Aalborg Universitet

AALBORG UNIVERSITY

DENMARK

Changes in cardiac repolarisation during hypoglycaemia in type 1 diabetes

Christensen, Toke Folke

Publication date:
2010

Document Version
Publisher's PDF, also known as Version of record

Link to publication from Aalborg University

CHANGES IN CARDIAC REPOLARISATION DURING HYPOGLYCAEMIA IN TYPE 1 DIABETES

PhD Thesis
by
Toke Folke Christensen

AALBORG UNIVERSITY

Department of Health Science and Technology

novo nordisk®

Device Research & Innovation

• 2010 •

Acknowledgements

Preface

This PhD thesis concludes my work through the last three years employed as an industrial PhD student at Novo Nordisk A/S. The project is a collaboration between Novo Nordisk A/S and Aalborg University and was co-funded by the Danish Ministry of Science, Technology and Innovation and Novo Nordisk A/S.

In this PhD thesis it is explored how hypoglycaemia in type 1 diabetes affects cardiac repolarisation. Furthermore, the physiological mechanisms governing this phenomenon are investigated.

In addition to this work, a substantial amount of time has been spent on exploiting the data and knowledge obtained through the PhD for development of technology for improving the treatment of diabetes. As this technology is bound by confidentiality agreements and intellectual property rights it will only be disclosed under confidentiality. A separate confidential technical report describing this work is thus included as a part of the PhD thesis.

The PhD thesis consists of the following in parts:
- An introduction where the problem background is described which is concluded by the aims and hypotheses of the PhD project.
- Four scientific articles describing the main scientific findings of the PhD project.
- A discussion of the findings in the articles and a conclusion on the hypotheses.
- A separate confidential technical report.

In the electronic edition of the thesis, part 2 and 4 are excluded due to copyright and confidentiality, respectively.

List of publications and related work

The PhD thesis is based on four scientific articles included as chapters in the thesis:

Paper 1   Christensen TF, Randløv J, Kristensen LE, Eldrup E, Hejlesen OK and Struijk JJ, "QT Measurement and Heart Rate Correction during Hypoglycemia: Is There a Bias?," Cardiology Research and Practice, vol. 2010, Article ID 961290
http://www.sage-hindawi.com/journals/crp/2010/961290.html

Paper 2   Christensen TF, Randløv J, Kristensen LE, Struijk JJ, Eldrup E, Hejlesen OK, "QT interval prolongation during spontaneous episodes of hypoglycaemia in type 1 diabetes: the impact of heart rate correction", Diabetologia. 2010 Sep;53(9):2036-41

http://www.springerlink.com/content/5170q8t1lq2p6704/

Paper 3   Christensen TF, Tarnow L, Randløv J, Kristensen LE, Struijk JJ, Hejlesen OK, "QTc during hypoglycaemia following subcutaneous insulin administration in people with type 1 diabetes"
(submitted to Diabetologia)

Paper 4   Christensen TF, Bækgaard M, Dideriksen JL, Steimle KL, Mogensen ML, Kildegaard J, Struijk JJ, Hejlesen OK, "A physiological model of the effect of hypoglycemia on plasma potassium"
J Diabetes Sci Technol 3:875-886, 2009
http://www.journalofdst.org/July2009/Articles/VOL-3-4-ORG9-CHRISTENSEN.pdf

In addition the author has contributed with the following scientific work during the PhD:

Paper   Kildegaard J, Christensen TF, Johansen MD, Randlov J, Hejlesen OK: Modeling the Effect of Blood Glucose and Physical Exercise on Plasma Adrenaline in People with Type 1 Diabetes. Diabetes Technol Ther 9:501-508, 2007

Paper   Kildegaard J, Christensen TF, Hejlesen OK: Sources of Glycemic Variability—What Type of Technology is Needed? J Diabetes Sci Technol 3:986-991, 2009

Conference Paper   Christensen TF, Lewinsky I, Kristensen LE, Randlov J, Poulsen JU, Eldrup E, Pater C, Hejlesen OK, Struijk JJ: QT Interval Prolongation during Rapid Fall in Blood Glucose in Type I Diabetes. *Comput Cardiol* 34:345-348, 2007

Conference Abstract   Christensen TF, Baekgaard M, Dideriksen JL, Steimle KL, Mogensen ML, Struijk JJ, Hejlesen OK: Modelling the Effect of Hypoglycemia on Serum Potassium Levels

Conference Abstract   Christensen TF, Struijk JJ, Tarnow L, Randlov J, Kristensen LE, Eldrup E, Hejlesen OK: Spontaneous Hypoglycemia Causes Significant Changes in Cardiac Repolarization in Type 1 Diabetes - 72 hours of CGM and Mobile ECG Monitoring

Abstract

The 'dead in bed' syndrome is a condition where otherwise healthy young people with type 1 diabetes are found dead in the morning in an undisturbed bed. It has been hypothesized that the phenomenon may be caused by hypoglycaemia triggering cardiac arrhythmia. This hypothesis has been strengthened with findings of QT interval prolongation during hypoglycaemia. QT interval prolongation has been associated with an increased risk of cardiac death in several subpopulations including patients with diabetes. This PhD thesis investigates changes in the QT interval and cardiac repolarisation during hypoglycaemia as well as the underlying physiological mechanisms.

In Paper I, different sources of variation when investigating the heart rate corrected QT interval (QTc) during hypoglycaemia were explored. Hypoglycaemia was induced by an intravenous bolus of insulin in persons with type 1 diabetes and the differences between QT interval measuring techniques, types of insulin and heart rate correction formulas were studied. The results suggested that the measurement technique has a profound effect on the QT interval prolongation seen during hypoglycaemia. Heart rate correction also affected the degree of prolongation during hypoglycaemia.

In Paper II, the changes in QTc during spontaneous hypoglycaemia were investigated. 21 adults with type 1 diabetes were monitored for 72 hours using a continuous glucose monitor and a Holter monitor with the aim of capturing spontaneous episodes of hypoglycaemia. In addition to quantifying the QTc during hypoglycaemia the performance of several QT interval correction formulas was explored. Spontaneous hypoglycaemia was only associated with significant prolongation of QTc using Bazett's formula, the most popular heart rate correction formula. The other applied correction formulas did not show a significant prolongation.

In Paper III, hypoglycaemia was induced by subcutaneous insulin injection to build a bridge between the results from hypoglycaemic clamp studies and studies of spontaneous hypoglycaemia. Ten adults with type 1 diabetes were studied and QTc, adrenaline and potassium were measured to investigate both the prolongation of QTc during hypoglycaemia and the underlying physiological mechanisms. The results showed significant prolongation of the QTc, however the prolongation was smaller than seen during typical clamp studies.

In Paper IV, we developed a physiological model of changes in potassium during hypoglycaemia to get a deeper understanding of the physiological mechanisms responsible for QT interval prolongation. The model was developed and tested on data from the literature. The tests showed that the model was able to simulate potassium in a range of situations, although rapid changes in insulin and adrenaline were associated with larger simulation errors.

In this PhD thesis I showed that the degree of QTc prolongation seen during hypoglycaemia is dependent on both measurement technique and heart rate correction (Paper I+II+III). I found that QTc prolongation during spontaneous hypoglycaemia may be due to overcorrection by Bazett's formula (Paper II). I presented a novel methodology for studying QTc during controlled but realistic episodes of hypoglycaemia, which showed significant QTc prolongation during hypoglycaemia compared with control episodes (Paper III). Lastly, I developed a physiological model of potassium to gain new knowledge on the physiological mechanisms behind the proposed QT interval prolongation during hypoglycaemia (Paper IV).

Contents

Abbreviations

ADA	American Diabetes Association
BG	Blood glucose (concentration)
BGM	Blood glucose measurement
BPM	Beats per minutes
CGM	Continuous Glucose Monitor.
ECG	Electrocardiogram.
IG	Interstitial glucose concentration
MA	Manual annotation (method for QT interval measurement)
QRS complex	Collective duration of the Q, R and S waves in the ECG. The QRS complex constitutes the duration of the depolarisation of the heart.
QT interval	The time from the onset of the Q wave to the end of the T wave in the ECG.
QTc	The heart rate corrected QT interval
QTcB	The heart rate corrected QT interval using Bazett's formula
QTcF	The heart rate corrected QT interval using Fridericia's formula
QTcN	The heart rate corrected QT interval using the Nomogram method
QTcS	The heart rate corrected QT interval using a subject specific method
RR interval	The time between two consecutive R waves in the ECG.
SI	Slope intersect (method for QT interval measurement)
SMBG	Self monitoring of blood glucose
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
T/R Ratio	The amplitude ratio between the T wave peak and the R wave peak in the ECG

INTRODUCTION

Diabetes Mellitus is a global epidemic. The prevalence of the disease is estimated to reach 285 million in 2010 corresponding to 6.6% of the world population.[1] By 2030, 7.8% of the world population or 438 million people are expected to have the disease.[1] In 2010, diabetes will be responsible for an estimated 4 million deaths or 6.8% of all deaths globally.[1] The estimated global healthcare expenditures to treat and prevent diabetes and its complications are expected to reach $376 billion in 2010.[1] In addition to economic costs, diabetes is associated with physical and psychological morbidity and decreased quality of life. The odds of depression double in the presence of diabetes.[2]

Diabetes

Diabetes is a collection of diseases characterised by insufficient insulin production and/or insulin resistance. Insulin is a hormone that mediates the uptake of glucose in liver, muscle and fat tissue. The lack of insulin or insulin resistance results in elevated blood glucose concentration (BG), hyperglycaemia, which is the cardinal symptom of diabetes. On the long term, hyperglycaemia causes damage to nerves and blood vessels resulting in a number of microvascular and macrovascular complications. The two major types of diabetes are type 1 (T1DM) and type 2 (T2DM) constituting approximately 10% and 90% of the total diabetes population, respectively. A small proportion (3-5%) of pregnant women develops gestational diabetes (GDM) that resembles T2DM in manifestation and aetiology.[3] GDM along with other types of the disease including pre-diabetes will not be discussed further in this report.

Type 1 diabetes

In T1DM, a progressive destruction of the insulin producing beta cells in the pancreas causes an absolute insulin deficiency. Therefore, people with T1DM are dependent on daily insulin injections. Without exogenous insulin people with T1DM will die from ketoacidosis within a short time. The incidence rate of T1DM peaks in the second decade of life and levels out in the third and fourth but increases again thereafter. The cumulative incidence rate by age 70 is 1%. The pathogenesis of T1DM is still largely unknown. A number of genetic mutations have been associated with the development of the disease but twin studies suggest that genetic factors can only partly explain the development of the disease. A number of environmental factors including viral infections have been suggested to trigger the disease.[4]

Type 2 diabetes

T2DM, the most common type of diabetes, is characterised by impaired insulin sensitivity and secretion. Unlike T1DM, persons with T2DM retain a certain production of insulin although insufficient to keep the BG within normal range. T2DM progresses slowly and as a result people can have the disease for years without knowing it. Treatment of T2DM starts with oral medication that increases insulin sensitivity but as the disease progresses it is often necessary to treat with insulin injections. The incidence rate increases with age with a cumulative incidence rate by age 70 of 11 %. The major risk factors for developing T2DM are obesity and a family history of the disease.[4]

Treatment of diabetes

The treatment of diabetes is focused on maintaining BG within the normal range of a healthy person (4-7mmol/l). In T1DM and in the later stages of T2DM, subcutaneous injections of insulin are needed several times daily. Insulin therapy aims at mimicking the insulin secretion of healthy people. The multiple daily injection (MDI) treatment regime recommended by the American Diabetes Association (ADA) comprises long acting insulin for maintaining a basal level and fast acting insulin in conjunction with meals.[5]

There is conclusive evidence that intensive insulin therapy aiming at elimination of hyperglycaemia reduce the risk of the long term complications associated with diabetes.[6] Effectiveness of insulin treatment is measured using the percentage of glycosylated hemoglobin, HbA1c, which indicates the average BG over the past few months. There is a close link between HbA1c and the risk of developing long term complications such as retinopathy, nephropathy and neuropathy. In Figure 1a, the rate of progression of retinopathy is shown to increase with increasing values of HbA1c. The ADA recommends a target level of HbA1c of < 7%, however this is achieved only for the minority of persons with T1DM.[5] The problem is that keeping a low HbA1c increases the risk of hypoglycaemia (Figure 1b).[6]
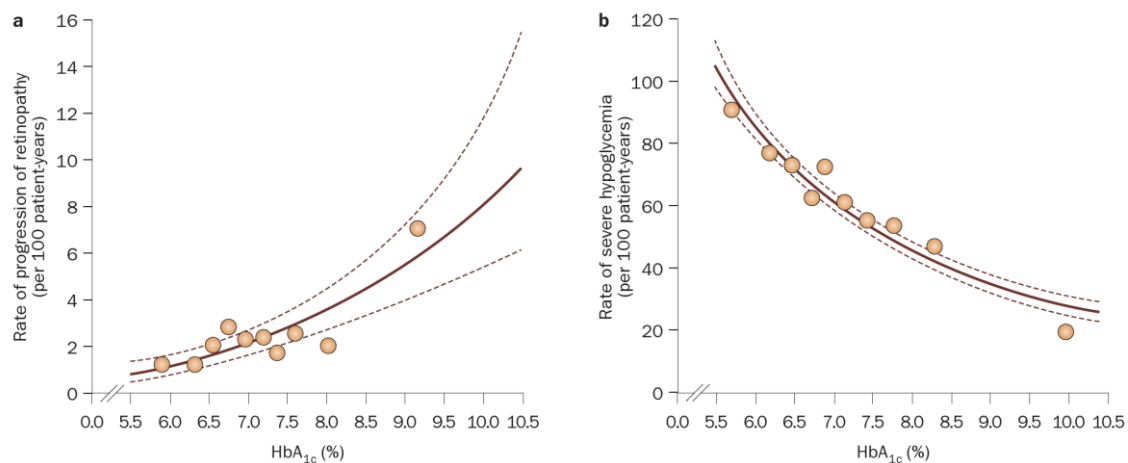
Figure 1. (a) The relationship between HbA1c and the rate of progression of retinopathy. (b) The relationship between HbA1c and the rate of severe hypoglycaemia.[6]

Hypoglycaemia

Hypoglycaemia is the limiting factor in achieving optimal glycaemic control in T1DM. If it was not for the risk of hypoglycaemia, people with diabetes could avoid high HbA1c levels and a normal life except for taking their medication.[7] The reality is however that the risk of hypoglycaemia imposes several limitations in the everyday life of people with diabetes. Alcohol, exercise, missed or skipped meals and bad timing of insulin injections are all frequent causes of hypoglycaemia in T1DM. The prospect of experiencing a severe episode of hypoglycaemia where help from others are needed causes psychological morbidity in both the person with diabetes as well as family and relatives. Although rare, hypoglycaemia can be fatal and it is estimated that 2%-6% of deaths in diabetes can be attributed to hypoglycaemia.[8] The problem faced by people with diabetes is that a lower HbA1c will reduce the risk of developing long term complications but it also increases the risk of severe hypoglycaemia as seen in Figure 1b.

Definition

There exists no clear definition of hypoglycaemia. ADA defines hypoglycaemia as BG of 3.9 mmol/l or below.[5] This definition is however more a guideline to which levels of BG should be avoided and not a strict definition of physiological hypoglycaemia.[9] Whipple's triad adapted for diabetes (Table 1) is more appropriate for the definition of hypoglycaemia.[10]

Table 1. Whipple's triad adapted for diabetes from Watkins et al.[10]

Criteria of Hypoglycaemia
1. Symptoms or signs compatible with low BG
2. Blood glucose <3.5mmol/L
3. Relief of symptoms and signs by restoration of circulating blood/plasma glucose concentrations

Ideally, to diagnose hypoglycaemia, all three criteria from Table 1 should be fulfilled. That is, there should be symptoms or signs of hypoglycaemia (including signs not recognised by the person with diabetes), a confirmatory blood glucose measurement and relief of symptoms and signs when the BG is restored to normal level. In reality, it may be difficult to ensure fulfilment of all three criteria in a clinical setting since symptoms and signs are subjective and different in individuals. Likewise the BG level at which a person experience symptoms and signs of hypoglycaemia has a large inter-person as well as intra-person variability, depending on several factors such as duration of diabetes, glycaemic control and antecedent hypoglycaemia.[11]

When assessing the severity of hypoglycaemia, episodes are often divided into the four categories listed in Table 2.[9] The definitions in Table 2 have gained wide acceptance when characterising episodes of hypoglycaemia both in diabetes research and in management of diabetes.

Table 2. Clinical definitions of hypoglycaemia from Strachan.[9]

Definition	Description
Asymptomatic	Low BG identified on routine blood test, with no associated symptoms
Mild	Symptoms suggestive of hypoglycaemia; episode successfully treated by the patient alone.
Severe	Assistance from a third party is required to effect treatment
Profound	Associated with permanent neurological deficits or death

Setting a specific biochemical definition of hypoglycaemia is not possible without the risk of false positives or negatives. However, when investigating asymptomatic episodes of hypoglycaemia a biochemical definition needs to be set.

Frequency

In persons with T1DM striving for glycaemic control, it is estimated that asymptomatic hypoglycaemia (< 3.3 mmol/l) may be present 10% of the time.[12] It is however difficult to estimate the frequency of asymptomatic hypoglycaemia because it depends on the sampling of BG or the use of continuous glucose monitors which may be inaccurate. In fact, the use of continuous glucose monitors might overestimate the frequency of or time in hypoglycaemia.[13,14] On average, in T1DM, mild episodes of hypoglycaemia are present two times every week while severe, temporally disabling episodes occur once every year.[9,12]

Frequency of hypoglycaemia is substantially lower in T2DM than in T1DM. In T2DM patients treated with oral agents only 2-3% experience severe hypoglycaemia while the number for insulin treated T2DM patients is 11%. For comparison, 65% of intensively treated T1DM patients experience severe hypoglycaemia.[12]

Counterregulation to hypoglycaemia

As BG falls below a certain level a series of physiological mechanisms occur to raise BG to normal levels. These mechanisms are collectively known as counterregulation to hypoglycaemia. In Figure 2 the different mechanisms of counterregulation are shown along with the glucose level at which they are initiated. The first counterregulatory mechanism in non-diabetic persons is a reduction in insulin secretion – a mechanism absent in T1DM due to absence of endogenous insulin production. The second mechanism in the counterregulatory response is the release of glucagon, a hormone that, antagonistic to insulin, stimulates glucogenolysis and gluconeogenesis while inhibiting glucogenesis. In T1DM, the glucagon secretory response to hypoglycaemia is lost within a few years of onset of the disease. The third counterregulatory response is the release of epinephrine, cortisol and growth hormone. Only epinephrine has an effect on acute hypoglycaemia by raising the BG and producing warning symptoms. For most people with T1DM an attenuated epinephrine response remains as the only counterregulatory response before BG drops to a level where cognitive function is affected. When blood glucose gets below 2 mmol/l increased cerebral blood and hepatic autoregulation set in. The consequence of an impaired counterregulation is that people with T1DM will develop hypoglycaemia much faster with less warning symptoms resulting in a significantly increased risk of severe or profound hypoglycaemia.[11]
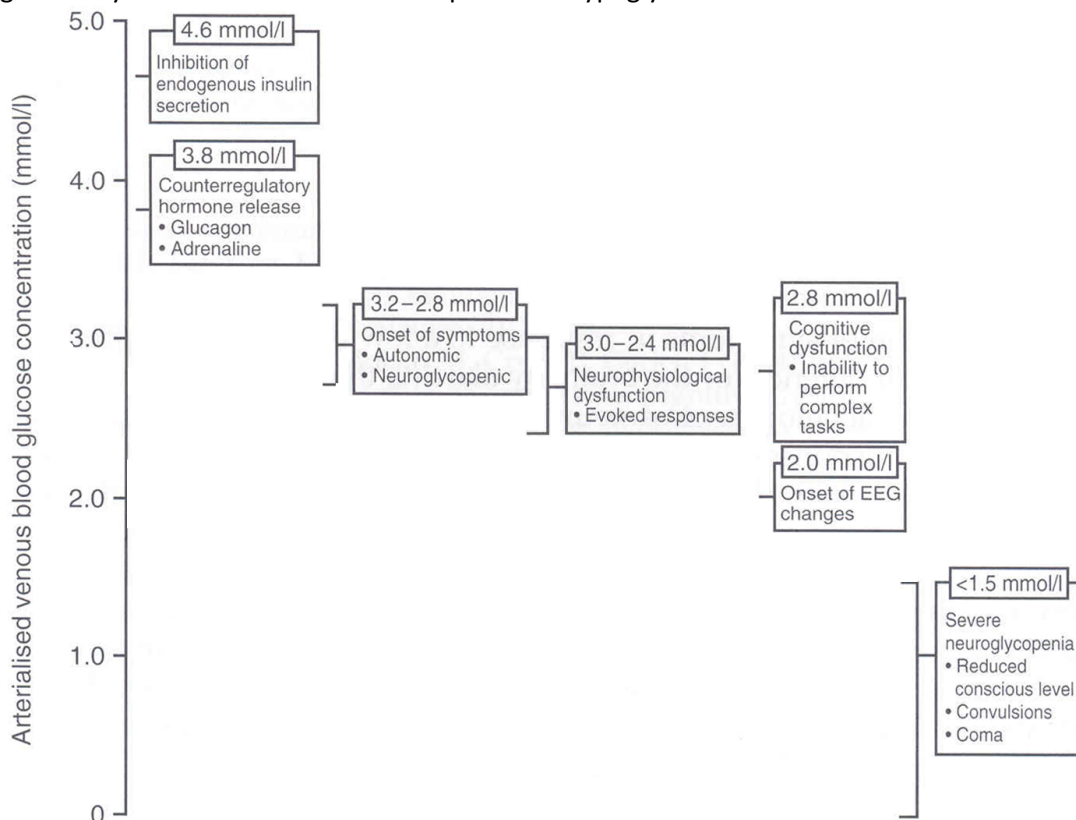

Figure 2. BG thresholds for counterregulatory mechanisms including release of hormones and onset of warning symptoms and cognitive impairment.[15]

Cardiac Arrhythmia and hypoglycaemia

Tattersall and Gill reported in the early 1990's a series of unexplained nocturnal sudden deaths of young people with T1DM.[16] This phenomenon was named the 'dead in bed' syndrome and evidence suggested that it was caused by hypoglycaemia, since the patients had a history of severe hypoglycaemia. The patients were found in an undisturbed bed which led to the hypothesis that fatal cardiac arrhythmia had been triggered by hypoglycaemia.[17] Since the phenomenon is very rare (an estimated 2-6 cases per 10.000 patient years[18]) it is impossible to make observational studies on actual cases. In stead several studies have investigated the effect of induced and spontaneous hypoglycaemia on the cardiovascular function and in the electrocardiogram. Hypoglycaemia has been associated with atrial fibrillation[19] but the most significant finding has been an altered cardiac repolarisation during hypoglycaemia manifest as a prolonged QT interval.[20,21,22,23,24,25,26,27,28] A prolonged QT interval during hypoglycaemia is an important finding since prolongation of the QT interval is known as an independent cardiac risk factor for sudden death and Torsade the Pointes (TdP) tachycardia.[29,30,31,32,33,34,35]

The QT interval

The QT interval is measured on the surface ECG and is defined as the onset of the Q wave to the end of the T wave (Figure 3).
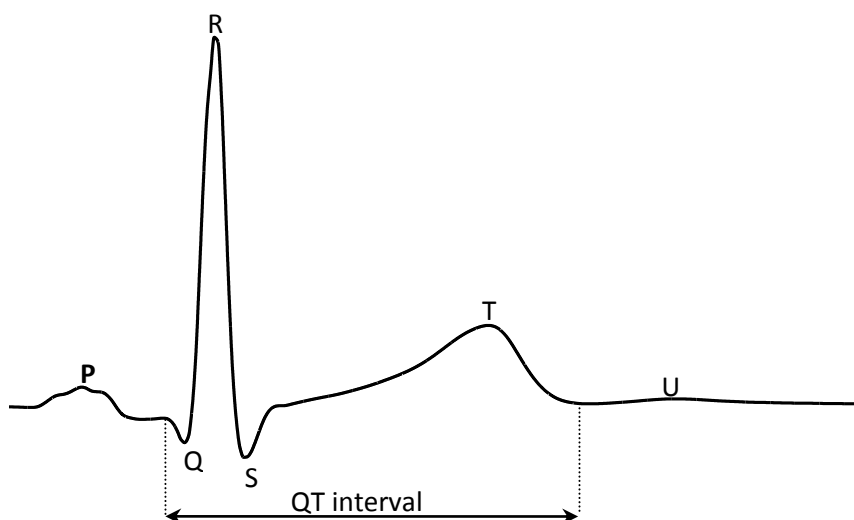
Figure 3. **Illustration of the ECG from one beat cycle with the associated waves and the QT interval.**

The QT interval comprises the QRS interval (depolarisation of the ventricles) and the T wave (repolarisation of the ventricles) and is in general perceived as a measure of the repolarisation duration of the ventricles as the QRS interval shows little variation in the normal ECG.

The heart rate corrected QT interval (QTc) has been subject of intense research for decades. Prolongation of the QT interval has been reported as a risk factor for cardiac death in patients with heart failure[36], myocardial infarction[37], type 1 diabetes[35,38,39], type 2 diabetes[33] and in the general population[40,34,29]. There is thus evidence to suggest a correlation between prolongation of the QT interval and cardiac death although others have questioned the prognostic value of a QT prolongation in the general population[31,41,42]. Prolongation of the QT interval can be either congenital or acquired. The congenital long QT syndrome is a rare disease caused by mutations of genes affecting the ion channels involved in the repolarisation of cardiac myocytes. Acquired long QT on the other hand is more common and is mostly caused by different medications[32,43] (in particular antiarrhythmic and antipsychotic drugs) although conditions as hypokalaemia[44] and hypoglycaemia[22] also have been reported to cause acquired long QT.

Measurement of the QT interval

The QT interval is traditionally measured manually using lead II, V5 or V6 in the 12-lead ECG.[45] Manual measurements of QT interval are usually done using manual annotations, today often done with on-screen computerised methods.[45] Modern electrocardiographs have software with the ability to automatically measure the QT interval, but manual ECG readings remain the golden standard for measuring the QT interval. Automatic measurements are associated with larger variability than manual measurements and the variability is dependent on pathological conditions affecting the ECG.[46,47] Therefore the use of automatic methods should be supplemented by manual review of the measurements.[45] In a study by Molnar et al.[48] the reproducibility of automatic measurements of QT was significantly improved when supplemented by manual review. The PhysioNet/Computers in Cardiology Challenge on QT interval estimation also revealed superiority of manual readings opposed to automatic algorithms, although some automatic algorithms came close to the performance of manual readings.[49] Nevertheless, QT interval measurement carries a large degree of subjectivity (manual measurements) or unexplained variability (automatic measurements).[50] Especially in the presence of odd morphologies including biphasic T wave, large U waves and fused T-U waves is the accurate measurement of QT interval difficult.[50] This is especially the case during e.g. hypokalaemia where large U waves and fused T-U waves are seen as shown in Figure 4. Hypokalaemia is present during hypoglycaemia which makes measurements of QT interval during hypoglycaemia difficult.
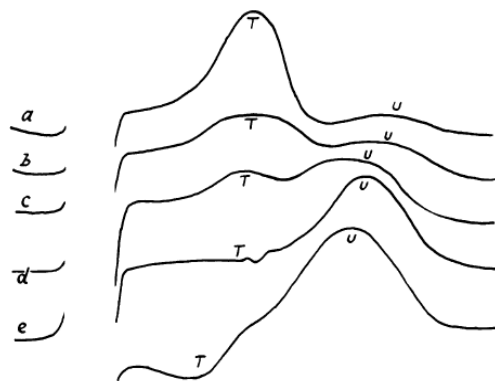
Figure 4. Lead V3 at different levels of serum potassium but similar heart rates. a: 4.6 mmol/l, b: 3.1 mmol/l, c: 2.7 mmol/l, d: 2.6 mmol/l.[50]

The end of the T wave is formally defined as the return of the terminal limb to the isoelectric baseline, as defined by manual annotation (MA).[45] This definition also applies in presence of a distinct U wave, however when the T and U waves are fused it must be interpreted as either a biphasic T wave or an early occurring U wave. If the former then the QT interval is measured to the end of the TU-complex but if the latter the QT interval is measured to the nadir between the T and U wave.[45,51] As seen in Figure 5 this distinction becomes increasingly difficult to define as the U wave occurs closer to the T wave. In studies of changes in the QT interval during hypoglycaemia a frequently used method for measuring the QT interval is the 'slope intersect' (SI) method.[27,52,53,23] With this method the end of the T wave is determined as the intersection between the isoelectric line and a tangent fitted to the steepest part of the terminal limb of the T wave. The SI method has gained popularity because of its simplicity, reduced subjectivity and ease of implementation in an automatic algorithm. It is however evident that the method consistently underestimates the QT interval[46] and is sensitive to the amplitude of the T wave.[47] Low amplitude T waves cause an increased variability and overestimation of QT with the SI method.[47]

Figure 5. a: Lead V3 from a normal person. b-e: Theoretical constructions of fused T-U wave morphologies as the U wave occurs earlier. The vertical line indicates the notch between the T and U wave while the tangents as used in the SI method are also shown.[50]

Ireland et al.[54] studied the differences between the SI method and the MA method during hypoglycaemia where fused T-U waves are common. They found that the SI method underestimated the QT interval at baseline and overestimated the QT at hypoglycaemia. Nevertheless, they recommended the use 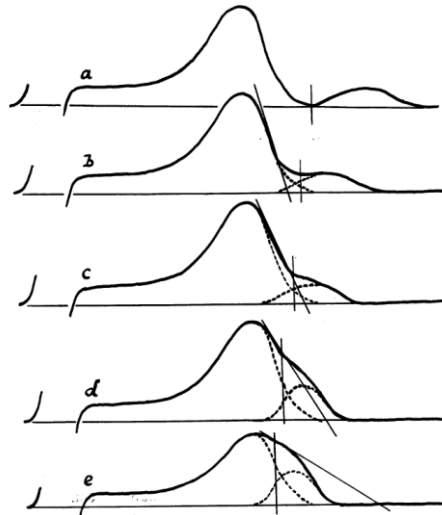of the SI method when investigating changes in the QT interval during hypoglycaemia because the method is less subjective and provides greater distinction between euglycaemia and hypoglycaemia.[54]

The heart rate corrected QT interval - QTc

The QT interval changes with the heart rate – the higher heart rate the shorter QT interval. Ideally, investigations of the effect of a drug or condition on the QT intervals should be done at a constant heart rate. While this might be possible with measurements on the same subjects it is difficult to do when studies involve several subjects.

To be able to compare the QT intervals at different heart rates it is therefore necessary to calculate the heart rate corrected QT interval (QTc). The QTc is obtained by a formula that normalises the QT interval to the expected equivalent at an RR interval of 1 second (a heart rate of 60 bpm). The RR interval is the distance between two consecutive R peaks in the ECG. The RR interval is thus the instantaneous heart rate but usually the average RR interval over a number of beats is used for heart rate correction. The correction formula can be based on several models of QT-RR relationship including linear[1], Parabolic[2], logarithmic[3] and exponential[4] models:

$$QTc = QT + a \cdot (1 - RR) \qquad [1]$$
$$QTc = QT / RR^{a} \qquad [2]$$
$$QTc = a \cdot \ln(RR) \qquad [3]$$
$$QTc = a \cdot (e^{-RR} - 1/e) \qquad [4]$$

The most widely used formula in studies investigating changes in the QT interval is the square root formula by Bazett[55] [5].

$$QTc = QT / RR^{\frac{1}{2}} \qquad [5]$$

Bazett's formula has gained popularity mainly due to its simplicity but is frequently criticised for its tendency to overcorrect the QT interval at high heart rates and undercorrect it at lower

heart rates.[45,56,57] The cube root formula by Fridericia[58] [6] is often stated as being superior to Bazett's formula[45,59] but in a study by Rautaharju[56], Fridericias formula was found to have the worst performance in comparison with several multiparametrical formulas (Bazett's formula was the next worst formula).

$$QTc = QT / RR^{\frac{1}{3}}$$ [6]

The Framingham formula[60][7] and the Karjalainen nomogram method[31] are two additional heart rate correction methods frequently used.

$$QTc = QT + 1.54 \cdot (1 - RR)$$ [7]

The performance of all the mentioned formulas suffer from the fact that the QT/RR relationship is highly individual and thus no universal heart rate correction formula can give exact corrections of QT intervals in all subjects.[57] If the heart rates are within a narrow range around 60 bpm most correction formulas give satisfactory results. However if the heart rates are low or high, the errors from a given universal correction formula can result in erroneous results and conclusions. The problem can be mitigated by using subject specific heart rate correction formulas – i.e. correction formulas that are fitted to the individual placebo QT/RR data points. However, this procedure requires QT/RR data points in a certain range of heart rates which are not available in most studies. In Figure 6 the QT/RR data points from two different subjects are shown. It is seen how Bazett's formula overcorrects the QTc at high heart rates (short RR Intervals) in one subject (left) while a good correction is obtained in another subject (right).
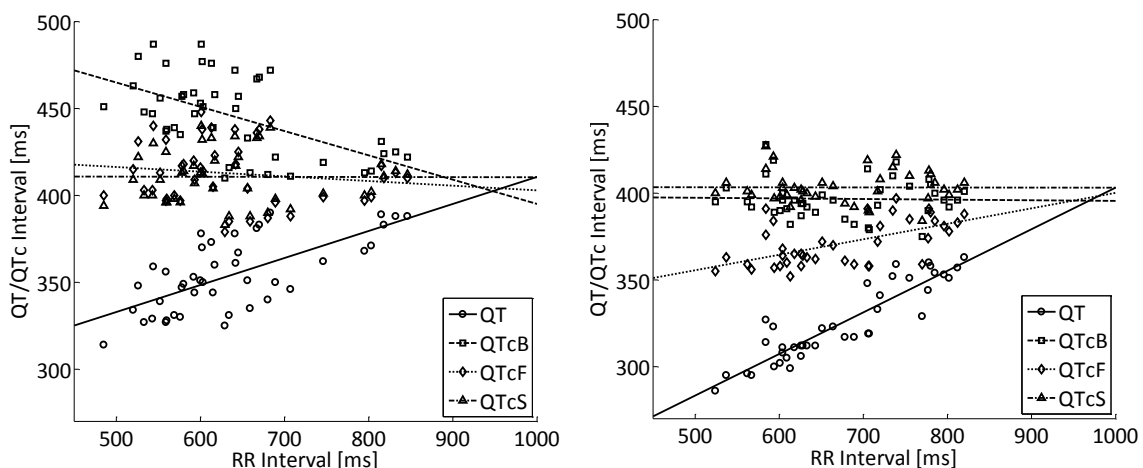
Figure 6. QT/QTc vs. RR interval relationship using Bazett's (QTcB), Fridericia's (QTcF), and a linear subject-specific method (QTcN) for euglycaemic measurements from subject 1 (left) and subject 7 (right). On the left, overcorrection of Bazett's formula is seen at low RR intervals (high heart rate), while Fridericia's formula is closer to the subjects specific method. The opposite is seen on the right side, where Fridericia's formula undercorrects the QTc, while Bazett's formula is closer to the subject specific method.

Changes in QTc during hypoglycaemia

The most common method for investigating changes in the QT interval during hypoglycaemia is the glucose clamp technique, where the blood glucose is clamped at either euglycaemia or hypoglycaemia using a constant intravenous infusion of insulin and a variable infusion of glucose.[61] Table 3 summarises the findings of eight studies of the effect of hypoglycaemia on the QT interval. The study populations include both healthy subjects, subjects with T1DM and subjects with T2DM. Marques[21] found a significant prolongation of the QTc during

hypoglycaemia of 583 [425–620] ms (median [range]) compared 429 [411–445] ms during euglycaemia in subjects with T1DM. A prolongation of this magnitude has however not been reproduced by other studies of patients with T1DM. Lee et al. showed a QTc prolongation from 407±34 at euglycaemia to 448±34 ms at hypoglycaemia in T1DM subjects. On the other hand Koivikko and colleagues[62] found a QTc of 410±31 at euglycaemia and 419±35 ms at hypoglycaemia, also in subjects with T1DM. In studies of healthy subjects the QTc during euglycaemia is typically 399-406 ms increasing to 450-480 ms during hypoglycaemia. Studies using both Bazett's and Fridericia's formulas indicate that the former might produce higher increases in QTc during hypoglycaemia than the latter, possibly indicating an overcorrection of the QT interval at the typically higher heart rates during hypoglycaemia.[27,62] It should be noted that the first five studies in the table are done by the same study group headed by Simon R. Heller. These studies in general show longer QT interval prolongations during hypoglycaemia than other studies.

Table 3. Studies of changes in the QTc during euglycaemic and hypoglycaemic glucose clamps.

Study	Population	QT Method	HR Correction	Euglycaemia		Hypoglycaemia	
				QTc[a]	ΔQTc[b]	QTc[c]	ΔQTc[d]
Marques[21]	T1DM	MA	Bazett	429	18	583	162
Robinson[23]	Healthy	SI	Bazett	400	20	450	70
Robinson[53]	Healthy	SI	Bazett	406	16	480	90
Lee[52]	T1DM	SI	Fridericia	407	16	448	57
Ireland[54]	Healthy	SI	Bazett	399	7	459	67
		MA	Bazett	414	-5	446	27
Koivikko[62]	T1DM	MA	Bazett	410	10	419	13
			Fridericia	397	4	403	5
	Healthy		Bazett	428	8	448	32
			Fridericia	426	11	440	22
Laitinen[27]	Healthy	SI	Bazett	408	9	429	30
			Fridericia	399	7	417	25
Landstedt-Hallin[63]	T2DM	MA	Bazett	438	11	491	61
			Mean:	412	10	452	49

[a]QTc [ms] measured at the end of euglycaemic clamp
[b]Change in QTc [ms] from baseline to the end of euglycaemic clamp
[c]QTc [ms] measured at the end of hypoglycaemic clamp
[d]Change in QTc [ms] from baseline to the end of hypoglycaemic clamp

Methodology for studying hypoglycaemia

While the glucose clamp technique provides excellent control and reproducibility it is not a realistic model of the clinical spontaneous hypoglycaemic episode experienced by persons with T1DM. Only a few studies have investigated the change in QTc during episodes of spontaneous hypoglycaemia in T1DM. Murphy et al.[25] studied the QTc in children and adolescents with T1DM during the night in an in-hospital setting where hourly blood samples were drawn during the night. During nights with hypoglycaemia (<3.5mmol/l) QTc was 412±22 ms compared to 401±19 ms during nights with no hypoglycaemia. In a similar setting with adult subjects, Robinson and colleagues[24] found an increase in QTc from baseline to nights with

hypoglycaemia (<2.5 mmol/l) of 27±15 ms whereas the increase from baseline to nights with no hypoglycaemia was 9±19 ms. Using Holter and CGM technology, Gill et al.[28] found a QTc on nights with hypoglycaemia (<3.5mmol/l) of 445±40 ms versus 415±23 ms on nights with no hypoglycaemia (>3.5mmol/l).

Mechanisms of changes in QTc during hypoglycaemia

Changes in QTc during hypoglycaemia are thought to be governed by two mechanisms: The sympatho-adrenal response to hypoglycaemia causing a release of catecholamines (adrenaline and noradrenaline) and lowered potassium concentration caused by elevated insulin and adrenaline levels.[64] Studies using both healthy subjects and subjects with T1DM have shown that blocking the effect of the catecholamines using a beta-blocker largely prevents QTc prolongation during hypoglycaemia.[52,23] Robinson and colleagues[23] found that infusion of potassium only reduced the QTc prolongation during hypoglycaemia slightly and concluded that QTc prolongation during hypoglycaemia was primarily caused by adrenaline.

However in the study by Murphy et al.[25] of spontaneous episodes of hypoglycaemia, adrenaline concentrations did not increase during nights with hypoglycaemia compared with nights without hypoglycaemia. Therefore the increase in QTc during hypoglycaemia in this study was more likely due to lowering of potassium.

Aim of PhD study

Changes in cardiac repolarisation during hypoglycaemia in T1DM are still far from being well understood. Although rare, the dead-in-bed syndrome is still a feared condition by people with diabetes. It is thus of importance to clarify the pathophysiological mechanisms.

Recent studies show no[62] or modest[27] increases in QTc during insulin induced hypoglycaemia whereas other studies show marked[23,52,53,54,63] or very large[21] increases in QTc. We suspect that methodological issues including QT measurement and heart rate correction may partly explain the differences.

Studies on cardiac repolarisation during spontaneous episodes of hypoglycaemia are few and are limited by difficulties in study design including the need for overnight sampling of blood glucose. The use of modern techniques including digital Holter and CGM might give a better insight into the changes in cardiac repolarisation during hypoglycaemia.

The glucose clamp technique for investigating changes in cardiac repolarisation does not resemble clinical episodes of hypoglycaemia. If, instead, hypoglycaemia was induced by a subcutaneous injection of insulin, the development of hypoglycaemia would resemble a spontaneous episode of hypoglycaemia more and at the same time enable controlled conditions.

We have previously published a physiological model of the effect of blood glucose and exercise on adrenaline.[65] Adrenaline constitutes together with potassium the main mechanisms behind the alterations in cardiac repolarisation. A physiological model of plasma potassium during hypoglycaemia might gain further insight into the dynamics of changes in cardiac repolarisation during hypoglycaemia.

Aims

The aim of the PhD thesis is thus to:
- Investigate the impact of different QT measurement techniques and heart rate correction methods on the QTc during hypoglycaemia
- Investigate changes in the QTc during spontaneous episodes of hypoglycaemia using Holter and CGM technology
- Investigate changes in QTc during simulated spontaneous episodes of hypoglycaemia by inducing hypoglycaemia by a subcutaneous injection of insulin.
- Investigate modelling approaches to gain further insight into the physiology behind ECG alterations during hypoglycaemia

Hypotheses

The hypotheses are:
1. The SI and MA techniques for measuring the QT interval can lead to different conclusions regarding the prolongation of QT during hypoglycaemia (investigated in *Paper I*).
2. Different methods of heart rate correction produce significantly different results when estimating the change in QTc from euglycaemia to hypoglycaemia (investigated in *Paper I+II+III*).
3. Spontaneous episodes of hypoglycaemia cause prolongation of QTc during hypoglycaemia (investigated in *Paper II*).
4. Hypoglycaemia induced by subcutaneous injection of insulin causes a prolongation of the QT interval (investigated in *Paper III*).
5. It is possible to model changes in potassium during episodes of hypoglycaemia (investigated in *Paper IV*).

References (introduction)

1. International Diabetes Federation: IDF Diabetes Atlas. 4th Edition:2009

2. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ: The prevalence of comorbid depression in adults with diabetes. *Diabetes Care* 24:1069-1078, 2001

3. Ben-Haroush A, Yogev Y, Hod M: Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. *Diabet Med* 21:103-113, 2004

4. Bennett PH and Knowler WC: Definition, Diagnosis, and Classification of Diabetes Mellitus and Glucose Homeostasis. In *Joslin's Diabetes Mellitus* (pp. 331-339), edited by Kahn CR, King GL, Moses AC, Weir GC, Jacobson AM, and Smith RJ, Boston, Lippincott Williams & Wilkins, 2005

5. American Diabetes Association: Standards of medical care in diabetes. *Diabetes Care* 31:12-54, 2008

6. "The Diabetes Control and Complications Trial Research Group": The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993

7. Cryer PE: Hypoglycaemia: The limiting factor in the glycaemic management of Type I and Type II Diabetes. *Diabetologia* 45:937-948, 2002

8. Fisher M and Heller SR: Mortality, Cardiovascular Morbidity and Possible Effects of Hypoglycaemia on Diabetic Complications. In *Hypoglycaemia in Clinical Diabetes* (pp. 265-283), edited by Frier BM and Fisher M, Chichester, Wiley, 2007

9. Strachan MWJ: Frequency, Causes and Risk Factors for Hypoglycaemia in Type 1 Diabetes. In *Hypoglycaemia in Clinical Diabetes* (pp. 49-81), edited by Frier BM and Fisher M, Chichester, Wiley, 2007

10. Watkins PJ, Amiel SA, Howell SL, and Turner E: Hypoglycaemia. In *Diabetes and its Management* (pp. 79-87), edited by Watkins PJ, Amiel SA, Howell SL, and Turner E, Oxford, Blackwell Publishing, 2003

11. Kerr D and Richardson T: Counterregulatory Deficiencies in Diabetes. In *Hypoglycaemia in Clinical Diabetes* (pp. 121-140), edited by Frier BM and Fisher M, Chichester, Wiley, 2007

12. Cryer PE, Davis SN, Shamoon H: Hypoglycemia in diabetes. *Diabetes Care* 26:1902-1912, 2003

13. Fiallo-Scharer R: Diabetes Research in Children Network Study Group. Eight-point glucose testing versus the continuous glucose monitoring system in evaluation of glycemic control in type 1 diabetes. *J Clin Endocrinol Metab* 90:3387-3391, 2005

14. Larsen J, Ford T, Lyden E, Colling C, Mack-Shipman L, Lane J: What is hypoglycemia in patients with well-controlled type 1 diabetes treated by subcutaneous insulin pump with use of the continuous glucose monitoring system? *Endocr Pract* 10:324-329, 2004

15. Frier BM and Fisher BM: Impaired hypoglycaemia awareness (pp. 111-146), edited by Frier BM and Fisher BM, Chichester, U.K., John Wiley and Sons, 1999

16. Tattersall RB, Gill GV: Unexplained deaths of type 1 diabetic patients. *Diabet Med* 8:49-58, 1991

17. Weston PJ, Gill GV: Is undetected autonomic dysfunction responsible for sudden death in Type 1 diabetes mellitus? The'dead in bed'syndrome revisited. *Diabet Med* 16:626-631, 1999

18. Sovik O, Thordarson H: Dead-in-bed syndrome in young diabetic patients. *Diabetes Care* 22:B40-2, 1999

19. Collier A, Matthews DM, Young RJ, Clarke BF: Transient atrial fibrillation precipitated by hypoglycaemia: two case reports. *Br Med J* 63:895-897, 1987

20. Lindström T, Jorfeldt L, Tegler L, Arnqvist HJ: Hypoglycaemia and cardiac arrhythmias in patients with type 2 diabetes mellitus. *Diabet Med* 9:536-541, 1992

21. Marques JLB, George E, Peacey SR, Harris ND, Macdonald IA, Cochrane T, Heller SR: Altered ventricular repolarization during hypoglycaemia in patients with diabetes. *Diabet Med* 14:648-654, 1997

22. Eckert B, Agardh CD: Hypoglycaemia leads to an increased QT interval in normal men. *Clin Physiol* 18:570-575, 1998

23. Robinson RT, Harris ND, Ireland RH, Lee S, Newman C, Heller SR: Mechanisms of abnormal cardiac repolarization during insulin-induced hypoglycemia. *Diabetes* 52:1469-1474, 2003

24. Robinson RT, Harris ND, Ireland RH, Macdonald IA, Heller SR: Changes in cardiac repolarization during clinical episodes of nocturnal hypoglycaemia in adults with Type 1 diabetes. *Diabetologia* 47:312-315, 2004

25. Murphy NP, Ford-Adams ME, Ong KK, Harris ND, Keane SM, Davies C, Ireland RH, Macdonald IA, Knight EJ, Edge JA: Prolonged cardiac repolarisation during spontaneous nocturnal hypoglycaemia in children and adolescents with type 1 diabetes. *Diabetologia* 47:1940-1947, 2004

26. Suys B, Heuten S, De Wolf D, Verherstraeten M, de Beeck LO, Matthys D, Vrints C, Rooman R: Glycemia and Corrected QT Interval Prolongation in Young Type 1 Diabetic Patients What is the relation? *Diabetes Care* 29:427-429, 2006

27. Laitinen T, Lyyra-Laitinen T, Huopio H, Vauhkonen I, Halonen T, Hartikainen J, Niskanen L, Laakso M: Electrocardiographic alterations during hyperinsulinemic

hypoglycemia in healthy subjects. *Annals of Noninvasive Electrocardiology* 13:97-105, 2008

28. Gill GV, Woodward A, Casson IF, Weston PJ: Cardiac arrhythmia and nocturnal hypoglycaemia in type 1 diabetes - the 'dead in bed' syndrome revisited. *Diabetologia* 52:42-45, 2009

29. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J: QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest. *Circulation* 83:1888-1894, 1991

30. Moss AJ: Measurement of the QT interval and the risk associated with QTc interval prolongation: a review. *Am J Cardiol* 72:23B-25B, 1993

31. Karjalainen J, Reunanen A, Ristola P, Viitasalo M: QT interval as a cardiac risk factor in a middle aged population. *Br Med J* 77:543-548, 1997

32. Yap YG, Camm AJ: Drug induced QT prolongation and torsades de pointes. *Br Med J* 89:1363-1372, 2003

33. Whitsel EA, Boyko EJ, Rautaharju PM, Raghunathan TE, Lin D, Pearce RM, Weinmann SA, Siscovick DS: Electrocardiographic QT interval prolongation and risk of primary cardiac arrest in diabetic patients. *Diabetes Care* 28:2045-2047, 2005

34. Straus SMJM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MCJM, Stricker BHC: Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J Am Coll Cardiol* 47:362-367, 2006

35. Veglio M, Sivieri R, Chinaglia A, Scaglione L, Cavallo-Perin P: QT interval prolongation and mortality in type 1 diabetic patients: a 5-year cohort prospective study. Neuropathy Study Group of the Italian Society of the Study of Diabetes, Piemonte Affiliate. *Diabetes Care* 23:1381-1383, 2000

36. Padmanabhan S, Silvet H, Amin J, Pai RG: Prognostic value of QT interval and QT dispersion in patients with left ventricular systolic dysfunction: results from a cohort of 2265 patients with an ejection fraction of 40%. *Am Heart J* 145:132-138, 2003

37. Schwartz PJ, Wolf S: QT interval prolongation as predictor of sudden death in patients with myocardial infarction. *Circulation* 57:1074-1077, 1978

38. Rana BS, Lim PO, Naas AAO, Ogston SA, Newton RW, Jung RT, Morris AD, Struthers AD: QT interval abnormalities are often present at diagnosis in diabetes and are better predictors of cardiac death than ankle brachial pressure index and autonomic function tests. *Br Med J* 91:44-50, 2005

39. Rossing P, Breum L, Major-Pedersen A, Sato A, Winding H, Pietersen A, Kastrup J, Parving HH: Prolonged QTc interval predicts mortality in patients with Type 1 diabetes mellitus. *Diabet Med* 18:199-205, 2001

40. Elming H, Holm E, Jun L, Torp-Pedersen C, Kober L, Kircshoff M, Malik M, Camm J: The prognostic value of the QT interval and QT interval dispersion in all-cause and cardiac mortality and morbidity in a population of Danish citizens. *Eur Heart J* 19:1391-1400, 1998

41. Montanez A, Ruskin JN, Hebert PR, Lamas GA, Hennekens CH: Prolonged QTc interval and risks of total and cardiovascular mortality and sudden death in the general population: a review and qualitative overview of the prospective cohort studies. *Archives of Internal Medicine* 164:943-948, 2004

42. Goldberg RJ, Bengtson J, Chen ZY, Anderson KM, Locati E, Levy D: Duration of the QT interval and total and cardiovascular mortality in healthy persons (The Framingham Heart Study experience). *Am J Cardiol* 67:55-58, 1991

43. Roden DM: Drug-induced prolongation of the QT interval. *N Engl J Med* 350:1013-1022, 2004

44. Diercks DB, Shumaik GM, Harrigan RA, Brady WJ, Chan TC: Electrocardiographic manifestations: electrolyte abnormalities. *J Emerg Med* 27:153-160, 2004

45. Goldenberg I, Moss AJ, Zareba W: QT interval: how to measure it and what is" normal". *J Cardiovasc Electrophysiol* 17:333-336, 2006

46. McLaughlin NB, Campbell RW, Murray A: Comparison of automatic QT measurement techniques in the normal 12 lead electrocardiogram. *Br Med J* 74:84-89, 1995

47. McLaughlin NB, Campbell RW, Murray A: Accuracy of four automatic QT measurement techniques in cardiac patients and healthy subjects. *Br Med J* 76:422-426, 1996

48. Molnar J, Ranade V, Cvetanovic I, Molnar Z, Somberg JC: Evaluation of a 12-Lead Digital Holter System for 24-Hour QT Interval Assessment. *Cardiology* 106:224-232, 2006

49. Moody GB, Koch H, Steinhoff U: The physionet/computers in cardiology challenge 2006: Qt interval measurement. *Comput Cardiol* Volume 33:2006

50. Lepeschkin E, Surawicz B: The measurement of the QT interval of the electrocardiogram. *Circulation* 6:378-388, 1952

51. Malik M and Batchvarov V: Measurement of the QT interval. In *QT dispersion* (pp. 5-9), edited by Malik M and Batchvarov V, New York, Futura Publishing Company, Inc, 2000

52. Lee SP, Harris ND, Robinson RT, Davies C, Ireland R, Macdonald IA, Heller SR: Effect of atenolol on QTc interval lengthening during hypoglycaemia in type 1 diabetes. *Diabetologia* 48:1269-1272, 2005

53. Robinson RT, Harris ND, Ireland RH, Lindholm A, Heller SR: Comparative effect of human soluble insulin and insulin aspart upon hypoglycaemia-induced alterations in cardiac repolarization. *Br J Clin Pharmacol* 55:246-251, 2003

54. Ireland RH, Robinson RT, Heller SR, Marques JL, Harris ND: Measurement of high resolution ECG QT interval during controlled euglycaemia and hypoglycaemia. *Physiol Meas* 21:295-303, 2000

55. Bazett HC: An analysis of the time relations of electrocardiograms. *Heart* 7:353-370, 1920

56. Rautaharju PM, Warren JW, Calhoun HP: Estimation of QT prolongation. A persistent, avoidable error in computer electrocardiography. *J Electrocardiol* 23:111-117, 1990

57. Malik M, Farbom P, Batchvarov V, Hnatkova K, Camm AJ: Relation between QT and RR intervals is highly individual among healthy subjects: implications for heart rate correction of the QT interval. *Br Med J* 87:220-228, 2002

58. Fridericia L: Die Systolendauer im Electrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Med Scand* 53:469-486, 1920

59. Funck-Brentano C, Jaillon P: Rate-corrected QT interval: techniques and limitations. *Am J Cardiol* 72:17-22, 1993

60. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D: An improved method for adjusting the QT interval for heart rate(the framingham heart study). *Am J Cardiol* 70:797-801, 1992

61. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol Gastrointest Liver Physiol* 237:214-223, 1979

62. Koivikko ML, Karsikas M, Salmela PI, Tapanainen JS, Ruokonen A, Seppänen T, Huikuri HV, Perkiömäki JS: Effects of controlled hypoglycaemia on cardiac repolarisation in patients with type 1 diabetes. *Diabetologia* 51:426-435, 2008

63. Landstedt-Hallin L, Englund A, Adamson U, Lins PE: Increased QT dispersion during hypoglycaemia in patients with type 2 diabetes mellitus. *J Intern Med* 246:299-1999

64. Lee S, Harris ND, Robinson RT, Yeoh L, Macdonald IA, Heller SR: Effects of adrenaline and potassium on QTc interval and QT dispersion in man. *Eur J Clin Invest* 33:93-98, 2003

65. Kildegaard J, Christensen TF, Johansen MD, Randlov J, Hejlesen OK: Modeling the Effect of Blood Glucose and Physical Exercise on Plasma Adrenaline in People with Type 1 Diabetes. *Diabetes Technol Ther* 9:501-508, 2007

PAPER I

QT MEASUREMENT AND HEART RATE CORRECTION DURING HYPOGLYCEMIA: IS THERE A BIAS?

Christensen TF[1,2], Randløv J[2], Kristensen LE[2], Eldrup E[3], Hejlesen OK[1], Struijk JJ[1]
[1]*Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*
[2]*Novo Nordisk A/S, Hillerød, Denmark*
[3]*Steno Diabetes Center, Gentofte, Denmark*

PAPER II

QT PROLONGATION DURING SPONTANEOUS EPISODES OF HYPOGLYCAEMIA IN TYPE 1 DIABETES – THE IMPACT OF HEART RATE CORRECTION

Christensen TF[1,2], Tarnov L[3], Randløv J[2], Kristensen LE[2], Struijk JJ[1], Eldrup E[3], Hejlesen OK[1]

[1]*Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*
[2]*Novo Nordisk A/S, Hillerød, Denmark*
[3]Steno Diabetes Center, Gentofte, Denmark

PAPER III

QTC DURING HYPOGLYCAEMIA FOLLOWING SUBCUTANEOUS INSULIN ADMINISTRATION IN PEOPLE WITH TYPE 1 DIABETES

Christensen TF[1,2], Tarnow L[3], Randløv J[2], Kristensen LE[2], Struijk JJ[1], Eldrup E[3], Hejlesen OK[1]

[1]*Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*
[2]*Novo Nordisk A/S, Hillerød, Denmark*
[3]*Clinical Research Unit, Steno Diabetes Center, Gentofte, Denmark*

PAPER IV

A PHYSIOLOGICAL MODEL OF THE EFFECT OF HYPOGLYCAEMIA ON PLASMA POTASSIUM

Christensen TF[1,2], Bækgaard M[1], Dideriksen JL[1], Steimle KL[1], Mogensen ML[1], Kildegaard J[2], Struijk JJ[1], Eldrup E[3], Hejlesen OK[1]
[1]*Department of Health Science and Technology, Aalborg University, Aalborg, Denmark*
[2]*Novo Nordisk A/S, Hillerød, Denmark*

DISCUSSION AND CONCLUSIONS

Several studies have reported a prolongation of the QTc during hypoglycaemia which has been seen as an indication of hypoglycaemia-induced cardiac arrhythmia being responsible for the 'dead-in-bed' syndrome. In the present PhD project we have investigated several issues regarding the changes in the QTc interval during hypoglycaemia.

QT measurement

In *Paper I* we compared two methods, the semi-automatic slope-intersect technique and the manual annotation for measuring the QT interval. The two methods produced very different results. The slope-intersect method showed a significant increase in QTc corrected by Bazett's formula (QTcB) during hypoglycaemia of 40 ms while the increase using the manual annotation was merely 7 ms. The fact that two acknowledged methods for measuring the QT interval can differ to such a degree clearly shows one of the issues associated with the QT interval as a parameter for quantifying cardiac repolarisation. Part of the explanation of the differences between the two methods can be found in the morphological change of the ECG during hypoglycaemia and hyperinsulinaemia. Hypoglycaemia and hyperinsulinaemia both cause a lowering of extracellular potassium known to reduce T wave amplitude and to cause pronounced U waves. The low amplitude T waves and U waves make it more difficult to measure the end of the T wave and when the slope-intersect method is employed an overestimation of the end of the T-wave is difficult to avoid. The advantage of the slope-intersect method over the manual annotation is that it is much easier to define resulting in lower inter-observer differences.[1] It cannot from our results be concluded which method is the more correct. As long as a detailed description of the methodology for measuring the QT interval is provided, the reader can incorporate it in the interpretation of the result. So when e.g. the slope-intersect method is used to measure the QT interval during the insulin induced hypoglycaemia it is known that the result might overestimate the actual QT prolongation due to hypokalaemia. For this reason, proprietary fully automatic QT measurement techniques as used in some studies of hypoglycaemia[2,3] can make the interpretation of the results difficult.

In *Paper II* and *Paper III* we decided to use the slope-intersect method for two reasons. First, the method is easy to implement in a computer algorithm which increased the speed of data analysis significantly. Second the manual measurements as used in *Paper I* were expensive and not readily available. Third and most important, the majority of studies of QT interval changes during hypoglycaemia employ the slope-intersect method. The results from *Paper I* indicate that comparing results from studies using different QT interval measurement techniques is associated with uncertainties. By using the slope-intersect method in our investigations comparisons of our results to the literature can be made with a higher degree of certainty.

Spontaneous hypoglycaemia

In *Paper II* we investigated the change in QTc during spontaneous hypoglycaemia in an ambulant setting using a CGM and a high resolution digital Holter monitor. We suspected that previous findings of modest QTc prolongation during spontaneous hypoglycaemia[4,5] could partly be caused by overcorrection of Bazett's formula. In accordance with the literature we found a significant albeit modest increase in QTcB of 11 ms. However, using other popular

correction methods including the allegedly superior subject specific correction (QTcS) we did not find a significant increase in QTc. As we included all measurements of hypoglycaemia in our analysis and not only measurements of nocturnal hypoglycaemia our results can not be directly compared with previous studies. However, the difference in heart rate between euglycaemia and hypoglycaemia is stated in none of the studies of spontaneous hypoglycaemia.[2,3,4,5] Thus, overcorrection of Bazett's formula can not be ruled out as an explanation of the observed difference in QTc between euglycaemia and hypoglycaemia. Our findings regarding heart rate correction in *Paper II* are supported by the results in *Paper I* and partly by the results in *Paper III*. In *Paper I* the increase in QTcB using the manual annotation method is significant while the increase in QTcF is not. In *Paper III* there is a significant increase in both QTcB and QTcF, however the increase in QTcF is smaller than the increase in QTcB. Since it was not possible in these studies to calculate QTcS, we do not know if the difference between Bazett's and Fridericia's formula is overcorrection by the former or undercorrection by the latter. However the results underline the problems associated with heart rate correction in studies of hypoglycaemia.

Subcutaneous insulin induced hypoglycaemia

Paper III describes a novel way of investigating physiological changes during induced hypoglycaemia that resembles clinical episodes of hypoglycaemia. The study filled a gap between the hyperinsulinaemic glucose clamp technique and the observational studies of spontaneous hypoglycaemia. The clamp technique provides reproducible episodes of hypoglyaemia with low variability but uses supraphysiological levels of insulin and does not resemble clinical episodes of hypoglycaemia. On the other hand, true spontaneous episodes of hypoglycaemia are difficult to investigate because a large number of participants are needed (to obtain a sufficient number of episodes), the episodes have great variability and it is difficult to obtain controlled conditions. In *Paper III,* to fill this gap, we used subcutaneous administration of insulin to induce hypoglycaemia in adults with T1DM. The amount of insulin was realistic and the changes in blood glucose thus resembled a clinical episode of hypoglycaemia. The method allowed us to do true control episodes where all conditions were held equal except the blood glucose which was maintained at euglycaemia by administration of glucose. The results from the study showed that the increase in QTcB from baseline to hypoglycaemia was 30 ms of which 18 ms could be attributable to hypoglycaemia per se when correcting for other factors using the control episode. With QTcF the prolongation attributable to hypoglycaemia was 11 ms. The results from *Paper III* also showed that while the decrease in potassium caused QTc prolongation during both hypoglycaemia and control, the likely mechanism for the additional prolongation during hypoglycaemia was mediated by an increase in adrenaline levels. Thus, the results indicate that hypoglycaemia induced by a subcutaneous bolus of insulin causes QT interval prolongation but that the prolongation is not of the magnitude seen in most clamp studies.

The design of the study in *Paper III* is not without issues. We chose a design where the amount of insulin was adjusted to the fasting glucose and the estimated insulin sensitivity. The estimations were carried out by an experienced diabetologist and the resulting blood glucose levels during hypoglycaemia were within target in all but one case. The alternative to the chosen approach would be to use a constant dose of e.g. 0.15 U/kg. We did not choose this approach because we wanted the episodes to resemble spontaneous episodes with insulin levels as realistic as possible. If we had used a constant dose of 0.15 U/kg it would have resulted in higher insulin concentrations and in many cases glucose infusion would be required. The fact that the amount of insulin was estimated does however make the design of

the study more difficult to reproduce. We chose not to randomise the hypoglycaemia control sequence because we wanted the procedures during the control episodes to be carried out exactly as during the hypoglycaemia day. This would not be possible if the control sequence were first, as the time of hypoglycaemia following insulin injection would be unknown. The close proximity of hypoglycaemia and control was not optimal. The design with only one day between hypoglycaemia and control was chosen to reduce the number of study visits and required time to run the study. A longer period between the two visits would mean that an additional visit was required since one CGM sensor only lasts 72 hours. However, the significant change in baseline blood glucose, QTcB and QTcF could indicate that one day between hypoglycaemia and control was too short.

Physiological modelling of potassium

In **Paper IV** we describe the, to our knowledge, first attempt to model changes in the potassium during episodes of hypoglycaemia. As we showed in **Paper III**, potassium plays a major role in the change of cardiac repolarisation during insulin-induced hypoglycaemia. A physiological model of potassium thus provides a way to analyse the physiology behind the changes in cardiac repolarisation during hypoglycaemia. The model in general simulated potassium levels accurately although the test material for the model was limited. Our initial idea was to combine the potassium model with a model of adrenaline as we have previously published.[6] When we are able to model the effect of hypoglycaemia on both hypokalaemia and adrenaline, we might be able to simulate the potential effect of hypoglycaemia on cardiac repolarisation in a wide range of situations. Another possibility would be to simulate the changes QTc based on measured or simulated potassium and adrenaline levels.

The model was built solely on data obtained from the literature by decoding graphs. As such the model becomes only as accurate as the data used for estimating the model parameters. Also the use of the potassium model in people with diabetes might prove difficult because of variations in insulin sensitivity that could affect the stimulatory effect of insulin on potassium levels.

An obvious next step would be to use the data from **Paper III** in further tests and development of the potassium model and the adrenaline model. This would enable better modelling and hopefully deeper understanding of the physiological mechanisms responsible for the QT interval prolongation during hypoglycaemia

Conclusions

The aim of the present thesis was to investigate the change in repolarisation and QTc during both induced and spontaneous hypoglycaemia as well as to understand the underlying physiology. In addition, I wanted to investigate how the methodology of measuring the QT interval, heart rate correcting the QT interval and inducing hypoglycaemia affected the change in QTc during hypoglycaemia. To answer these questions two studies were designed (Paper II and Paper III) and data from a previous study (Paper I) was used. The conclusions to the hypotheses tested are:

Hypothesis 1:	*Conclusion:*
The semi-automatic slope intersect and the manual annotation techniques for measuring the QT interval can lead to different conclusions regarding the prolongation of QT during hypoglycaemia	Yes, indeed. In one of the studies slope-intersect method showed a QTc prolongation of 40 ms, while the manual annotation method showed a prolongation of 7ms. Both methods have advantaged but comparisons of studies using the different methods should be done with caution.

Hypothesis 2:	*Conclusion:*
Different methods of heart rate correction produce significantly different results when estimating the change in QTc from euglycaemia to hypoglycaemia.	Yes, in two of our studies the use of Bazett's formula resulted in different conclusions than the use of Fridericia's formula. In one study the two formulas produced different degrees of prolongation but resulted in the same conclusion. It is not clear whether this difference is due to overcorrection by Bazett's or undercorrection by Fridericia's formula. We recommend the use of both formula and a subject specific formula when possible.

Hypothesis 3:	*Conclusion:*
Spontaneous episodes of hypoglycaemia cause prolongation of QTc during hypoglycaemia.	Yes and no, spontaneous hypoglycaemia in our study cause changes in the QTcB, but not QTcF or QTcS. We therefore conclude that the increase in QTcB during spontaneous hypoglycaemia may be due to overcorrection by Bazett's formula.

Hypothesis 4:	*Conclusion:*
Hypoglycaemia induced by subcutaneous injection of insulin cause a prolongation of the QT interval.	Yes, we observed significant changes in both QTcB and QTcF during hypoglycaemia after controlling for other factors using control episodes.

Hypothesis 5:	*Conclusion:*
It is possible to model changes in potassium during episodes of hypoglycaemia	Yes, we developed a model of potassium changes during hypoglycaemia that was reasonably accurate. However, further validation of the model is needed on more episodes of hypoglycaema.

References (discussion)

1. Ireland RH, Robinson RT, Heller SR, Marques JL, Harris ND: Measurement of high resolution ECG QT interval during controlled euglycaemia and hypoglycaemia. *Physiol Meas* 21:295-303, 2000

2. Gill GV, Woodward A, Casson IF, Weston PJ: Cardiac arrhythmia and nocturnal hypoglycaemia in type 1 diabetes - the 'dead in bed' syndrome revisited. *Diabetologia* 52:42-45, 2009

3. Suys B, Heuten S, De Wolf D, Verherstraeten M, de Beeck LO, Matthys D, Vrints C, Rooman R: Glycemia and Corrected QT Interval Prolongation in Young Type 1 Diabetic Patients What is the relation? *Diabetes Care* 29:427-429, 2006

4. Murphy NP, Ford-Adams ME, Ong KK, Harris ND, Keane SM, Davies C, Ireland RH, Macdonald IA, Knight EJ, Edge JA: Prolonged cardiac repolarisation during spontaneous nocturnal hypoglycaemia in children and adolescents with type 1 diabetes. *Diabetologia* 47:1940-1947, 2004

5. Robinson RT, Harris ND, Ireland RH, Macdonald IA, Heller SR: Changes in cardiac repolarization during clinical episodes of nocturnal hypoglycaemia in adults with Type 1 diabetes. *Diabetologia* 47:312-315, 2004

6. Kildegaard J, Christensen TF, Johansen MD, Randlov J, Hejlesen OK: Modeling the Effect of Blood Glucose and Physical Exercise on Plasma Adrenaline in People with Type 1 Diabetes. *Diabetes Technol Ther* 9:501-508, 2007

COMPLETE LIST OF REFERENCES

1. "The Diabetes Control and Complications Trial Research Group": The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993

2. Afonso VX, Tompkins WJ, Nguyen TQ, Luo S, Inc ES, Saint Paul MN: ECG beat detection using filter banks. *IEEE Trans Biomed Eng* 46:192-202, 1999

3. Algra A, Tijssen JG, Roelandt JR, Pool J, Lubsen J: QTc prolongation measured by standard 12-lead electrocardiography is an independent risk factor for sudden death due to cardiac arrest. *Circulation* 83:1888-1894, 1991

4. American Diabetes Association: Standards of medical care in diabetes. *Diabetes Care* 31:12-54, 2008

5. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ: The prevalence of comorbid depression in adults with diabetes. *Diabetes Care* 24:1069-1078, 2001

6. Aytemir K, Maarouf N, Gallagher MM, Yap YG, Waktare JE, Malik M: Comparison of formulae for heart rate correction of QT interval in exercise electrocardiograms. *Pacing Clin Electrophysiol* 22:1397-1401, 1999

7. Bazett HC: An analysis of the time relations of electrocardiograms. *Heart* 7:353-370, 1920

8. Bell DSH: Dead in bed syndrome - a hypothesis. *Diabetes Obes Metab* 8:261-263, 2006

9. Ben-Haroush A, Yogev Y, Hod M: Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. *Diabet Med* 21:103-113, 2004

10. Benatar A, Decraene T: Comparison of formulae for heart rate correction of QT interval in exercise ECGs from healthy children. *Br Med J* 86:199-202, 2001

11. Bennett PH and Knowler WC: Definition, Diagnosis, and Classification of Diabetes Mellitus and Glucose Homeostasis. In *Joslin's Diabetes Mellitus* (pp. 331-339), edited by Kahn CR, King GL, Moses AC, Weir GC, Jacobson AM, and Smith RJ, Boston, Lippincott Williams & Wilkins, 2005

12. Beretta-Piccoli C, Weidmann P, Flammer J, Glück Z, Bachmann C: Effects of standard oral glucose loading on the renin-angiotensin-aldosterone system and its relationship to circulating insulin. *J Mol Med* 58:467-474, 1980

13. Bode B, Gross K, Rikalo N, Schwartz S, Wahl T, Page C, Gross T, Mastrototaro J: Alarms based on real-time sensor glucose values alert patients to hypo-and hyperglycemia: the guardian continuous monitoring system. *Diabetes Technol Ther* 6:105-113, 2004

14. Boyle PJ, Nagy RJ, O'Connor AM, Kempers SF, Yeo RA, Qualls C: Adaptation in brain glucose uptake following recurrent hypoglycemia. *Proc Natl Acad Sci U S A* 91:9352-9356, 1994

15. Brown MJ, Brown DC, Murphy MB: Hypokalemia from beta2-receptor stimulation by circulating epinephrine. *N Engl J Med* 309:1414-1419, 1983

16. Campbell I: Dead in bed syndrome: a new manifestation of nocturnal hypoglycaemia? *Diabet Med* 8:3-4, 1991

17. Christensen TF, Lewinsky I, Kristensen LE, Randlov J, Poulsen JU, Eldrup E, Pater C, Hejlesen OK, Struijk JJ: QT Interval Prolongation during Rapid Fall in Blood Glucose in Type I Diabetes. *Comput Cardiol* 34:345-348, 2007

18. Clausen T, Flatman JA: The effect of catecholamines on Na-K transport and membrane potential in rat soleus muscle. *J Physiol* 270:383-414, 1977

19. Collier A, Matthews DM, Young RJ, Clarke BF: Transient atrial fibrillation precipitated by hypoglycaemia: two case reports. *Br Med J* 63:895-897, 1987

20. Cryer PE: Hypoglycaemia: The limiting factor in the glycaemic management of Type I and Type II Diabetes. *Diabetologia* 45:937-948, 2002

21. Cryer PE: Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. *Diabetes* 54:3592-3601, 2005

22. Cryer PE, Davis SN, Shamoon H: Hypoglycemia in diabetes. *Diabetes Care* 26:1902-1912, 2003

23. DeFronzo RA, Felig P, Ferrannini E, Wahren J: Effect of graded doses of insulin on splanchnic and peripheral potassium metabolism in man. *Am J Physiol Endocrinol Metab* 238:421-427, 1980

24. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol Gastrointest Liver Physiol* 237:214-223, 1979

25. Diercks DB, Shumaik GM, Harrigan RA, Brady WJ, Chan TC: Electrocardiographic manifestations: electrolyte abnormalities. *J Emerg Med* 27:153-160, 2004

26. Due-Andersen R, Hoi-Hansen T, Larroude CE, Olsen NV, Kanters JK, Boomsma F, Pedersen-Bjergaard U, Thorsteinsson B: Cardiac repolarization during hypoglycaemia in type 1 diabetes: impact of basal renin-angiotensin system activity. *Europace* 10:860-867, 2008

27. Due-Andersen R, Hoi-Hansen T, Olsen NV, Larroude CE, Kanters JK, Boomsma F, Pedersen-Bjergaard U, Thorsteinsson B: Cardiac repolarization during hypoglycaemia and hypoxaemia in healthy males: impact of renin-angiotensin system activity. *Europace* 10:219-226, 2008

28. Eckert B, Agardh CD: Hypoglycaemia leads to an increased QT interval in normal men. *Clin Physiol* 18:570-575, 1998

29. Elming H, Holm E, Jun L, Torp-Pedersen C, Kober L, Kircshoff M, Malik M, Camm J: The prognostic value of the QT interval and QT interval dispersion in all-cause and cardiac mortality and morbidity in a population of Danish citizens. *Eur Heart J* 19:1391-1400, 1998

30. Elming H, Sonne J, Lublin HKF: The importance of the QT interval: a review of the literature. *Acta Psychiatr Scand* 107:96-101, 2003

31. Fiallo-Scharer R: Diabetes Research in Children Network Study Group. Eight-point glucose testing versus the continuous glucose monitoring system in evaluation of glycemic control in type 1 diabetes. *J Clin Endocrinol Metab* 90:3387-3391, 2005

32. Field MJ, Giebisch GJ: Hormonal control of renal potassium excretion. *Kidney Int* 27:379-387, 1985

33. Fisher BM, Thomson I, Hepburn DA, Frier BM: Effects of adrenergic blockade on serum potassium changes in response to acute insulin-induced hypoglycemia in nondiabetic humans. *Diabetes Care* 14:548-552, 1991

34. Fisher M and Heller SR: Mortality, Cardiovascular Morbidity and Possible Effects of Hypoglycaemia on Diabetic Complications. In *Hypoglycaemia in Clinical Diabetes* (pp. 265-283), edited by Frier BM and Fisher M, Chichester, Wiley, 2007

35. Fridericia L: Die Systolendauer im Electrokardiogramm bei normalen Menschen und bei Herzkranken. *Acta Med Scand* 53:469-486, 1920

36. Frier BM and Fisher BM: Impaired hypoglycaemia awareness (pp. 111-146), edited by Frier BM and Fisher BM, Chichester, U.K., John Wiley and Sons, 1999

37. Funck-Brentano C, Jaillon P: Rate-corrected QT interval: techniques and limitations. *Am J Cardiol* 72:17-22, 1993

38. Garson AJ: How to measure the QT interval: what is normal? *Am J Cardiol* 72:14-16, 1993

39. Gastaldelli A, Emdin M, Conforti F, Camastra S, Ferrannini E: Insulin prolongs the QTc interval in humans. *Am J Physiol Regul Integr Comp Physiol* 279:2022-2025, 2000

40. Gavryck WA, Moore RD, Thompson RC: Effect of insulin upon membrane-bound (Na++ K+)-ATPase extracted from frog skeletal muscle. *J Physiol* 252:43-58, 1975

41. Gill GV, Woodward A, Casson IF, Weston PJ: Cardiac arrhythmia and nocturnal hypoglycaemia in type 1 diabetes - the 'dead in bed' syndrome revisited. *Diabetologia* 52:42-45, 2009

42. Goldberg RJ, Bengtson J, Chen ZY, Anderson KM, Locati E, Levy D: Duration of the QT interval and total and cardiovascular mortality in healthy persons (The Framingham Heart Study experience). *Am J Cardiol* 67:55-58, 1991

43. Goldenberg I, Moss AJ, Zareba W: QT interval: how to measure it and what is" normal". *J Cardiovasc Electrophysiol* 17:333-336, 2006

44. Gordon RD, Bachmann AW, Ballantine DM, Thompson RE: Potassium, glucose, insulin interrelationships during adrenaline infusion in normotensive and hypertensive humans. *Clin Exp Pharmacol Physiol* 18:291-294, 1991

45. Heller SR, Robinson RT: Hypoglycaemia and associated hypokalaemia in diabetes: mechanisms, clinical implications and prevention. *Diabetes Obes.Metab* 2:75-82, 2000

46. International Diabetes Federation: IDF Diabetes Atlas. 4th Edition:2009

47. Ireland RH, Robinson RT, Heller SR, Marques JL, Harris ND: Measurement of high resolution ECG QT interval during controlled euglycaemia and hypoglycaemia. *Physiol Meas* 21:295-303, 2000

48. Karjalainen J, Reunanen A, Ristola P, Viitasalo M: QT interval as a cardiac risk factor in a middle aged population. *Br Med J* 77:543-548, 1997

49. Karjalainen J, Viitasalo M, Manttari M, Manninen V: Relation between QT intervals and heart rates from 40 to 120 beats/min in rest electrocardiograms of men and a simple method to adjust QT interval values. *J Am Coll Cardiol* 23:1547-1553, 1994

50. Kerr D and Richardson T: Counterregulatory Deficiencies in Diabetes. In *Hypoglycaemia in Clinical Diabetes* (pp. 121-140), edited by Frier BM and Fisher M, Chichester, Wiley, 2007

51. Kildegaard J, Christensen TF, Johansen MD, Randlov J, Hejlesen OK: Modeling the Effect of Blood Glucose and Physical Exercise on Plasma Adrenaline in People with Type 1 Diabetes. *Diabetes Technol Ther* 9:501-508, 2007

52. Koivikko ML, Karsikas M, Salmela PI, Tapanainen JS, Ruokonen A, Seppänen T, Huikuri HV, Perkiömäki JS: Effects of controlled hypoglycaemia on cardiac repolarisation in patients with type 1 diabetes. *Diabetologia* 51:426-435, 2008

53. Kolloch RE, Kruse HJ, Friedrich R, Ruppert M, Overlack A, Stumpe KO: Role of epinephrine-induced hypokalemia in the regulation of renin and aldosterone in humans. *J Lab Clin Med* 127:50-56, 1996

54. Koltin D, Daneman D: Dead-in-bed syndrome -a diabetes nightmare. *Pediatr Diabetes* 9:504-507, 2008

55. Laitinen T, Lyyra-Laitinen T, Huopio H, Vauhkonen I, Halonen T, Hartikainen J, Niskanen L, Laakso M: Electrocardiographic alterations during hyperinsulinemic

hypoglycemia in healthy subjects. *Annals of Noninvasive Electrocardiology* 13:97-105, 2008

56. Landstedt-Hallin L, Englund A, Adamson U, Lins PE: Increased QT dispersion during hypoglycaemia in patients with type 2 diabetes mellitus. *J Intern Med* 246:299-1999

57. Larsen J, Ford T, Lyden E, Colling C, Mack-Shipman L, Lane J: What is hypoglycemia in patients with well-controlled type 1 diabetes treated by subcutaneous insulin pump with use of the continuous glucose monitoring system? *Endocr Pract* 10:324-329, 2004

58. Lau CP, FREEDMAN AR, FLEMING S, Malik M, Camm AJ, WARD DE: Hysteresis of the ventricular paced QT interval in response to abrupt changes in pacing rate. *Cardiovasc Res* 22:67-72, 1988

59. Lee S, Harris ND, Robinson RT, Yeoh L, Macdonald IA, Heller SR: Effects of adrenaline and potassium on QTc interval and QT dispersion in man. *Eur J Clin Invest* 33:93-98, 2003

60. Lee SP, Harris ND, Robinson RT, Davies C, Ireland R, Macdonald IA, Heller SR: Effect of atenolol on QTc interval lengthening during hypoglycaemia in type 1 diabetes. *Diabetologia* 48:1269-1272, 2005

61. Lee SP, Yeoh L, Harris ND, Davies CM, Robinson RT, Leathard A, Newman C, Macdonald IA, Heller SR: Influence of autonomic neuropathy on QTc interval lengthening during hypoglycemia in type 1 diabetes. *Diabetes* 53:1535-1542, 2004

62. Lepeschkin E, Surawicz B: The measurement of the QT interval of the electrocardiogram. *Circulation* 6:378-388, 1952

63. Lindström T, Jorfeldt L, Tegler L, Arnqvist HJ: Hypoglycaemia and cardiac arrhythmias in patients with type 2 diabetes mellitus. *Diabet Med* 9:536-541, 1992

64. Malik M and Batchvarov V: Measurement of the QT interval. In *QT dispersion* (pp. 5-9), edited by Malik M and Batchvarov V, New York, Futura Publishing Company, Inc, 2000

65. Malik M, Farbom P, Batchvarov V, Hnatkova K, Camm AJ: Relation between QT and RR intervals is highly individual among healthy subjects: implications for heart rate correction of the QT interval. *Br Med J* 87:220-228, 2002

66. Malik M, Hnatkova K, Batchvarov V: Differences between study-specific and subject-specific heart rate corrections of the QT interval in investigations of drug induced QTc prolongation. *Pacing Clin Electrophysiol* 27:791-800, 2004

67. Marques JLB, George E, Peacey SR, Harris ND, Macdonald IA, Cochrane T, Heller SR: Altered ventricular repolarization during hypoglycaemia in patients with diabetes. *Diabet Med* 14:648-654, 1997

68. McLaughlin NB, Campbell RW, Murray A: Accuracy of four automatic QT measurement techniques in cardiac patients and healthy subjects. *Br Med J* 76:422-426, 1996

69. McLaughlin NB, Campbell RW, Murray A: Comparison of automatic QT measurement techniques in the normal 12 lead electrocardiogram. *Br Med J* 74:84-89, 1995

70. Meinhold J, Heise T, Rave K, Heinemann L: Electrocardiographic changes during insulin-induced hypoglycemia in healthy subjects. *Horm Metab Res* 30:694-697, 1998

71. Meinhold J, Heise T, Rave K, Heinemann L: Electrocardiographic Changes During Insulin-Induced Hypoglycemia in Healthy Subjects. *Horm Metab Res* 30:694-697, 1998

72. Minaker KL, Rowe JW: Potassium homeostasis during hyperinsulinemia: effect of insulin level, beta-blockade, and age. *Am J Physiol Endocrinol Metab* 242:373-377, 1982

73. Molnar J, Ranade V, Cvetanovic I, Molnar Z, Somberg JC: Evaluation of a 12-Lead Digital Holter System for 24-Hour QT Interval Assessment. *Cardiology* 106:224-232, 2006

74. Montanez A, Ruskin JN, Hebert PR, Lamas GA, Hennekens CH: Prolonged QTc interval and risks of total and cardiovascular mortality and sudden death in the general population: a review and qualitative overview of the prospective cohort studies. *Archives of Internal Medicine* 164:943-948, 2004

75. Moody GB, Koch H, Steinhoff U: The physionet/computers in cardiology challenge 2006: Qt interval measurement. *Comput Cardiol* Volume 33:2006

76. Morganroth J, Brown AM, Critz S, Crumb WJ, Kunze DL, Lacerda AE, Lopez H: Variability of the QTc interval: impact on defining drug effect and low-frequency cardiac event. *Am J Cardiol* 72:26B-31B, 1993

77. Moss AJ: Measurement of the QT interval and the risk associated with QTc interval prolongation: a review. *Am J Cardiol* 72:23B-25B, 1993

78. Murphy NP, Ford-Adams ME, Ong KK, Harris ND, Keane SM, Davies C, Ireland RH, Macdonald IA, Knight EJ, Edge JA: Prolonged cardiac repolarisation during spontaneous nocturnal hypoglycaemia in children and adolescents with type 1 diabetes. *Diabetologia* 47:1940-1947, 2004

79. O'Brien IA, O'hare P, Corrall RJ: Heart rate variability in healthy subjects: effect of age and the derivation of normal ranges for tests of autonomic function. *Br Med J* 55:348-354, 1986

80. Padmanabhan S, Silvet H, Amin J, Pai RG: Prognostic value of QT interval and QT dispersion in patients with left ventricular systolic dysfunction: results from a cohort of 2265 patients with an ejection fraction of 40%. *Am Heart J* 145:132-138, 2003

81. Pedersen-Bjergaard U, Pramming S, Thorsteinsson B: Recall of severe hypoglycaemia and self-estimated state of awareness in type 1 diabetes. *Diabetes Metab Rev* 19:232-240, 2003

82. Petersen KG, Schluter KJ, Kerp L: Regulation of serum potassium during insulin-induced hypoglycemia. *Diabetes* 31:615-617, 1982

83. Poole-Wilson PA: Potassium and the heart. *Clin Endocrinol Metab* 13:249-268, 1984

84. Rana BS, Lim PO, Naas AAO, Ogston SA, Newton RW, Jung RT, Morris AD, Struthers AD: QT interval abnormalities are often present at diagnosis in diabetes and are better predictors of cardiac death than ankle brachial pressure index and autonomic function tests. *Br Med J* 91:44-50, 2005

85. Rautaharju PM, Warren JW, Calhoun HP: Estimation of QT prolongation. A persistent, avoidable error in computer electrocardiography. *J Electrocardiol* 23:111-117, 1990

86. Robinson RT, Harris ND, Ireland RH, Lee S, Newman C, Heller SR: Mechanisms of abnormal cardiac repolarization during insulin-induced hypoglycemia. *Diabetes* 52:1469-1474, 2003

87. Robinson RT, Harris ND, Ireland RH, Lindholm A, Heller SR: Comparative effect of human soluble insulin and insulin aspart upon hypoglycaemia-induced alterations in cardiac repolarization. *Br J Clin Pharmacol* 55:246-251, 2003

88. Robinson RT, Harris ND, Ireland RH, Macdonald IA, Heller SR: Changes in cardiac repolarization during clinical episodes of nocturnal hypoglycaemia in adults with Type 1 diabetes. *Diabetologia* 47:312-315, 2004

89. Roden DM: Drug-induced prolongation of the QT interval. *N Engl J Med* 350:1013-1022, 2004

90. Rossing P, Breum L, Major-Pedersen A, Sato A, Winding H, Pietersen A, Kastrup J, Parving HH: Prolonged QTc interval predicts mortality in patients with Type 1 diabetes mellitus. *Diabet Med* 18:199-205, 2001

91. Rowe JW, Minaker KL, Pallotta JA, Flier JS: Characterization of the insulin resistance of aging. *J Clin Invest* 71:1581-1587, 1983

92. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D: An improved method for adjusting the QT interval for heart rate(the framingham heart study). *Am J Cardiol* 70:797-801, 1992

93. Schwartz PJ, Wolf S: QT interval prolongation as predictor of sudden death in patients with myocardial infarction. *Circulation* 57:1074-1077, 1978

94. Sörnmo L: Time-varying digital filtering of ECG baseline wander. *Med Biol Eng Comput* 31:503-508, 1993

95. Sovik O, Thordarson H: Dead-in-bed syndrome in young diabetic patients. *Diabetes Care* 22:B40-2, 1999

96. Stork ADM, Kemperman H, Erkelens DW, Veneman TF: Comparison of the accuracy of the hemocue glucose analyzer with the Yellow Springs Instrument glucose oxidase analyzer, particularly in hypoglycemia. *Eur J Endocrinol* 153:275-281, 2005

97. Strachan MWJ: Frequency, Causes and Risk Factors for Hypoglycaemia in Type 1 Diabetes. In *Hypoglycaemia in Clinical Diabetes* (pp. 49-81), edited by Frier BM and Fisher M, Chichester, Wiley, 2007

98. Straus SMJM, Kors JA, De Bruin ML, van der Hooft CS, Hofman A, Heeringa J, Deckers JW, Kingma JH, Sturkenboom MCJM, Stricker BHC: Prolonged QTc interval and risk of sudden cardiac death in a population of older adults. *J Am Coll Cardiol* 47:362-367, 2006

99. Suys B, Heuten S, De Wolf D, Verherstraeten M, de Beeck LO, Matthys D, Vrints C, Rooman R: Glycemia and Corrected QT Interval Prolongation in Young Type 1 Diabetic Patients What is the relation? *Diabetes Care* 29:427-429, 2006

100. Tattersall RB, Gill GV: Unexplained deaths of type 1 diabetic patients. *Diabet Med* 8:49-58, 1991

101. Veglio M, Sivieri R, Chinaglia A, Scaglione L, Cavallo-Perin P: QT interval prolongation and mortality in type 1 diabetic patients: a 5-year cohort prospective study. Neuropathy Study Group of the Italian Society of the Study of Diabetes, Piemonte Affiliate. *Diabetes Care* 23:1381-1383, 2000

102. Watkins PJ, Amiel SA, Howell SL, and Turner E: Hypoglycaemia. In *Diabetes and its Management* (pp. 79-87), edited by Watkins PJ, Amiel SA, Howell SL, and Turner E, Oxford, Blackwell Publishing, 2003

103. Weston PJ, Gill GV: Is undetected autonomic dysfunction responsible for sudden death in Type 1 diabetes mellitus? The'dead in bed'syndrome revisited. *Diabet Med* 16:626-631, 1999

104. Whitsel EA, Boyko EJ, Rautaharju PM, Raghunathan TE, Lin D, Pearce RM, Weinmann SA, Siscovick DS: Electrocardiographic QT interval prolongation and risk of primary cardiac arrest in diabetic patients. *Diabetes Care* 28:2045-2047, 2005

105. Whyte KF, Whitesmith R, Reid JL: The effect of diuretic therapy on adrenaline-induced hypokalaemia and hypomagnesaemia. *Eur J Clin Pharmacol* 34:333-337, 1988

106. Yap YG, Camm AJ: Drug induced QT prolongation and torsades de pointes. *Br Med J* 89:1363-1372, 2003

107. Zhu Y, Lee PJ, Pan J, Lardin HA: The relationship between ventricular repolarization duration and RR interval in normal subjects and patients with myocardial infarction. *Cardiology* 111:209-218, 2008

CONFIDENTIAL