated measurements belonging to the same individual must be to take into account.

Ordinary logistic regression does not address this, therefore the data in this study was explored using linear mixed models. This allows comparison of changes in the response variables over time and also accommodates repeated measurements and possible within-individual correlation.

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3. Possible limitations of this study

This study was small and used consecutive enrolment of the subjects. Multiple observers contributed to the measuring and charting of values. Although small, this could have introduced some discrepancies in the results.

Another limitation is that this analysis did not record or evaluate anion gap or lactate levels. In 2009, The American Diabetes Association (ADA) adopted the normalization of the anion gap into the new one criteria for the resolution of diabetic ketoacidosis.[27] This gives an indication of increased levels of exogenous or endogenous acids, either measured or unmeasured. The study was planned and executed before the changed 2009 criteria for resolution of DKA was available; therefor anion gap measurement was not included in the analysis. This inclusion could have improved study outcome.

Observations and measurements were only recorded until pH was normalised. If measurements, especially β- hydroxybuterate testing, were continued after normalisation of pH, normoglycaemia and resolution of ketonaemia, a better end point evaluation could have been performed.

4. Questions arising for further research

Measurement of β- Hydroxybuterate might help in the management of diabetic ketoacidosis although this remains to be demonstrated by future research. Further studies are needed to evaluate this in adults, as part of monitoring and guidance of insulin based treatment, and possible use as end point for intravenous insulin.

Studies evaluations on the use of capillary β- Hydroxybuterate, pH should include evaluation of anion gap and lactate measurements and evaluate the possible discrepancy between the various definitions of resolution.

5. Conclusion

In contrast with studies previously published studies, the present results did not show a linear association between serum β Hydroxybuterate measurement and serum pH values.

Complex mathematical equations were derived from the fitted models to predict pH values using capillary β-Hydroxybuterate levels. These formulas were also showed that the relationship between β-Hydroxybuterate and pH levels were dependant on time, limiting its applicability and use in clinical scenarios. Furthermore, the study data suggests that relying on β Hydroxybuterate capillary testing, as a possible end point evaluation for intravenous insulin therapy, was not sufficient.

Furthermore, a single cut-off value could not be determined and the analysis implicated that the cut-off values for resolution versus still ketoacidosis, using capillary ketones, is time dependent.

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   b. Prof Emmanuel Lesaffre (Chair of the Department of Biostatistics, Erasmus University MC Rotterdam, The Netherlands and Professor at School of Public Health, KU University of Leuven, Leuven Belgium
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ADDENDUM 1

PATIENT INFORMATION LEAFLET AND INFORMED CONSENT

TRIAL TITLE

Fluid management in Diabetic Keto-acidosis. Ringer's lactate versus Saline in normalization of plasma pH. (**DKA-RS study)**

INTRODUCTION

You are invited to volunteer for a research study. This information leaflet is to help you to decide if you would like to participate. Before you agree to take part in this study you should fully understand what is involved. If you have any questions, which are not fully explained in this leaflet, do not hesitate to ask the investigator. You should not agree to take part unless you are completely happy about all the procedures involved. In the best interests of your health, it is strongly recommended that you discuss with or inform your personal doctor that you participated in this study.

WHAT IS THE PURPOSE OF THIS TRIAL?

You have been diagnosed, as suffering from Diabetes Keto-acidosis and the investigator would like you to consider taking part in the research. You are one of 120 patients that will be partaking in this study. The study will recruit patients at Kalafong and Pretoria Academic hospitals.

During the study you will receive fluid replacement with either the usual Normal Saline or Ringers lactate solution.

WHAT IS THE DURATION OF THIS TRIAL?

If you decide to take part you will be one of approximately 120 patients. The study will last for up to 4 days. You will only be studied while you are in hospital and will not be admitted for longer than is necessary for your condition.

You will be continuously monitored while in hospital and a short drip needle will be inserted to allow the taking of blood frequently (Every 30 min to 4 hourly depending on your response to treatment. The amount of blood that will be taken amounts to 1 ml at a time and will be less than 30 ml in total. Your urine will also be tested every 2 to 4 hours.

HAS THE TRIAL RECEIVED ETHICAL APPROVAL?

This clinical trial Protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human/subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

WHAT ARE MY RIGHTS AS A PARTICIPANT IN THIS TRIAL?

Your participation in this trial is entirely voluntary and you can refuse to participate or stop at any time without stating any reason. Your withdrawal will not affect your access to other medical care. The investigator retains the right to withdraw you from the study if it is considered to be in your best interest.

IS ALTERNATIVE TREATMENT AVAILABLE?

Alternative treatment in the form of the usual fluid resuscitation with normal saline is used to treat Diabetes Keto-acidosis. If you decide not to take part in this study it is likely that your doctor will treat you with this, or other suitable treatment.

MAY ANY OF THESE TRIAL PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE?

Venepunctures (i.e. drawing blood) are normally done as part of routine medical care and present a slight risk of discomfort. Drawing blood may result in a bruise at the puncture site, or less commonly fainting or swelling of the vein, infection and bleeding from the site. Your protection is that the procedures are performed under sterile conditions by experienced personnel. A total of 40 ml of blood (i.e. 8 teaspoons) will be collected over the course of the entire study.

WHAT ARE THE RISKS INVOLVED IN THIS TRIAL?

All medicines carry some risk, however small. There might be a small risk of developing high potassium levels, this will however be constantly monitored.

ARE THERE ANY WARNINGS OR RESTRICTIONS CONCERNING MY PARTICIPATION IN THIS TRIAL? No restrictions to participation

DISCONTINUATION OF TRIAL TREATMENT

Uncontrolled discontinuation of trial fluids is inadvisable. You may request that usual therapy be continued but it is extremely dangerous to stop fluid resuscitation fluids altogether if you have a DKA. The investigator will supervise any discontinuation with your health as first priority.

FINANCIAL ARRANGEMENTS

You will not be paid to participate in this trial.

SOURCE OF ADDITIONAL INFORMATION

For the duration of the trial, you will be under the care of Dr …… . If at any time you feel that any of your symptoms are causing you any problems, or you have any questions during the trial, please do not hesitate to contact him/her. The telephone number is ……..……., through which you can reach him/her or another authorized person.

CONFIDENTIALITY

All information obtained during the course of this trial is strictly confidential. Data that may be reported in scientific journals will not include any information, which identifies you as a patient in this trial. In connection with this trial, it might be important for domestic and foreign regulatory health authorities and the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, as well as your personal doctor, to be able to review your medical records pertaining to this trial.

Any information uncovered regarding your test results or state of health as a result of your participation in this trial will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this trial but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission. The only exception to this rule will be cases in which a law exists compelling us to report individuals infected with communicable diseases. In this case, you will be informed of our intent to disclose such information to the authorized state agency.

INFORMED CONSENT

I hereby confirm that I have been informed by the investigator, Dr ……..……about the nature, conduct, benefits and risks of **DKA-RS study**. I have also received, read and understood the above written information (Patient Information Leaflet and Informed Consent) regarding the clinical trial.

I am aware that the results of the trial, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a trial report.

I may, at any stage, without prejudice, withdraw my consent and participation in the trial. I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the trial.

Patient's name _____
(Please print)
Patient's signature _____ Date _____

I, Dr …… herewith confirm that the above patient has been informed fully about the nature, conduct and risks of the above trial.

Investigator's name _____
(Please print)
Investigator's signature _____ Date _____

Witness's name* _____

Witness's signature _____ Date _____

VERBAL PATIENT INFORMED CONSENT (applicable when patients cannot read or write)

I, the undersigned, Dr ……., have read and have explained fully to the patient, named …….. and/or is/her relative, the patient information leaflet, which has indicated the nature and purpose of the **DKA-RS study**, in which I have asked the patient to participate. The explanation I have given has mentioned both the possible risks and benefits of the trial and the alternative treatments available for his/her illness. The patient indicated that he/she understands that he/she will be free to withdraw from the trial at any time for any reason and without jeopardizing his/her subsequent injury attributable to the drug(s) used in the clinical trial, to which he/she agrees.

I hereby certify that the patient has agreed to participate in this trial.

Patient's Name_____
(Please print)

Investigator's Name _____
(Please print)

Investigator's Signature _____ Date _____

Witness's Name _____

Witness's Signature _____ Date _____

Addendum 2

Diabetes DKA-RS study

Managed where? **Casualty** **Ward High care** **ICU** ward?_____
Internal medicine firm:_____

Patient name:_____

Hospital no:_____ DOB: YY / MM / DD Gender: M / F

Date enrolled: YY / MM / DD
Date discharged from high care: YY / MM / DD
Date discharged from hospital: YY / MM / DD

Newly diagnosed diabetic? Y / N If no when first diagnosed? YYYY
If no where did patient receive treatment for diabetes?_____
Diabetes type before DKA? 1 / 2 / sec
Precipitant for DKA? E.g. Non-compliance/pneumonia_____
Where does patient stay? E.g. town/ township

In hospital complications?

DKA Treatment and nursing observation sheet

Time (hours since initiation of treatment)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Time actual (hh:mm)															
Insulin administration (U/hour)															
Resusitation fluid administration (ml/h)															
Heart rate (beats/min)															
Systolic Blood pressure (mmHg)															
Diastolic Blood pressure (mmHg)															
Respiratory rate (breaths/min)															
Signature															

Time (hours since initiation of treatment)	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
Time actual (hh:mm)															
Insulin administration (U/hour)															
Resusitation fluid administration (ml/h)															
Heart rate (beats/min)															
Systolic Blood pressure (mmHg)															
Diastolic Blood pressure (mmHg)															
Respiratory rate (breaths/min)															
Signature															

DKA - RS study

Subject inclusion criteria

1) Previously known or unknown diabetic patients (Type 1 or type 2)
2) Older than 18 years of age
3) Venous pH 7.0 to 7.29
4) Urinary ketones on dipstix >2+
5) Capillary blood glucose or venous blood glucose > 13 mmol/L
6) Verbal informed consent given

Subject exclusion criteria

1) Patients not complying with the inclusion criteria
2) Acidosis due to other causes than DKA
3) Patients declining verbal consent, or unable to give verbal consent
4) Need for cardiovascular supportive agents
5) Need for ventilatory support

ADDENDUM 3

Instructions

All DKA patients should be managed to the accompanying protocol

Contents of kit:
4 liters of resuscitation fluid (unmarked/blinded)
10 Medisence Optium blood ketone measurement strips
Booklet with DKA management protocol, data sheets, Patient information leaflet and informed consent.
A4 envelope.
Treatment monitoring sheet

0 - indicates baseline, 1, 2, 3…..indicates sequential measurements

Observations: pH, cH+, HCO3, cK+, cNa+ and glucose and cap Ketones should be done at baseline and 1 hour later then **according to the last pH value** as indicated by the schedule.

Shedule:

Baseline (time 0)
Time 0 +1 hour
Then according to pH:
pH 6.9 – 7.2 repeat over 3 hours
pH 7.21 – 7.3 repeat over 2 hours
pH 7.31 – 7.33 repeat over 1 hour
pH 7.34 – 7.36 repeat over 30 min
pH > 7.36 stop

A **lab Na+, K+, Cl-, CO2, Urea, Creat** should be done at baseline (time 0), time 0 +1 hour and when pH is ≥7.36.
Serum **albumin and protein** should be done at time 0 and when pH is ≥7.36.
Urine Ketones should be done at baseline, at pH 7.21 – 7.3 and when pH ≥7.36.

Observations should be recorded on the attached table. The actual time when the blood / urine **are taken** should be noted.

Bloods: All bloods should preferably be venous, if not; it should be clearly marked arterial or capillary. Blood gas printouts should be timed and inserted in the attached envelope.

If any problems please **contact**:
Dr Delport: 0829243458 or Prof van Zyl: 0828232056

Patient Flow Chart (DKA-RS study)																
Measurement	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Time actual (hh:mm)																
pH																
cH+ (nmol/L)																
HCO3 (mmol/l)																
cK+ (mmol/L)																
cCl- (mmol/L)																
cAlbumin (mmol/L)																
cTot Prot (mmol/L)																
Cap Glucose (mmol/L)																
Cap Ketone (mmol/L)																
U Ketone																

Patient Flow Chart (DKA-RS study) Cont.

Measurement	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Time actual (hh:mm)																
pH																
cH+ (nmol/L)																
HCO3 (mmol/l)																
cK+ (mmol/L)																
cCl- (mmol/L)																
cAlbumin (mmol/L)																
cTot Prot (mmol/L)																
Cap Glucose (mmol/L)																
Cap Ketone (mmol/L)																
U Ketone																

DKA - RS study

Subject inclusion criteria

7) Previously known or unknown diabetic patients (Type 1 or type 2)
8) Older than 18 years of age
9) Venous pH 7.0 to 7.29
10) Urinary ketones on dipstix >2+
11) Capillary blood glucose or venous blood glucose > 13 mmol/L
12) Verbal informed consent given

Subject exclusion criteria

6) Patients not complying with the inclusion criteria
7) Acidosis due to other causes than DKA
8) Patients declining verbal consent, or unable to give verbal consent
9) Need for cardiovascular supportive agents
10) Need for ventilatory support

Addendum 4

University of Pretoria
Faculty of Health Sciences Research Ethics Committee
University of Pretoria
Tel: 012 354 1677 Fax to E-Mail: 086 6516047
E-Mail: deepeka.behari@up.ac.za
Date: 21/11/2007

31 Bophelo Road
HW Snyman South Building
Level 2, Room 2.33

P O Box 667
Pretoria
0001

Number : S274/2007

Title : Capillary β-Hydroxybuterate testing in patients with diabetic ketoacidosis

Investigator: Dr E F Delport, Department of Internal Medicine, University of Pretoria
(SUPERVISOR: PROF P RHEEDER)

Sponsor : Research Development Fund of Prof D G van Zyl & Funds from Contract Research

Study Degree: MSc (Clinical Epidemiology)

This Student Protocol has been considered by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 20/11/2007 and found to be acceptable.

Advocate AG Nienaber	(female)BA(Hons) (Wits); LLB; LLM (UP); Dipl.Datametrics (UNISA)
Prof V.O.L. Karusseit	MBChB; MFGP (SA); M.Med (Chir); FCS (SA); Surgeon
Prof M Kruger	(female) MB.ChB.(Pret); Mmed.Paed.(Pret); PhDo. (Leuven)
Dr N K Lukibi	MB.BCh.; Med.Adviser (Gauteng Dept.of Health)
Snr Sr J. Phatoli	(female) BCur (E.Ad) Senior Nursing-Sister
Dr L Schoeman	(female) Bpharm, BA Hons (Psy), PhD
Prof J.R. Snyman	MBChB, M.Pharm.Med. MD: Pharmacologist
Dr R Sommers	(female) MBChB; M.Med (Int): MPhar.Med;
Prof C W van Staden	MBChB; Mmed (Psych): MD; FTCL; UPLM: Dept of Psychiatry
Prof TJP Swart	BChD, MSc (Odont), MChD (Oral Path) Senior Specialist; Oral Pathology
Dr AP van der Walt	BChD, DGA (Pret) Director: Clinical Services, Pretoria Academic Hospital

Student Ethics Sub-Committee

Prof R S K Apetu	MBChB(Legon); PhD(Cambridge)
Dr A M Bergh	(female) BA (*cum laude*), Rand Afrikaans University BA (Hons) (Linguistics), University of Stellenbosch Secondary Education Diploma (*cum laude*), University of Stellenbosch BA (Hons) (German) (*cum laude*), University of South Africa (Unisa) BEd (Curriculum Research and Non-formal Education) (*cum laude*), University of Pretoria PhD (Curriculum Studies), University of Pretoria
Dr S I Cronje	DD (UP) - Old Testament Theology
Dr M M Geyser	(female) BSc; MBChB; BSc HONS (Pharm); Dip PEC; MpraxMed; FCEM(SA) and MSc (Clinical Epidemiology)
Mrs N Sriers	(female) BSc(Stell), BSc (Hons) (Pret),MSc (Pret) DHETP (Pret)
Dr S A S Clonmju	B.Sc Hons; M.Sc; Ph.D
Dr L Schoeman	(female) BPharm, BA Hons (Psy), PhD
Dr R Sommers	SECRETARIAT (female) MBChB, M.Med (Int): MPharMed

DR R SOMMERS; MBChB, M.Med (Int); MPhar.Med
SECRETARIAT of the Faculty of Health Sciences
Research Ethics Committee
University of Pretoria

DR L SCHOEMAN; Bpharm, BA Hons (Psy), PhD
CHAIRPERSON of the Faculty of Health Sciences Research
Students Ethics Committee – University of Pretoria