Minna Koivikko

CARDIAC AUTONOMIC REGULATION AND REPOLARISATION DURING HYPOGLYCAEMIA IN TYPE 1 DIABETES
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Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 7 of Oulu University Hospital, on 15 February 2013, at 12 noon
Abstract

Hypoglycaemia may contribute to the nocturnal occurrence of sudden death in type 1 diabetes. The impact of hypoglycaemia on cardiac autonomic regulation and electrical properties is a potential factor predisposing patients to fatal arrhythmic events. This study was designed to assess the effects of hypoglycaemia on cardiovascular autonomic regulation and cardiac repolarisation in type 1 diabetic patients and their non-diabetic counterparts during experimental and spontaneous hypoglycaemia.

Sixteen subjects with type 1 diabetes and eight healthy controls participated in experimental hypoglycaemia induced by using glucose clamp technique. Altogether 37 patients with type 1 diabetes were evaluated in real-life situation by using continuous glucose monitoring system. Ten of those individuals participated in an additional experiment to determine the effect of sympathetic activation on the cardiac autonomic regulation. Continuous electrogram recordings were used to analyze heart rate variability (HRV) and cardiac repolarisation.

During experimental hypoglycaemia, cardiac vagal activity, assessed by the high frequency (HF) component and beat-to-beat R-R interval variability (SD1), decreased progressively with no differences among diabetic or non-diabetic subjects. Controlled hypoglycaemia evoked profound changes in cardiac repolarisation. These changes tended to be even more evident in the diabetic subjects compared to those encountered in their healthy counterparts.

During spontaneous hypoglycaemia, the low frequency (LF) component of HRV decreased significantly and correlated positively with the change in the glucose concentration. The muscle sympathetic nerve activity study confirmed that the reduction in the LF spectral component resulted mainly from pure sympathetic activation without any concomitant vagal withdrawal. Spontaneous hypoglycaemia induced significant changes in T-wave loop morphology. The QT interval corrected for heart rate by Bazett’s formula and by the nomogram method shortened during hypoglycaemia.

The present observations indicate that hypoglycaemia has major impacts on cardiac autonomic regulation and repolarisation which may partly explain the vulnerability of these individuals to life-threatening cardiac arrhythmias and may have some clinical importance in contributing to the occurrence of ‘dead-in-bed’ syndrome.

Keywords: heart rate variability, hypoglycaemia, repolarisation, type 1 diabetes
Tiivistelmä

Hypoglykemia saattaa vaikuttaa yöllisten äkkikuolemien ilmaantuvuuteen tyypin 1 diabetesta sairastavilla. Hypoglykemian vaikutus sydämen autonomiseen toimintaan ja sähköisiin ominaisuuksiin voi altistaa potilaat kouluille johtaville rytmihäiriöille. Tämän tutkimuksen tarkoitus oli selvittää hypoglykemian vaikutuksia kardiovaskulaarisen autonomisen toiminnan säätelyyn ja sydämen repolarisaatioon tyypin 1 diabetesta sairastavilla ja heidän terveillä verrokeilla kokeellisen ja spontaanin hypoglykemian aikana.


Kokeellisen hypoglykemian aikana sydämen vagaalinen aktiivisuus määriteltynä korkean taajuuden (HF) komponentin osuutena ja R-R-välin vaihteluna (SD1) vähensi progressiivisesti sekä diabetesta sairastavilla että verrokeilla. Kontrolloitu hypoglykemia aiheutti huomattavia muutoksia sydämen repolarisaatioon. Nämä muutokset olivat jopa suurempia diabetesta sairastavilla kuin heidän terveillä verrokeilla.


Tehdyt havaintot osottavat, että hypoglykemialla on sellaisia merkittäviä vaikutuksia sydämen autonomisen toiminnan säätelyyn ja repolarisaatioon, jotka voivat osittain selittää näiden yksilöiden alttiuden henkeä uhkaaville rytmihäiriöille. Näillä muutoksilla voi olla kliinistä merkitystä ns. “dead in bed”-oireyhtymän esiintymisessä.

Asiakirjat: hypoglykemia, repolarisaatio, sykevaihtelu, tyypin 1 diabetes
Dedicated to my husband, Jukka and to my children,
Anni-Maria and Samu-Aleksi
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Oulu, December 2012 Minna Koivikko
Abbreviations

ACTH  adrenocorticotropic hormone
ADA  American Diabetes Association
AV  atrioventricular
BP  blood pressure
cAMP  cyclic adenosine monophosphate
CGMS  continuous glucose monitoring system
DCCT  Diabetes Control and Complications Trial
ECG  electrocardiogram
EEG  electroencephalogram
FFT  Fast Fourier transform
GH  growth hormone
HAAF  hypoglycaemia-associated autonomic failure
HbA1c  glycated haemoglobin
HF  high frequency
HR  heart rate
HRV  heart rate variability
HRVI  heart rate variability triangular index
LF  low frequency
MSNA  muscle sympathetic nerve activity
OGTT  oral glucose tolerance test
PCA  Principal Component Analysis
PSD  power spectrum density
QTc/QTc  QT interval corrected for heart rate by Bazett’s formula
QTfc  QT interval corrected for heart rate by Fridericia’s formula
QTnc  QT interval corrected for heart rate by the nomogram method
pNN50  percentage of the differences between adjacent normal R-R interval
       greater than 50 ms
RMSDD  square root of the mean of the squared differences between
       successive R-R intervals
R-R  R-peak-to-R-peak interval
SA  sinoatrial
SD1  standard deviation of beat-to-beat R-R interval variability
SD2  standard deviation of long term R-R interval variability
SDNN  standard deviation of all normal-to-normal R-R intervals
<table>
<thead>
<tr>
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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDANN</td>
<td>standard deviation of the average R-R intervals of analyzed segments</td>
</tr>
<tr>
<td>SR</td>
<td>sinus rhythm</td>
</tr>
<tr>
<td>TCRT</td>
<td>total-cosine-R-to-T</td>
</tr>
<tr>
<td>ULF</td>
<td>ultra low frequency</td>
</tr>
<tr>
<td>VCG</td>
<td>vectorcardiogram</td>
</tr>
<tr>
<td>VLF</td>
<td>very low frequency</td>
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List of original articles

This thesis is based on the following original articles, which are referred to in the text by their Roman numerals


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1 Introduction

Individuals with type 1 diabetes adhering to strict glycaemic control are prone to suffer severe hypoglycaemia. Other well-established risk factors for hypoglycaemia include a history of severe hypoglycaemia and impaired awareness of hypoglycaemia (Cryer 1999, Cryer et al. 2003, The Diabetes Control and Complications Trial Research Group 1997). During hypoglycaemia, the release of counterregulatory hormones serves to maintain blood glucose homeostasis. The response of catecholamines is normally not important but becomes critical when the response of glucagon is deficient, as in type 1 diabetic patients, who are largely dependent on adrenaline to correct and prevent hypoglycaemia (Bolli & Fanelli 1999, Cryer et al. 2003). Many studies have documented impaired secretory responses of adrenaline to plasma glucose decrements in type 1 diabetic patients. This response becomes impaired gradually over several years following the diagnosis of diabetes. In diabetic patients, the blunted response of counterregulatory hormones may result in a loss of the ability to recognise the development of hypoglycaemia. (Bolli et al. 1983, Cryer 1999). Recurrent iatrogenic hypoglycaemia also elevates the glycaemic thresholds for symptoms as well as for the activation of the glucose counterregulation system (Amiel et al. 1987b, Cryer 1999, Dagogo-Jack et al. 1993, Davis et al. 1992, Fanelli et al. 1993, Lingenfelser et al. 1995).

According to earlier studies, approximately 2–4% of deaths of type 1 diabetic subjects have been attributed to hypoglycaemia (Laing et al. 1999). However, more recent reports have indicated that as many as 6–10% of deaths in these patients were the result of hypoglycaemia (Feltbower et al. 2008, Skrivarhaug et al. 2006, The Diabetes Control and Complications Trial Research Group 1997). Despite advanced technology and the availability of new insulin analogues, the fear of hypoglycaemia is still a major problem complicating the management of diabetes mellitus (Cryer 2008b).

Since 1991, when Tattersall and Gill introduced the term ´dead in bed syndrome´ (Tattersall & Gill 1991) the role of hypoglycaemia as a factor predisposing young adults to sudden arrhythmic death has been debated (Bell 2006, Weston & Gill 1999). However, the pathophysiologic background of nocturnal sudden death has remained something of an enigma. It is evident that nocturnal hypoglycaemia plays an important role, but it is not well understood how hypoglycaemia itself affects the cardiac electrical properties predisposing the individual to sudden arrhythmia-induced death. Roles for the autonomic nervous
system and its effects on cardiac repolarisation have been proposed (Weston & Gill 1999).

Heart rate variability (HRV) has been increasingly used to assess autonomic function in various physiological and pathological settings, including the assessment of autonomic dysfunction in diabetic patients (Bellavere et al. 1992, Ewing et al. 1984, Ewing et al. 1991, Freeman et al. 1991, Malpas & Maling 1990, Pagani et al. 1988, Ziegler et al. 1992). There are some reports describing changes in HRV during hypoglycaemia among healthy subjects (Laitinen et al. 2003, Schächinger et al. 2004), but less is known about the hypoglycaemia-induced changes in cardiac autonomic regulation in diabetic patients.

There is increasing evidence that a prolonged QTc interval (a rate corrected QT interval using Bazett’s formula) is a significant predictor of mortality both in type1 (Rossing et al. 2001, Veglio et al. 2000) and type 2 diabetes (Okin et al. 2004, Rana et al. 2005, Salles et al. 2004). Several hyperinsulinaemic glucose clamp studies have demonstrated an increase in the QTc interval during hypoglycaemia in diabetic subjects (Due-Andersen et al. 2008a, Landstedt-Hallin et al. 1999, Lee et al. 2004, Lee et al. 2005, Marques et al. 1997, Rothenbuhler et al. 2008). Similar changes in cardiac repolarisation have been detected during spontaneous hypoglycaemia (Gill et al. 2009, Murphy et al. 2004, Robinson et al. 2004). It has been postulated that this hypoglycaemia-related prolongation of the QTc interval may contribute to the sudden nocturnal death of young individuals with type 1 diabetes (Bell 2006, Weston & Gill 1999).

Since the measurement of the QT interval and dispersion may include many methodological inaccuracies and does not provide any information on the morphology of the T-wave, new descriptors of cardiac repolarisation have been developed (Karjalainen et al. 1994, Malik et al. 2000). The analysis of the T-wave morphology and the spatial QRS-T angle are not subject to the inaccuracies inherent in QT heart rate correction formulas or to the intersubject variability in the measurement of QT interval. These novel descriptors of cardiac repolarisation have been demonstrated to possess well-established diagnostic and prognostic value in several populations and clinical settings (Anttonen et al. 2009, Huang et al. 2009, Kardys et al. 2003, Pavri et al. 2008, Perkiömäki et al. 2006, Priori et al. 1997, Rautaharju et al. 2006, Zabel et al. 2000, Zabel et al. 2002).

The present study was designed to evaluate hypoglycaemia-induced changes in cardiac autonomic regulation and repolarisation in type 1 diabetes by analyzing HRV and the novel descriptors of repolarisation during experimental and spontaneous hypoglycaemia.
2 Review of the literature

2.1 General aspects of type 1 diabetes

2.1.1 Definition of type 1 diabetes

Type 1 diabetes refers to the processes of autoimmune mediated beta-cell destruction that may ultimately lead to diabetes mellitus in which insulin is required for survival to prevent the development of ketoacidosis, coma and death. An individual with type 1 diabetes may be metabolically normal before the disease is clinically manifest, but, nonetheless the process of beta-cell destruction can be detected. Type 1 diabetes is usually characterised by the presence of glutamic acid decarboxylase antibodies (85–90%), islet cell or insulin antibodies which are involved in the autoimmune processes that lead to beta-cell destruction (Verge et al. 1996). The rate of destruction is quite variable (Zimmet et al. 1994). The rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey et al. 1998). The slowly progressive form generally occurs in adults and is referred to as latent autoimmune diabetes in adults (LADA). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease (Japan and Pittsburgh Childhood Diabetes Research Groups 1985). Others have modest fasting hyperglycaemia that can rapidly change to severe hyperglycaemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual beta-cell function, sufficient to prevent ketoacidosis, for many years (Zimmet 1995). Individuals with this form of type 1 diabetes often become dependent on insulin for survival and are at risk of suffering ketoacidosis (Willis et al. 1996). At this stage of the disease, there is little or no insulin secretion as manifested by low or undetectable levels of plasma C-peptide (Hother-Nielsen et al. 1988).

2.1.2 Diagnosis of type 1 diabetes

The clinical diagnosis of diabetes is often prompted by symptoms such as increased thirst and urine volume, recurrent infections, unexplained weight loss and, in severe cases, drowsiness and coma. In such cases, a single blood glucose estimation in excess of the diagnostic values may be sufficient to establish the
diagnosis (Alberti & Zimmet 1998). Diabetes mellitus is likely when plasma glucose is $\geq 11.1$ mmol/l in a random venous sample or $\geq 12.2$ mmol/l in a random capillary sample according to the WHO Study Group Report (World Health Organization 1985). The requirements for diagnostic confirmation for an individual presenting with severe symptoms and gross hyperglycaemia differ from those of the asymptomatic person with blood glucose values assayed as being just above the diagnostic cut-off value. The WHO consultation published in 1998 stated that the diagnosis of diabetes in an asymptomatic subject should not be made on the basis of a single abnormal blood glucose value. For the asymptomatic person, at least one additional plasma/blood glucose test result with a value in the diabetic range is essential, either from a fasting or a random sample, or from the results of an oral glucose tolerance test (OGTT). If an OGTT is performed, it is sufficient to measure the blood glucose values while fasting and at 2 hours after a 75 g oral glucose load. In type 1 diabetes, OGTT is not usually needed because of the rapid onset and progression of the disease. Severe hyperglycaemia detected under conditions of acute infective, traumatic, circulatory or some other stress may be transitory and should not in itself be regarded as confirming the diagnosis of diabetes. (Alberti & Zimmet 1998). In the 2011 WHO report, glycated haemoglobin (HbA1c) of 6.5% is recommended as the cut-off point for diagnosing diabetes. A value less than 6.5% does not exclude diabetes diagnosed using glucose tests.(World Health Organisation 2011).

### 2.1.3 Incidence and prevalence of type 1 diabetes

Type 1 diabetes usually accounts for only a minority of the total burden of diabetes in a population but the disease is increasing in incidence in both poor and rich countries (International Diabetes Federation 2009). It is the predominant form of the disease in younger age groups in most high-income countries. Two international collaborative projects, the Diabetes Mondiale study (The DIAMOND Project Group 2006) and the Europe and Diabetes study (Patterson et al. 2009) have monitored trends in the incidence through the establishment of population-based regional or national registries. The incidence of childhood onset type 1 diabetes is increasing in many countries in the world, at least in the under 15-year age group. There are clear indications of geographic differences in trends. In the DiaMond study, a greater than 350-fold difference in the incidence of type 1 diabetes among the 100 populations worldwide was reported with age-adjusted incidences ranging from a low of 0.1/100,000 per year in China and Venezuela to
a high of 36.5/100,000 in Finland and 36.8/100,000 per year in Sardinia. The lowest incidence (< 1/100,000 per year) has been reported in the populations from China and South America with the highest incidence (>20/100,000 per year) found in Sardinia, Finland, Sweden, Norway, Portugal, the UK, Canada, and New Zealand. The overall annual increase is estimated to be around 3%. The authors of the report hypothesised that the explanation for the variation within ethnic groups may be due to differences in genetic characteristics or environmental/behavioral factors. They also reported that in countries undergoing rapid social change, population exposure to putative etiologic factors for T1D may change rapidly. There is evidence that the incidence is increasing more steeply in some of the low prevalence countries such as those in central and eastern Europe and that the increases are greatest in young children. There are clear indications that similar trends exist in many other parts of the world, but in sub-Saharan Africa, incidence data are sparse or non–existent. According to the Diabetes Atlas 2009, it is estimated that annually some 76 000 children aged under 15 years develop type 1 diabetes worldwide. Of the estimated 480 000 children with type 1 diabetes, 24% live in the South-East Asian Region, but the European Region, where the most reliable and up-to-date estimates of incidence are available, comes a close second (23%).

According to The National Health Insurance (2012), 319 727 persons were being reimbursed for antidiabetic medication in Finland. Individuals with type 1 diabetes account for some 15% of all diabetes cases, thus there are some 48 000 Finns with type 1 diabetes. Moreover, diabetes is one of the most common chronic diseases among children (Niemi & Winell 2005). The number of new children with diabetes is currently increasing at an annual rate of 3% (Harjutsalo et al. 2008). The Diabetes in Finland (FinDM I) Study revealed that there are major regional differences in disease prevalence, i.e. prevalence is highest in the east of Finland and lowest in northern Finland. (Niemi & Winell 2005).

### 2.2 General aspects of hypoglycaemia

#### 2.2.1 Definition of hypoglycaemia

Traditionally, hypoglycaemia is documented by Whipples’s triad: symptoms compatible with hypoglycaemia, a low plasma glucose concentration, and relief of symptoms after the plasma glucose concentration is elevated (Whipple 1938).
The American Diabetes Association (ADA) Workgroup on Hypoglycaemia (2005) defined hypoglycaemia in people with diabetes as “all episodes of abnormally low plasma glucose concentration that expose the individual to potential harm”. The description includes also episodes of asymptomatic hypoglycaemia. Though it is difficult to set a specific plasma glucose concentration that would define hypoglycaemia because of its dynamic state, the ADA Workgroup recommended that people with type 1 diabetes become concerned about the possibility of hypoglycaemia at a self-monitored (or glucose sensing device estimated) plasma glucose concentration of 3.9 mmol/l. The rationale for that cut-off value is that 3.9 mmol/l approximates to the lower limit of the postabsorptive plasma glucose concentration range and the glycaemic threshold for activation of glucose counterregulatory systems. It is also the highest low level reported to reduce counterregulatory responses to subsequent hypoglycaemia in nondiabetic individuals. The workgroup also suggested a classification of hypoglycaemia in diabetes, i.e. 1) severe hypoglycaemia (an event requiring assistance of another person to raise glucose levels and promote neurological recovery), 2) documented symptomatic hypoglycaemia (symptoms plus low glucose levels) and 3) asymptomatic hypoglycaemia (low glucose levels without symptoms) as well as 4) probable symptomatic hypoglycaemia (symptoms without a glucose estimate) and 5) relative hypoglycaemia (symptoms with glucose levels that are not low but are approaching that level). (Workgroup on Hypoglycemia,American Diabetes Association 2005).

2.2.2 Incidence of hypoglycaemia

Individuals with type 1 diabetes suffer an average of two episodes of symptomatic hypoglycaemia every week and an average of approximately one episode of severe, at least temporarily disabling, hypoglycaemia, often with a seizure or coma, per year (Cryer 2008a). In the Diabetes Control and Complications Trial (DCCT), a landmark study into the treatment of type 1 diabetes, the relative risk of severe hypoglycaemia was tripled in the intensive treatment group compared with the conservative therapy group (61.2 episodes per 100 patient-years vs. 18.7 per 100 patient-years) (The Diabetes Control and Complications Trial Research Group 1997) and 55% of incidents of severe hypoglycaemia occurred during sleep (The DCCT Research Group 1991). In 2007, the U.K. Hypoglycaemia Study Group reported an incidence of severe hypoglycaemia of 110 episodes per 100 patient-years (nearly twice that in the DCCT) in patients with type 1 diabetes.
for less than 5 years and an incidence of 320 episodes per 100 patient-years in those with type 1 diabetes for more than 15 years (UK Hypoglycaemia Study 2007). In a more recent study using subcutaneous glucose sensing in type 1 diabetes reported that glucose levels were < 3.9 mmol/l 1.5 hours per day (i.e., 6.3% of the time) (Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group 2009). In a study where nocturnal plasma glucose measurements were taken every 15 minutes in type 1 diabetes, glucose levels were < 3.9 mmol/l in 57% (12 of 21) of the patients (Raju et al. 2006).

In type 2 diabetes, population-based studies have indicated that severe hypoglycaemic episodes occur at rates comparable to those in type 1 diabetes (Leese et al. 2003). The UK Hypoglycaemia Study showed that individuals with type 2 diabetes who had been treated with insulin for over 5 years and whose endogenous insulin production as measured by stimulated C-peptide was low, had significantly high rates of hypoglycaemia including severe episodes (UK Hypoglycaemia Study 2007).

2.2.3 Physiology of glucose counterregulation

During hypoglycaemia, the release of counterregulatory hormones attempts to maintain blood glucose homeostasis. Falling plasma glucose concentrations elicit a sequence of responses that normally prevent or correct hypoglycaemia (Cryer et al. 1989) (Table 1.). First, pancreatic β-cells decrease insulin secretion. Secondly, pancreatic α-cells increase glucagon secretion and thirdly, adrenomedullary adrenaline secretion increases. Insulin, glucagon and adrenaline act rapidly, within minutes. The reduced insulin levels favour increased glucose production which is also stimulated by glucagon and adrenaline. Adrenaline also limits glucose utilisation, mobilises gluconeogenic precursors and inhibits insulin secretion. Over a longer time period, 3–4 hours, cortisol and growth hormone both limit glucose utilisation by insulin-sensitive tissues such as muscles and stimulate glucose production. Hypoglycaemia is prevented by the dynamic regulation of endogenous glucose production by the liver (and the kidneys) as well as by changes in glucose utilisation to ensure an adequate supply of metabolic fuel for the brain.
Table 1. Physiologic responses to decreasing plasma glucose concentrations according to Williams Textbook of Endocrinology, 11th Edition 2008.

<table>
<thead>
<tr>
<th>Response</th>
<th>Glycaemic threshold (mmol/l)</th>
<th>Role in prevention of hypoglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased insulin secretion</td>
<td>4.4-4.7</td>
<td>Primary regulatory factor, first defence</td>
</tr>
<tr>
<td>Increased glucagon secretion</td>
<td>3.6-3.9</td>
<td>Primary regulatory factor, second defense</td>
</tr>
<tr>
<td>Increased adrenaline secretion</td>
<td>3.6-3.9</td>
<td>Critical when glucagon is deficient, third defence</td>
</tr>
<tr>
<td>Increased cortisol and growth hormone secretion</td>
<td>3.6-3.9</td>
<td>Involved, not critical</td>
</tr>
<tr>
<td>Symptoms</td>
<td>2.8-3.1</td>
<td>Behavioral defence, food ingestion</td>
</tr>
<tr>
<td>Cognitive dysfunction</td>
<td>&lt; 2.8</td>
<td></td>
</tr>
</tbody>
</table>

The symptoms of hypoglycaemia are categorised as neuroglycopenic (the direct result of brain glucose deprivation) and neurogenic (or autonomic), those that are largely the result of the perception of physiological changes caused by the sympathoadrenal discharge triggered by hypoglycaemia (Amiel 1991, Cryer 1993, Cryer 2008b, Cryer 2010). Neuroglycopenic manifestations include cognitive impairments, behavioral changes and psychomotor abnormalities, and, at lower plasma glucose concentrations, seizure and coma. Adrenergic neurogenic symptoms include palpitations, tremor, and anxiety/arousal. Cholinergic neurogenic symptoms include sweating, hunger, and paresthesias. The awareness of hypoglycaemia is largely the result of the perception of neurogenic symptoms. (Towler et al. 1993). According to questionnaires, sweating is the first symptom of hypoglycaemia; sweating and trembling are the most reliable hypoglycaemic warning symptoms (Muhlhauser et al. 1991). Neuroglycopenic manifestations are often observable.

2.2.4 Pathophysiology of glucose counterregulation in type 1 diabetes

Hypoglycaemia in people with diabetes is typically the result of the interplay of relative or absolute therapeutic hyperinsulinaemia and blunted physiological and behavioral defences against the falling plasma glucose concentrations (Amiel 1991, Cryer 1993, Cryer 2008b, Cryer 2010). In type 1 diabetes, the first and
second lines of physiological defences against hypoglycaemia – a decrease in insulin and an increase in glucagon – are lost, and the third physiological defence mechanism – an increase in adrenaline – is often attenuated (Bolli et al. 1983, Bolli et al. 1984). Loss of the endogenous insulin response is the result of β-cell failure and loss of the glucagon response is also likely the result of β-cell failure since a decrease in β-cell secretion of insulin, perhaps in conjunction with other secretory products, together with hypoglycaemia, normally signals increased α-cell glucagon secretion (Cooperberg & Cryer 2009, Raju & Cryer 2005).

Although it can be evoked by recent antecedent hypoglycaemia (Heller & Cryer 1991), or by prior exercise (Ertl & Davis 2004) or by sleep (Banarer & Cryer 2003), the mechanism of the attenuated sympathoadrenal response is not known.

In the setting of therapeutic hyperinsulinaemia and falling plasma glucose concentrations and absent insulin and glucagon secretory responses, the blunted adrenaline response is responsible for the clinical syndrome of defective glucose counterregulation (Dagogo-Jack et al. 1993, Heller & Cryer 1991) which is associated with a 25-fold (White et al. 1983) or greater (Bolli et al. 1984) increased risk of severe iatrogenic hypoglycaemia. The attenuated sympathoadrenal response causes the clinical syndrome of hypoglycaemia unawareness and, thus, loss of the behavioural defence. Hypoglycaemia unawareness is associated with a 6-fold increased risk of iatrogenic hypoglycaemia (Geddes et al. 2008).

The concept of hypoglycaemia-associated autonomic failure (HAAF) in diabetes claims that recent antecedent hypoglycaemia, or prior exercise or sleep, causes both defective glucose counterregulation and hypoglycaemia unawareness and, thus, a vicious cycle of recurrent hypoglycaemia. Perhaps the most convincing evidence of the clinical relevance of HAAF is the finding that avoidance of hypoglycaemia for 2–3 weeks can reverse hypoglycaemia unawareness, and improve the deficient adrenaline excretion of defective glucose counterregulation, in most affected patients (Cranston et al. 1994, Dagogo-Jack et al. 1993, Fanelli et al. 1994, Fanelli et al. 1993).

Risk factors for HAAF include absolute endogenous insulin deficiency which determines that insulin levels do not decrease and glucagon levels do not increase as glucose levels fall in response to therapeutic hyperinsulinemia, a history of severe hypoglycaemia, hypoglycaemia unawareness or both as well as aggressive glycaemic therapy (lower HbA1c levels, lower glycaemic goals). (Cryer 2010).
2.2.5 Impacts of hypoglycaemia in type 1 diabetes

**Neurological effects**

Since the brain cannot synthesise glucose or store substantial amounts as glycogen, it requires a virtually continuous supply of glucose from the circulation. Facilitated diffusion of glucose from the blood into the brain is a direct function of the arterial plasma glucose concentration. The rate of blood-to-brain glucose transport exceeds the rate of brain glucose metabolism at normal plasma glucose levels, but it falls and becomes the limiting factor in brain glucose metabolism when arterial glucose concentrations fall to low levels (Blomqvist et al. 1991). Functional brain failure caused by hypoglycaemia is typically corrected after the plasma glucose concentration is elevated (Cryer 2007). In severe cases, hypoglycaemia can promote convulsions and coma but permanent neurological damage is rare (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group 2007).

Twenty years ago it was demonstrated that significant changes in the low frequency range of the electroencephalogram (EEG) appeared and disappeared within a narrow range of blood glucose concentration (Pramming et al. 1988, Tallroth et al. 1990). When blood glucose levels reach 1.8–2.0 mmol/l, alpha activity decreases rapidly and a significant increase in delta and theta activities is observed in the EEG. Following a return to euglycaemia, the EEG changes are fully reversible and the frequency content rapidly normalises. When EEG changes in response to hypoglycaemia were detected by an automated mathematical algorithm, these changes were found to precede the effects on cognitive function by at least 9 minutes (Juhl et al. 2010).

Profound, prolonged hypoglycaemia can cause brain death. In studies of insulin-induced hypoglycaemia in monkeys, 5–6 hours of blood glucose concentrations of less than 1.1 mmol/l were required for the reliable production of neurological damage (Kahn & Myers 1971); the average blood glucose level was 0.7 mmol/l. In individuals with diabetes, hypoglycaemia of that magnitude and duration occurs rarely.

**Cardiovascular effects**

Hypoglycaemia stimulates the profuse release of large amounts of adrenaline as a consequence of sympathetic neural activation, and this hormone has many direct
cardiovascular effects. Heart rate and systolic blood pressure rise while a small decrement in diastolic blood pressure occurs (Wright & Frier 2008). Total cerebral blood flow increases at blood glucose concentrations $< 2.0$ mmol/l and the regional distribution of blood flow within the brain is altered to provide glucose to those areas that are most vulnerable to neuroglycopenia, such as the cortex and the basal ganglia (MacLeod et al. 1994, Tallroth et al. 1992). Total splanchnic blood flow increases (Braatvedt et al. 1993), and a relative increase in hepatic blood flow occurs which should enhance the production of glucose in the liver. Blood flow to skeletal muscles increases (Allwood et al. 1959), while blood flow to the kidneys declines (Patrick et al. 1989). This redistribution of blood flow has two main hypothetical roles: 1) to protect vital organs such as the brain, and 2) to maintain a supply of glucose by increasing the delivery of gluconeogenic precursors to the liver (Wright & Frier 2008).

Hypoglycaemia-induced hemodynamic changes produce a sustained effect on cardiac function, as stroke volume, cardiac output, and left ventricular ejection fractions remain elevated for at least 90 min after the onset of acute hypoglycaemia (Fisher et al. 1987). In subjects with type 1 diabetes of over 15 years’ duration, arterial wall stiffness per se is greater and the arteries are less elastic in response to hypoglycaemia, which is manifested in a lesser fall in central arterial pressure than healthy controls (Sommerfield et al. 2007). Progressive stiffening of the arterial walls accelerates the return of the reflected pressure wave, causing its earlier arrival during late systole. This pathophysiological effect may interfere with coronary arterial perfusion and promote myocardial ischemia together with increased cardiac stress (Frier et al. 2011).

Hypoglycaemia has also pronounced effects on intravascular coagulability and viscosity. Increased plasma viscosity occurs during hypoglycaemia because there is an increase in erythrocyte concentration (Frier et al. 1983, Hilsted et al. 1985), whereas coagulation is promoted by platelet activation (Hutton et al. 1979) and an increment in factor VIII (Corrall et al. 1980) and von Willebrand factor (Frier et al. 1991). Endothelial function may be compromised during hypoglycaemia because of the increase in plasma levels of endothelin (Wright et al. 2007) and C-reactive protein (Galloway et al. 2000) and the mobilisation and activation of neutrophils (Frier et al. 1983) and platelet activation. These changes may promote intravascular coagulation and thrombosis and encourage the development of tissue ischemia, with the myocardium being potentially vulnerable (Wright & Frier 2008). However, evidence for cardiovascular
morbidity associated with hypoglycaemia in type 1 diabetes has been predominantly hypothetical and anecdotal based on short case reports (Frier et al. 2011, Wright & Frier 2008).

Mortality

Insulin-treated diabetes carries an increased mortality risk when compared with the general population, particularly for cardiovascular disease. The standardised mortality ratio has been 2.8–12.9 in different populations (Asao et al. 2003, Dawson et al. 2008, Feltbower et al. 2008, Harjutsalo et al. 2011, Laron-Kenet et al. 2001, Skrivarhaug et al. 2006). According to earlier studies, approximately 2–4% of deaths of type 1 diabetic subjects have been attributed to hypoglycaemia (Laing et al. 1999). However, more recent reports have indicated that as many as 6–10% of deaths in individuals with type 1 diabetes were the result of hypoglycaemia (Dawson et al. 2008, Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group 2007, Feltbower et al. 2008, Skrivarhaug et al. 2006).

Fear of hypoglycaemia

Given the unpleasant symptoms of hypoglycaemia and the potentially life threatening nature of severe hypoglycaemia, it is not surprising that many people with type 1 diabetes carry a significant fear of developing hypoglycaemia (Irvine et al. 1992, Wild et al. 2007). With intensified insulin therapy being linked to a threefold increase in frequency of severe hypoglycaemia (The Diabetes Control and Complications Trial Research Group 1997), the problem of fear of hypoglycaemia might also have increased since the implementation of intensive treatment strategies. There have been reports of a relationship between previous events of severe hypoglycaemia and the development of fear of hypoglycaemia (Gold et al. 1997, ter Braak et al. 2000, Wild et al. 2007). Anderbro et al. (2010) identified the frequency of severe hypoglycaemia as the most important factor associated with fear of hypoglycaemia. Other links between fear of hypoglycaemia and disease-specific factors include variability in blood glucose level and length of time since first insulin treatment (Irvine et al. 1992, Wild et al. 2007), as well as reduced hypoglycaemic awareness (Hepburn et al. 1994). Extreme fear of self injecting and fear of self testing are other problems related to fear of hypoglycaemia (Mollema et al. 2001). Anderbro et al. (2010) documented
gender differences in the fear of hypoglycaemia, suggesting that females are more affected by fear of hypoglycaemia. Fear of hypoglycaemia may lead to negative consequences in relation not only to the quality of life but also to diabetes management, metabolic control and subsequent health outcomes (Cryer 2008a, Wild et al. 2007).

2.3 Experimental models of hypoglycaemia

Our current understanding of glucose homeostasis has emerged from the observations of hypoglycaemia induced in experimental settings by the administration of exogenous insulin. When interpreting data acquired in a laboratory, it is important to remember that the method used to achieve hypoglycaemia may influence the results. (Amiel 1991).

2.3.1 Model of acute hypoglycaemia

Early studies of hypoglycaemia examined responses to a single intravenous bolus injection of insulin (Garber et al. 1976, Gerich et al. 1980, Rizza et al. 1979). In this model of acute hypoglycaemia, plasma insulin peaks to supraphysiologic levels and the initial decrease in plasma glucose is abrupt. Thereafter, recovery of plasma glucose occurs during the subsequent, rapid waning of the insulin level. Such unopposed administration of insulin has the advantage of allowing the observation of the responses of counterregulatory hormones. When the secretion or action of a single counterregulatory hormone is blocked, it is possible to establish its individual role in the recovery from hypoglycaemia. (Amiel 1991, Bolli & Fanelli 1999).

However, the conclusions derived from such a model do not necessarily apply to the clinical situation of hypoglycaemia induced by sustained hyperinsulinaemia. In addition, differences in counterregulation will result in different degrees of hypoglycaemia, and the different stimuli complicate the comparison of other responses.

2.3.2 Model of prolonged hypoglycaemia

In order to mimic clinical hypoglycaemia and to conduct relevant comparisons, it is recommended to use a sustained, reproducible hypoglycaemic stimulus. For these purposes, the modified insulin clamp is often used (Amiel et al. 1987a,
Predictable hyperinsulinaemia is produced by means of an intravenous insulin infusion. The plasma glucose level can then be controlled by means of simultaneous infusion of exogenous glucose, adjusted regularly on the basis of frequent estimations of plasma glucose concentrations. Clamps using step-wise slow falls of plasma glucose are particularly useful since they allow the investigation of blood glucose levels of hypoglycaemia associated with a given response (Bolli et al. 1985, De Feo et al. 1986, Sacca et al. 1979). In addition, a clear dose response can be observed. However, absolute values should only be applied to the clinical situation with care.

2.3.3 Studies on spontaneous hypoglycaemia

When considering the clinical implications of clamp studies, it should be taken into consideration that the responses to hypoglycemia have been assessed during a hyperinsulinaemic clamp. The experimental conditions have been artificial and thus differ from real life, where the rate and depth of hypoglycaemia are not controlled. There is also evidence that supraphysiological concentrations of insulin per se have effects on neuroendocrine responses. At comparable levels of hypoglycaemia, adrenaline, noradrenaline, cortisol and GH responses appear to increase in parallel with greater hyperinsulinaemia (Galassetti & Davis 2000).

The development of continuous glucose monitoring systems (CGMS) has now provided a tool that may enable more detailed insight into glucose fluctuations. CGMS assess blood glucose fluctuations indirectly by measuring the concentration of abdominal or forearm interstitial glucose but are calibrated via self-monitoring to approximate to the blood glucose level. The system has been validated at low glucose levels (Hoi-Hansen et al. 2005, Kovatchev et al. 2008) and during rapid blood glucose changes (Wilhelm et al. 2006). It has been used to detect nocturnal hypoglycaemia (Guillod et al. 2007). Gill et al. (2009) reported a real-life ambulatory observational study of a group of type 1 diabetic patients in whom they recorded simultaneous 24 h ECG and continuous glucose monitoring, aiming to capture nocturnal hypoglycaemic events and to record the QT interval and cardiac rhythm simultaneously.

2.4 General aspects of cardiac autonomic regulation

The regulation of the peripheral circulation is intended to distribute cardiac output to the various organs and tissues according to their individual metabolic or
functional needs while maintaining arterial blood pressure within a relatively
narrow range. Regional blood flows can be efficiently regulated at the local level
by the intrinsic ability of vessels to respond to various mechanical forces (e.g.,
wall tension and shear stress) as well as chemical stimuli (e.g., tissue metabolites
and O2). Superimposed on this local control system is another level of regulation
governed by changes in central neural activity that adjust cardiovascular function
to meet the needs of the body as a whole.

The autonomic nervous system is responsible for involuntary control of most
visceral organs, including the heart and blood vessels. Autonomic motor control is
effected by preganglionic fibers originating within the central nervous system at
the level of the brainstem or sacral spinal cord in the case of the parasympathetic
division and the thoracic or lumbar spinal cord in the case of the sympathetic
neurones. Axons of these preganglionic fibers synapse in autonomic ganglia
located outside of the central nervous system on the cell bodies of postganglionic
fibers, which, in turn, innervate the effector tissues. Both parasympathetic and
sympathetic preganglionic fibers are cholinergic, releasing the fast excitatory
neurotransmitter, acetylcholine. The parasympathetic arm consists of long
preganglionic fibers that synapse on short postganglionic fibers arising from
ganglia located close to the effector targets. In contrast, the sympathetic division
consists of short preganglionic fibers that synapse on long postganglionic fibers
arising from the paravertebral chain ganglia or collateral ganglia. Consequently,
sympathetic discharge can cause diffuse responses involving multiple regional
effectors while parasympathetic discharge causes fairly localized responses. The
axons of the postganglionic neurons that innervate cardiovascular tissues (cardiac
and smooth muscles) branch extensively, and each branch comes in close contact
with numerous effector cells. The principal neurotransmitter released from the
postganglionic parasympathetic fibers is acetylcholine, which binds to muscarinic
acetylcholine receptors on target tissues. The effects of acetylcholine are discrete
and short lived due to high local concentrations of acetylcholinesterase, which
rapidly degrades the neurotransmitter and prevents its access to the bloodstream.
Sympathetic fibers release noradrenaline, which binds to either α- or β-adrenergic
receptors. Noradrenaline has more prolonged and wider ranging effects than

The autonomic nervous system exerts a profound influence on the heart due
to its ability to modulate cardiac rate (chronotropy), conduction velocity
(dromotropy), contraction (inotropy), and relaxation (lusitropy). The chronotropic
and dromotropic effects are mediated by both parasympathetic and sympathetic
fibers innervating the sinoatrial (SA) and atrioventricular (AV) nodes, whereas the inotropic and lusitropic effects are mediated mainly by sympathetic fibers innervating atrial and ventricular myocytes. The parasympathetic fibers travel in the vagus nerve. The right vagus targets mainly the SA node with only a minor innervation to the AV node whereas the left vagus goes primarily to the AV node with only a small outflow to the SA node. (Noble et al. 2010). Parasympathetic fibers release acetylcholine, which activates M2 muscarinic acetylcholine receptors to reduce the production of cAMP which reduces the rate of depolarisation and slows the heart rate. The activation of M2 muscarinic receptors also increases the K+ conductance of nodal cells. The resulting membrane hyperpolarisation decreases the spontaneous firing rate of the SA node and slows conduction in the AV node, thereby slowing the intrinsic heart rate. The sympathetic innervation of the heart is routed via the cervical and stellate sympathetic ganglia. It is the sympathetic fibers on the right side of the body which have the major effects on heart rate. The fibers from the left side of the sympathetic nervous system are more concerned with the regulation of the cardiac contractility. The sympathetic fibers release noradrenaline, which binds to β1-adrenergic receptors and causes cAMP mediated phosphorylation of membrane proteins and a subsequent increase in the inward Ca2+ current resulting in accelerated slow diastolic depolarisation and increasing heart rate. In myocytes, noradrenaline increases membrane Ca2+ currents and Ca2+ release from the sarcoplasmic reticulum during each action potential, resulting in increased force production. In addition, Ca2+ reuptake into the SR is enhanced, thereby accelerating relaxation. Together, the inotropic and lusitropic effects of sympathetic stimulation result in increased stroke volume. (Noble et al. 2010, Thomas 2011).

Given the ability to modulate both cardiac rate and stroke volume, the autonomic nerves provide an important remote mechanism to rapidly adjust cardiac output to meet short-term changes in the body’s needs. During rest, there is a little sympathetic efferent input with a low release of catecholamines. At rest, heart rate is about 30% lower than the intrinsic heart rate of 90–100 beats/min. Additional vagal discharge can further reduce heart rate and decrease cardiac output. With any movement away from the resting state, sympathetic activity increases and parasympathetic activity decreases. On termination of exertion, the recovery of resting heart state is again largely governed by parasympathetic dominance. (Noble et al. 2010, Somers 2011, Thomas 2011).
In addition to the rapid, direct effects of the sympathetic nerves, the sympathetic nervous system also exerts more prolonged, indirect effects on the cardiovascular system by activation of humoral systems such as the sympathoadrenal system. Sympathetic preganglionic neurons innervate the chromaffin cells in the adrenal medulla, which essentially act as postganglionic neurons to synthesize and release mainly adrenaline (80%) along with noradrenaline (20%) into the bloodstream. These circulating catecholamines contribute to cardiovascular regulation by activating cardiac and vascular adrenergic receptors. The physiological effects of circulating noradrenaline are similar to those of neurally released noradrenaline. (Noble et al. 2010, Thomas 2011).

The activity of the autonomic nerves that regulate cardiovascular function is determined by a network of neurons located in the medulla oblongata that receive inputs from other central structures and peripheral reflexes arising from baroreceptor, chemoreceptor, mechanoreceptor, thermoreceptor, and nociceptor afferents located in the blood vessels, heart, lungs, skeletal muscles, skin, and viscera (Guyenet 2006). In the humans, the central autonomic network is made up of the limbic/paralimbic systems including the insular cortex, amygdala, and anterior cingulate gyrus which appear to play an important role in regulating the cardiovascular system. Furthermore, the right insular cortex appears to play a major role in establishing sympathetic tone and the left insular cortex in modulating parasympathetic tone (Nagai et al. 2010). The descending signals from higher brain centers and afferent sensory signals from the large systemic arteries, cardiopulmonary region, and some of the viscera make their first synapse in the nucleus tractus solitarius in the dorsomedial region of the medulla. Other afferent inputs from the skin and skeletal muscles are transmitted to the medullary vasomotor centers via the spinal cord. Neural pathways from the nucleus tractus solitarius project to the ventrolateral medulla, which is the primary central site that regulates the sympathetic outflow. The rostral ventrolateral medulla contains excitatory neurons that synapse on the sympathetic preganglionic neurons in the intermediolateral gray column of the spinal cord, whereas the caudal ventrolateral medulla contains inhibitory neurons that project to the rostral ventrolateral medulla. Medullary control of vagal outflow to the heart is also mediated by nucleus tractus solitarius neurons that synapse on preganglionic parasympathetic neurons in the dorsal motor nucleus of the vagus and the nucleus ambiguus. (Thomas 2011).
The arterial baroreflex is a classic example of a negative feedback system and is designed to buffer beat-to-beat fluctuations in arterial blood pressure from an internal set point or baseline. This sympathoinhibitory reflex is stimulated by acute changes in arterial blood pressure that are sensed by stretch receptors (baroreceptors) in the vessel wall of the carotid sinus and aortic arch. The afferent baroreceptor discharge is relayed from the carotid sinus via the glossopharyngeal nerve and from the aorta via the vagus nerve, together these being commonly referred to as the buffer nerves, to the nucleus tractus solitarius, which evokes changes in efferent sympathetic and parasympathetic outflow to the heart and blood vessels in order to adjust cardiac output and vascular resistance to restore blood pressure to its original baseline. (Noble et al. 2010, Somers 2011, Thomas 2011). The arterial baroreflex can be reset to operate around a new baseline blood pressure. This resetting can be acute or temporary, for example, that occurring during exercise, or it can be chronic as encountered during the development of hypertension. (Thomas 2011).

Stretch receptors are also found in the walls of the atria and pulmonary arteries, where they respond to changes in central blood volume. Similar to the baroreceptors in the large systemic arteries, these receptors are activated by distension of the vessel wall giving rise to afferent signals transmitted via the vagus nerve to the vasomotor center and resulting in reflex inhibition of the efferent sympathetic outflow. Thus, the cardiopulmonary baroreceptors serve to minimize changes in arterial blood pressure in response to changes in blood volume. (Noble et al. 2010, Somers 2011, Thomas 2011).

Chemoreceptors located in carotid bodies at the bifurcation of the common carotid arteries and in aortic bodies in the region of the aortic arch respond to changes in arterial PO2, PCO2, and pH. Although primarily involved in regulating ventilation, they can also influence systemic vascular resistance because afferent signals are conveyed to both the respiratory and vasomotor centers in the medulla. Decreases in PO2 and pH or increases in PCO2 trigger increased afferent chemoreceptor discharge, which excites the respiratory center to reflexly increase ventilatory rate and volume and they excite the vasomotor center to reflexly increase sympathetic outflow. (Noble et al. 2010, Somers 2011, Thomas 2011).

2.5 Heart rate variability analysis methods

Heart rate variability (HRV) describes the complex regulatory system between heart rate and the autonomic nervous system. HRV is usually measured in two
different settings of the HRV signal or ECG recording. First, HRV signal recordings can be obtained under controlled laboratory conditions in response to different events such as the tilt, metronomic ventilation, drugs, or other physiological maneuvers that have an effect on the autonomic nervous system. These kinds of laboratory settings usually involve short-term ECG measurements. Secondly, the HRV signal can be obtained from long term ECG recordings lasting up to 24-hour or even longer. Diurnal ECG recordings are normally taken while the individual is conducting his/her daily activities. Short-term recordings may fail to detect some very low frequency oscillations, while long-term recordings are affected by changing environmental conditions that may confound the results of the HRV analysis.

There are several methods available to measure HRV (Task Force 1996). Variation in the HR can be evaluated in different ways, which can be categorized as time domain methods, frequency domain methods, geometric methods and methods based on the nonlinear dynamics of the HR. In addition, HR turbulence and baroreflex sensitivity are commonly analyzed HRV parameters.

HRV has been increasingly used to assess autonomic function in various physiological and pathological settings, including the assessment of autonomic dysfunction in diabetic patients. Diabetic autonomic neuropathy can produce severe autonomic dysfunction and is one cause of morbidity and mortality among diabetic patients. Diabetic neuropathy is characterized by a widespread neurological degeneration affecting the small nerve fibers of the parasympathetic and sympathetic branches of the autonomic nervous system. The decreased beat-to-beat variability in diabetic patients during sleep was first documented in 1973 (Wheeler & Watkins, 1973) and later, this reduction in HRV has been confirmed in several other studies (Bellavere et al. 1992, Ewing et al. 1984, Ewing et al. 1991, Freeman et al. 1991, Malpas & Maling 1990, Pagani et al. 1988, Ziegler et al. 1992). Cohorts which have examined large patient populations have demonstrated the presence of association between a low HRV and the prevalence of diabetes (Gerritsen et al. 2000, Singh et al. 2000).

2.5.1 Time domain measures of heart rate variability

Time domain indices are often considered as the simplest HRV analysis. They are based on either statistical or geometrical analyses of the HR or the intervals between successive normal complexes (Task Force 1996). The simplest time domain variables include the following HRV parameters: the mean value of the
normal-to-normal (N-N) intervals, usually called as the mean HR, the difference between the longest and the shortest N-N interval and the difference in the HR between night and day. More complex time domain methods involve a higher degree of statistical analysis or geometrical methods. Statistical time domain analyses can be divided into two classes, 1) methods derived from the measurements of the N-N intervals directly, and 2) methods derived from the differences between N-N intervals. Both of these methods can be derived from both short-term and long-term ECG recordings. One of the most straightforward statistical time domain variables to calculate is the standard deviation of the N-N intervals (SDNN). The SDNN reflects all the cyclic components responsible for the variability in the period of recording, and the durations of the recordings used to determine the SDNN need to be standardised, as the SDNN depends on the period length. In most studies, SDNN is computed over a 24-hour period. The variation of the SDNN is the standard deviation of the average N-N interval (SDANN), which is computed over short periods, usually around five minutes, of R-R interval data. Another variation of the SDNN is the mean value of the five minute standard deviation of the N-N intervals called the SDNN index. The SDNN index is computed over 24 hours in segments of five minutes. The square root of the mean squared differences of successive R-R intervals (RMSSD), and the NN50, i.e. the number of interval differences of successive R-R intervals greater than 50 ms (Ewing et al. 1984), and the proportion of these events (pNN50), are the most commonly used measures derived from R-R interval differences, and are estimates of high frequency (HF) variations of the HR (Task Force 1996). All of the above mentioned short-term time domain HRV measurements tend to estimate the HF fluctuations in the HR and are highly correlated (Task Force 1996).

Geometrical methods categorize R-R intervals into geometrical patterns and various methods exist to derive HRV measures from these patterns. One approach is to quantitatively analyse the Poincaré plots (Huikuri et al. 1996, Tulppo et al. 1996), which are scattergrams plotting each R-R interval as a function of the previous R-R interval. The Poincaré plot is useful and an easy method for visualising the regularities or randomness that may exist in the beat-to-beat variability (Brennan et al. 2001, Kamen et al. 1996). An R-R interval time series with a high R-R interval variability produces a plot with a widespread pattern around a straight line at 45 degrees from the horizontal axis. For an HRV signal with low variability, the Poincaré pattern will accumulate in a small-sized area. The standard descriptors of the Poincaré plot are SD1 and SD2 (Brennan et al.
The line of identity is the 45 degree imaginary diagonal line on the Poincaré plot. SD1 measures the dispersion of data points perpendicular to the line of identity and it represents the instantaneous beat-to-beat variability. SD2 measures the dispersion of data points along the line of identity and it represents the continuous long-term beat-to-beat variability. The ratio of these parameters, SD1/SD2, represents a measure of heart activity (Tulppo et al. 1996). Another geometrical method is the triangular index (HRVI), where the total number of the NN intervals is divided by the largest number of equally long NN intervals. The HRVI is based on the concept that when the major peak of the histogram is a triangle, its baseline width is equal to its area divided by its height (Malik et al. 1989). In the HRVI representation, the durations of the NN intervals are placed in the x-axis and the number of each interval length serves as the y-axis. The triangular index is considered as being insensitive to artifacts, especially to the presence of many ectopic beats, because these beats are left outside the histogram triangle as outlier samples (Malik et al. 1989). However, the major advantage of the geometrical methods is attributable to the lack of the sensitivity to the analytical quality of the NN interval series.

2.5.2 Frequency domain measures of heart rate variability

Various spectral methods have been used in the analysis of the HRV. Power spectral density (PSD) analysis provides the basic information about how variance distributes as a function of frequency, though only an estimate can be obtained by the appropriate mathematical algorithms (Task Force 1996).

The power spectrum is commonly divided into three or four frequency bands i.e.: ultra low frequency (ULF) < 0.0033 Hz, very low frequency (VLF) 0.0033–0.04 Hz, low frequency LF 0.04–0.15 Hz and HF 0.15–0.40 Hz (Task Force 1996). The power of the different components along with the total power is expressed as absolute units (ms$^2$). LF and HF components can also be expressed as normalised units and the LF:HF -ratio is also commonly used in order to depict the controlled and balanced behaviour of the two branches of the autonomic nervous system (Malliani et al. 1991, Pagani et al. 1986).

The origin of VLF components is not understood in detail, but they are suggested as being affected by the thermoregulation systems, the renin-angiotensin system and perhaps other humoral factors (Kitney & Rompelman 1977).
The LF component is created in a heart rhythm that is usually observed around 0.1 Hz. This slow oscillation of the heart rhythm has been suggested as being caused by the mechanisms regulating the blood pressure (Kamath & Fallen 1993, Kitney et al. 1985). However, the physiological interpretation of LF rhythms is controversial. Contributions from both parasympathetic and sympathetic systems are involved in the LF rhythms. An increase in the LF power has been proposed as being a marker for sympathetic activation (Kamath & Fallen 1993, Malliani et al. 1991). On the other hand, also the parasympathetic regulation has been reported as having an influence on the LF power (Akselrod et al. 1985, Pomeranz et al. 1985).

The HF component of the HRV spectrum is usually identified in the frequency range between 0.15 Hz to 0.4 Hz, which is related to the respiratory frequency. Respiration related HF rhythm is affected by the changes in the intrathoracic pressure and the mechanical changes due to the breathing. This activity, which is mediated by the vagus nerve, is considered to be a marker of parasympathetic activation (Hirsch & Bishop 1981, Katona & Jih 1975, Pagani et al. 1986).

In addition to the above three main frequency components, the ultra low frequency (ULF) component can be determined. The ULF component is situated in the frequency range \( f < 0.0033 \) Hz (Kamath & Fallen 1993). The physiological background of the ULF component has still not been specified in detail, but the major underlying factors of the ULF are speculated as being involved in the day to night variation, with large infrequent events such as awakening and falling asleep (Roach et al. 1998).

The methods for the calculation of the PSD are generally classified as nonparametric (e.g. FFT) and parametric (e.g. the autoregressive model approach). Nonparametric methods have the advantages of being handled by simple algorithms and thus have a high processing speed, whereas the benefits of parametric methods are smoother spectral components, easy post-processing and the accurate estimation of the PSD even from a small number of samples. The basic disadvantage of the parametric method is the need for verification of the suitability of the chosen model, and variations of this model order within or between subjects may invalidate quantitative comparisons of the results.
2.5.3 Non-linear measures of heart rate variability

Non-linear methods related to chaos tend to deal with autonomous systems, i.e. systems where there is no input or where the input has a very simple form (Hoyer et al. 1998). It has been suggested that a healthy heart rhythm is chaotic and exhibits a fractal form that may be disrupted by a disease (Goldberger et al. 1990, Goldberger 1996). The basic concept of the nonlinear HRV methods is to try to capture the non-periodic behaviour of the HRV and to reveal the complexity that exists inside the R-R interval dynamics. Various different nonlinear methods including return maps, fractal scaling analysis and different complexity measures have been tested in various sets of R-R interval data (Bigger Jr. et al. 1996, Huikuri et al. 1998, Huikuri et al. 2000, Lombardi et al. 1996, Mäkikallio et al. 1997, Mäkikallio et al. 1998, Mäkikallio et al. 1999a, Mäkikallio et al. 1999b).

Mandelbrot (1982) introduced the term ‘fractal’. A fractal describes a set of points that resembles the whole set even when examined at very small scales. The quantification of fractal properties assesses the self-similarity of the HR oscillation over multiple time scales. The presence or absence of the fractal correlation properties in an R-R interval time series can be examined with the detrended fluctuation analysis (DFA) technique (Peng et al. 1995). The scaling exponent $\alpha$ represents the ‘roughness’ of the time series. Large values of $\alpha$ indicate a smooth time series. The values of the scaling exponent for the normal healthy subject are typically near to 1, which is indicative of fractal-like HR behaviour, while patients with cardiovascular diseases or with advancing age have been reported as displaying altered fractal-like behaviour in their HR (Goldberger et al. 2002).

Other nonlinear HRV analysis methods are power-law correlation, approximate entropy (ApEn), Lyapunov exponent, correlation dimension ($D_2$) and Kolmogorov entropy ($K$). The last three of the nonlinear HRV analysis methods require long-term data and therefore ApEn has become a more common method in order to classify the complexity of relatively short-term HR data (Pincus & Goldberger 1994).
2.6 Repolarisation analysis methods

2.6.1 QT interval

The QT interval measures the time from ventricular depolarisation onset to the end of ventricular repolarisation. Since repolarisation does not extend beyond the end of the QT-interval, it is commonly accepted that the QT interval is a valid reflector of the end of myocardial repolarisation (Surawicz & Knoebel 1984). The measurement of the QT interval is fraught with potential problems. It has been recognised that the QT interval measurements vary greatly depending on the leads that are being used (Campbell Cowan et al. 1988). In addition, there are many different methods that can be used for making the measurement. The QT interval has been calculated from a variety of leads but there is no clear consensus about the best method and best leads, even among experts (Kautzner 2002). Technically the QT interval can be measured using a simple ruler, both using manual or electronic calipers connected to a digitizing pad. Whatever method is used, the problem is the great inter- and intraindividual variability between observers. The end of the T-wave can be defined as the point where the descending limb of the T-wave intersects the baseline. The end of the T-wave may also be determined with a tangent fitted to the steepest slope of the descending T-wave. The occurrence of U-wave can also cause confusion; i.e. should it be included into the T-wave or not. Traditionally it has been recommended that the measurements are made from leads II and V5 but also V2 or V3 are claimed to provide a close approximation to the maximum QT interval.

There are several factors influencing the QT interval. The QT interval tends to be longer in females than in males (Bazett 1920, Lepeschkin & Surawicz 1953). Hypocalcemia prolongs the QT interval and conversely hypercalcemia shortens it. Hyperkalemia tends to shorten the QT interval but in hypokalemia, the measurement is more difficult due to commonly occurring T-U complex (Surawicz 1967). Additionally, body temperature has an effect on QT interval with fever apparently shortening it (Karjalainen & Viitasalo 1986). Many drugs can affect the QT interval. In addition, changes in autonomic nervous system tone can alter the QT interval both via heart rate modulation or more directly by affecting depolarisation and repolarisation kinetics in the myocyte. The QT interval is significantly longer during sleep than during the waking state at the same heart rate (Viitasalo & Karjalainen 1992). Heart rate greatly influences the QT interval. For example, under normal physiologic conditions a normal heart
has a longer QT interval at a lower heart rate than at a higher heart rate. Reproducibility and comparability of measurements of the QT interval in
different studies or in clinical assessment of an individual subject requires
compensation for this variation. Usually the QT interval is corrected for the heart
rate by applying a mathematical correction formula.

There are various different formulae, exponential, linear and logarithmic that
have been proposed for adjusting the QT interval for heart rate (Moss 1993). The
major weakness in most of these formulae is that they are based on the
predominant heart rate from a resting ECG in the studied population making
conversion unreliable at both slow and high heart rates. The most commonly used
correction method for the QT interval is Bazett’s square root formula (QT
measurement/√ R-R interval) which is rooted in medical practice so deeply that it
is considered as a gold standard despite its well known deficiencies at low and
high heart rates (Bazett 1920). Although it is widely used, it is also clearly
understood that the QT interval corrected by Bazett is artificially prolonged at
heart rates > 60/min and shortened at heart rates < 60/min meaning that the use of
this formula is unsatisfactory in many instances (Malik 2001). The cube root
Fridericia formula (QT measurement / 3√ R-R-interval) has the same limitations
at high heart rates but is considered to perform better at low rates (Fridericia
1920). In addition, the widely used Framingham linear correction formula (Sagie
et al. 1992) tends to give too low values at slow heart rates compared to the
formula believed to be most accurate at the present time (Karjalainen et al. 1994).
This favoured technique corrects heart rate well over all sub ranges of heart rates
using a nomogram method where a correction factor is added to each specific QT
interval.

It has become obvious that the QT interval is a major risk factor for serious
cardiac arrhythmias and sudden cardiac death. There is data revealing that a long
QT interval predicts a risk of malignant ventricular tachyarrhythmias and sudden
death in postmyocardial infarction patients (Ahnve et al. 1980, Ahnve et al. 1984,
Wolf 1978). In addition, the prolongation of the QTc interval (a rate corrected QT
interval using Bazett’s formula) has predicted total mortality (Schouten et al.
1991), coronary artery disease and sudden death (Dekker et al. 1994) in
apparently healthy population. There is increasing evidence that a prolonged QTc
interval is a significant predictor of mortality both in type 1 (Rossing et al. 2001,
Veglio et al. 2000) and type 2 diabetes (Okin et al. 2004, Rana et al. 2005, Salles
et al. 2004).
2.6.2 QT dispersion

Originally QT dispersion was defined as the difference between the longest and shortest QT-interval measured from a 12-lead ECG. These differences between ECG leads were believed to reflect regional differences in repolarisation (Day et al. 1990). Without clear clinical or experimental verification, this interlead variability was widely accepted as a marker of arrhythmogenicity. However, the observed lack of reproducibility in measuring the QT dispersion (Kautzner & Malik 1997) and the negative results of some major clinical studies investigating the association between QT dispersion and mortality (Brendorp et al. 2001, Zabel et al. 1998) suggest that different QT intervals in different 12-lead ECG leads do not reflect true dispersion of repolarisation of the ventricular myocardium. In addition, studies examining technical aspects of QT interval projection on the body surface have increased doubts regarding this method (Macfarlane et al. 1998) to the extent, that generally this method is no longer in use.

Since the measurement of the QT interval and dispersion may contain many methodological inaccuracies and does not provide any information about the morphology of the T-wave, new descriptors of cardiac repolarisation have been developed.

2.6.3 Vector loop analysis of repolarisation

Vectorcardiogram (VCG) can be measured by using a VCG lead system or it can be derived from the standard 12-lead ECG. The orthogonal lead systems are based on the principle that electric activity in the three-dimensional heart at one particular instant can be summed into one resultant vector. The most commonly used orthogonal lead system is the Frank lead system (Frank 1956). Frank leads are derived from seven electrodes: four leads on the chest, one lead on the back, one on the neck, and one on the left foot. The three leads derived from the system are called X, Y, and Z. The X and Y directions determine a frontal plane, X and Z directions refer to a horizontal plane, and Z and Y directions determine a sagital plane. When the standard 12 lead ECG is measured, all the chest leads are on the front side of the body, in contrast to the Frank lead system, where one of the chest leads is located on the back. Therefore the measurement by the Frank lead system can be considered as being more three dimensional than can be obtained with the standard 12 lead ECG.
During the last decade, Principal Component Analysis (PCA) has been increasingly used for reducing the standard 12 ECG leads to three orthogonal VCG leads. PCA is a statistical technique, which is intended to compress the information of a large set of correlated variables into a few variables, i.e. principal components, while not discarding the variability present in the data. When the ECG-signal is processed by PCA, the resulting three largest principal components will be a description of this single vector in three dimensional space. All the components of higher order, i.e. nondipolar components, describe the part of the signal that cannot be explained by the single vector of the model. 96–99.9% of all information in the QRS-complex has been retrieved in the first three factors (Scher et al. 1960).

The software automatically creates T-wave and QRS loops in the Frank’s 3-dimensional space using matrix modification and the singular value decomposition technique. The software calculates the plane where the loop displays the maximum first and second dimensions. The loop is rotated until its longest axis is parallel to the x-axis. The longest axis of the loop defines its width, and the second longest axis perpendicular to the longest axis defines its height. The height/width ratio reflects the shape of the loop. The calculated E parameter describes the roughness of the plane, i.e. how well the loop can be adjusted in the plane. Subsequently, a rectangle is adjusted around the loop and this is divided into 100 subdivisions. T-wave loop dispersion (TWLD) and QRS loop dispersion (QRSLD) are defined as the number of subrectangles traversed by the borderline of the corresponding loop (Zabel et al. 2000). The relationship between the QRS complex and the T wave, expressed as an angle in three dimensional space, has been a subject of interest during the last decade. One of the most often used parameters for measuring the QRS-T angle was developed by Acar et al. (1999). That parameter is the averaged cosine of the QRS-T angle, ‘total cosine R-to-T’ (TCRT). It measures the vector deviation between the depolarisation and repolarisation fronts. Small values correspond to large differences in the orientation of the two loops.

The dynamical behaviour of the vectorcardiographic features in general is largely unknown. Postural changes and respiration, HR and autonomic effects are all believed to influence TCRT (Batchvarov et al. 2002). On average, women have been shown to have a predominantly higher cosine of the QRS/T angle and TCRT-parameters regardless of the heart rate (Rautaharju et al. 2006, Smetana et al. 2002, Smetana et al. 2004, Zhang et al. 2007). The rate dependence of TCRT
has been previously reported by Smetana et al. (2004). Their study indicated that TCRT has a tendency towards lower values at a higher HR.

Especially vectorcardiography has proved its potential during the last decade because of its ability to handle the digital ECG signal. The spatial QRS/T angle has been shown to be a strong and independent marker of cardiovascular mortality in both general populations and cardiac patients (De Torbal et al. 2004, Kardys et al. 2003, Rautaharju et al. 2006, Yamazaki et al. 2005). There are studies which have examined the association of TCRT with adverse cardiac events, mainly arrhythmias, and death (Kenttä et al. 2011, Malik et al. 2004, Perkiömäki et al. 2006, Zabel et al. 2000).

2.7 Cardiovascular autonomic regulation during hypoglycaemia

2.7.1 Experimental hypoglycaemia

There are some reports describing changes in HRV during hypoglycaemia in healthy subjects (Laitinen et al. 2003, Schächinger et al. 2004), but less is known about hypoglycaemia-induced changes in cardiac autonomic regulation in diabetic patients. The previous studies tended to provide conflicting results regarding the effect of hypoglycaemia on HR and HRV. Premature ventricular beats, severe nodal bradycardia and an increase in HR have been observed (Lindström et al. 1992, Shimada et al. 1984). Russell III et al. (2001) described an increase in HR in non-diabetic and intensively treated type 1 diabetic patients during both hypoglycaemic and euglycaemic hyperinsulinaemia without any statistically significant difference between the euglycaemic and hypoglycaemic conditions. Laitinen et al. (2003) did not observe any responses in cardiac parasympathetic regulation during a hyperinsulinaemic hypoglycaemic clamp in healthy, non-diabetic subjects. In this study, they used a single-day study protocol where hypoglycaemia followed euglycaemia. They also examined higher blood glucose values (3.0 mmol/l) with correspondingly weaker stimulation of the autonomic nervous system. Schächinger et al. (2004) did not detect any significant changes in HR during a hyperinsulinaemic hypoglycaemic clamp but they did observe a minor increase in the HF spectral component. They used a two-day single-blinded crossover design and they targeted a glucose level of 2.7 mmol/l. In both studies, a 5-minute-period was used for the analysis of changes in HR and HR variability.
In addition, the effects of insulin \textit{per se} on cardiac autonomic regulation have been studied although the results have been conflicting. Bellavere \textit{et al}. (1996) and Van De Borne \textit{et al}. (1999) observed marked reductions in the HF band during a hyperinsulinaemic, euglycaemic clamp in healthy individuals. Later, Stockhorst \textit{et al}. (2011) reported a significant increase in HF component and parasympathetic tone after insulin or glucose induced hyperinsulinaemia in healthy men. Insulin has also been shown to have a vasodilatory effect on the skeletal circulation, leading to an increase in skeletal blood flow (Anderson \textit{et al}. 1991, Creager \textit{et al}. 1985).

2.7.2 \textbf{Spontaneous hypoglycaemia}

There is only one case report concerning spontaneous hypoglycaemia and HRV in type 1 diabetes. Malpas & Maling (1989) described a chance observation of increased HRV during hypoglycaemia in a diabetic patient with parasympathetic neuropathy undergoing 24-hour ECG monitoring.

2.8 \textbf{Cardiac repolarisation during hypoglycaemia}

2.8.1 \textbf{Experimental hypoglycaemia}

The fact that hypoglycaemia can produce ECG changes has been known since the 1930s, when insulin-induced hypoglycaemia was used in attempts to treat schizophrenic patients. Subsequently, Goldman observed electrocardiographic changes in these unconscious hypoglycaemic patients using standard limb leads and one chest lead. He noted changes in rhythm (sinus bradycardia, sinus tachycardia, atrial fibrillation) and minor alterations in the P wave and QRS morphology, as well as depression and the occasional elevation of the ST segment. Most patients, however, showed flattening or inversion of the T wave, which could take months to return to normal (Goldman 1940). Since then, the flattening of the T-wave during insulin-induced hypoglycaemia in healthy subjects has been well documented in several studies (Eckert & Agardh 1998, Laitinen \textit{et al}. 2008, Meinhold \textit{et al}. 1998). Nonetheless, recent studies investigating individuals with diabetes have focused on the QT interval and QT dispersion and only a few studies have been concerned with changes in T-wave morphology during experimental hypoglycaemia (Lindström \textit{et al}. 1992).
There are several studies indicating QTc interval prolongation during hypoglycaemia in healthy subjects (Due-Andersen et al. 2008b, Eckert & Agardh 1998, Laitinen et al. 2008, Robinson et al. 2003a, Robinson et al. 2003b). Robinson et al. (2003a, 2003b) measured also the QT dispersion and observed a significant increase of the QT dispersion during experimental hypoglycaemia. In these studies, the most widely used correction formula was the Bazett’s formula but Laitinen et al. (2008) used three different formulas (Bazett’s formula, the Fridericia formula and the nomogram method).

The earliest study concerning subjects with diabetes was performed by Marques et al. (1997). The study included patients with both type 1 and type 2 diabetes. A significant lengthening of the QTc interval was observed during hypoglycaemic clamp. Two years later, Landstedt-Hallin et al. (1999) examined patients with type 2 diabetes and observed a significant increase in both the noncorrected and the corrected mean QT intervals as well as the QT dispersion. In 2004, Lee et al. (2004) demonstrated that in adults with type 1 diabetes and cardiac autonomic neuropathy, the peak QTc interval during insulin-induced hypoglycaemia tended to be lower than in healthy controls or patients with type 1 diabetes without cardiac autonomic neuropathy. One year later, this group reported that hypoglycaemic QT interval lengthening corrected using the Fridericia formula was blunted by atenolol in individuals with type 1 diabetes (Lee et al. 2005). Due-Andersen et al. (2008a) demonstrated a prolongation of the QT interval in subjects with type 1 diabetes during hypoglycaemia. Bazett’s formula and the Fridericia formula were used for the rate correction of the QT interval. Rothenbuhler et al. (2008) observed that a hypoglycaemic clamp increased the QT interval in all explored diabetic adolescents. The increase was less extensive with the Fridericia formula than with the Bazett’s formula.

### 2.8.2 Spontaneous hypoglycaemia

In a case report, Skyrme-Jones & Gribbin (2001) described a 46-year-old diabetic male with a deep anterolateral T-wave inversion and a ST-T wave abnormality in the inferior leads during severe hypoglycaemia. Angiography revealed smooth vessels with normal left ventricular function. The resting ECG progressively returned towards normal. In the studies of Gill et al. (2009), Murphy et al. (2004), Robinson et al. (2004), lengthening of QT interval during spontaneous hypoglycaemia was observed. Murphy et al. (2004) and Robinson et al. (2004) used Bazett’s formula for the correction of QT interval for heart rate and in the
study of Gill et al. (2009) the correction formula was not stated. In the study of Christensen et al. (2010), Bazett’s formula, the Fridericia formula, the nomogram method and a linear subject-specific method were used for correction of the QT interval. QT intervals corrected with formulas other than Bazett’s were not associated with any significant change in the QT interval. The protocol and the study population of the studies differed markedly. In the first two studies, the participants were hospitalized and blood samples and ECG recordings were taken at regular intervals. In the study of Robinson et al. (2004) and Christensen et al. (2010), the subjects were older and some of them had hypertension or coronary heart disease. In the study of Murphy et al. (2004), the subjects were children or adolescents. Additionally, the analysis of the data differed considerably. In the study of Robinson et al. (2004), 4 a.m. was selected as the start time for collecting control data. Murphy et al. (2004) compared mean overnight QTc values between nights with and without hypoglycaemia. In the studies of Gill et al. (2009) and Christensen et al. (2010), continuous glucose monitoring and ECG recording were used. In the first study, the QT interval was analyzed throughout the entire hypoglycaemic period and expressed as a mean QT interval and a control period was selected from a normoglycaemic period immediately prior to hypoglycaemia. In the latter study, the QT interval was analysed at every measurement of hypoglycaemia, measurements of QT interval during euglycaemia were taken at intervals of 30 minutes and median QT interval durations were calculated.

2.9 ‘Dead in bed syndrome’

2.9.1 History of ‘dead in bed syndrome’

In 1989 it was suggested that there had been an increase in sudden deaths of young people with type 1 diabetes in the United Kingdom, and that they might be related to the increasing use of human insulin. Tattersall & Gill (1991) investigated all such cases of individuals under age 50 years and reported 22 of 50 sudden deaths in 1989 where no cause of death or anatomical lesions were found at autopsy. Nineteen of the 22 were sleeping alone at the time of death and 20 were found lying in an undisturbed bed. Most had uncomplicated diabetes. The investigators hypothesized that hypoglycaemia or a hypoglycaemia-associated event was responsible for the deaths because of the timing of death and other circumstantial evidence (most of the subjects had a history of recurrent severe
nocturnal hypoglycaemia). There was nothing to implicate the species of insulin as a factor in these deaths. Tattersall and Gill classified this group of cases as 'dead in bed'. The criteria of so-called 'dead in bed syndrome' were that a patient would be found dead in an undisturbed bed, the patient had been observed to be in good health condition on the previous day, he or she did not have any clinical evidence of late complications and that autopsy did not reveal any cause of death.

2.9.2 Epidemiology of 'dead in bed syndrome'

Borch-Johnsen & Helweg-Larsen (1993) investigated all cases of sudden death in Denmark in younger insulin-treated diabetic patients, age at death below 50 years. The total number of cases fulfilling the inclusion criteria was 226. Ketoacidosis or unknown cause of death (including found dead in bed) was identified in 15 (7%) patients. The annual number of sudden deaths did not change during the study period. The number of deaths due to hypoglycaemia and cases with unexplained cause of death also remained constant.

The incidence of unexplained deaths was investigated in Norwegian diabetic patients under the age of 40 during the period of 1981–1990 (Thordarson & Søvik 1995). Sixteen of 240 deaths (7%) fulfilled the criteria of so-called ‘dead in bed syndrome’. Twelve of these patients were reported as having had frequent episodes of hypoglycaemia, with nocturnal episodes in 10 cases.

Edge et al. (1999) determined the mortality rate and causes of death in children with type 1 diabetes. The Office of National Statistics (England and Wales) and the General Register Office (Scotland) notified all deaths under 20 years of age from 1990 to 1996 with diabetes on the certificate. 116 deaths were notified and nine of these (8%) were found ‘dead in bed’.

In Sweden, Dahlquist & Kallen (2005) reported 78 deaths of young type 1 diabetic subjects in 1977–2000. Seventeen (22%) diabetic case subjects were found deceased in bed without any cause of death diagnosed at forensic autopsy. Wibell et al. (2001) studied prospectively, in young, Swedish adult patients, the mortality during the first 10 years after the diagnosis of diabetes. During that period, 4097 new cases were registered and classified as type 1 diabetes (73%), type 2 (16%), secondary (2%) and unclassified (9%). The median follow-up was 5 years. Fifty-eight patients died but unexplained ‘dead in bed’ was found only once.

Tu et al. (2008) documented 67 deaths of Australians under the age of 40 with type 1 diabetes in 1994–2006. Acute complications of diabetes, unnatural
deaths, and sudden unexpected deaths were the predominant causes of death in young individuals with diabetes. Of the 15 (22%) sudden unexpected deaths, 10 (15%) people were found dead in an undisturbed bed with no cause of death diagnosed at autopsy.

In the U.S., Secrest et al. (2011) examined all unwitnessed deaths in two related registries (the Children’s Hospital of Pittsburgh and Allegheny County) yielding 1319 persons with childhood-onset (age < 18 years) type 1 diabetes diagnosed between 1965 and 1979. Of the 329 participants who had died, the Mortality Classification Committee reviewed and assigned a final cause of death in 255 of cases (78%). Nineteen (8%) of these were sudden unexplained deaths (13 male) and seven (3%) met dead-in-bed criteria.

Thus, about 100 sudden unexplained deaths that fit the dead-in-bed syndrome criteria have been reported in United Kingdom, Norway, Sweden, Denmark, Australia and U.S.A. Some of these individuals were found to be taking multiple daily insulin doses and experiencing frequent episodes of hypoglycaemia prior to death (Thordarson & Søvik 1995). Two studies have reported that sudden deaths occur in > 20% of all young type 1 diabetes deaths (age < 50 years), compared with 1–5% of the similar general populations (Dahlquist & Kallen 2005, Tu et al. 2008). Recently, Koltin & Daneman (2008) estimated that 5–6% of all type 1 diabetes deaths below 40 years of age fitted the criteria of dead-in-bed syndrome. According to these reports, ‘dead in bed’ syndrome remains a rare but important cause of death in type 1 diabetic patients under the age of 40 years.

2.9.3 Etiology of ‘dead in bed syndrome’

The underlying causes of ‘dead in bed syndrome’ have remained unclear. Several hypotheses have been proposed over the years, although none has been proven. The most common theory is that there is a preceding profound hypoglycaemic episode as the causal event. Indeed, many of these patients demonstrated a history of severe hypoglycaemic events in the period leading to their death. However, the absence of cerebral damage at autopsy and the undisturbed bed makes a seizure or a confused state less likely.

Another theory is that the death is due to a cardiac event initiated either by hypoglycaemia or by autonomic dysfunction. The most consistent cardiac finding correlating to increased mortality in individuals with diabetes is a prolonged QT interval, which may lead to dysrhythmias. In 1991, Ewing et al. demonstrated a connection between QT prolongation and autonomic neuropathy in diabetes.
However, most of the patients who died unexpectedly were young, with a relatively short disease duration, and would not be expected to exhibit diabetic neuropathy. Weston et al. (1996) compared a group of young patients with diabetes with a group of nondiabetic subjects. On bedside examination, none of the subjects in either group demonstrated findings compatible with cardiac autonomic neuropathy; however, when tested for the baroreflex response to pharmacologic agents, there was already a significant reduction in both sympathetic and parasympathetic responses in the group with diabetes compared with the non-diabetics, pointing to a cardiac autonomic dysfunction.

Several studies have demonstrated a prolonged QT interval during hypoglycaemia amongst type 1 diabetes patients (Due-Andersen et al. 2008a, Gill et al. 2009, Landstedt-Hallin et al. 1999, Lee et al. 2004, Lee et al. 2005, Marques et al. 1997, Murphy et al. 2004, Robinson et al. 2004, Rothenbuhler et al. 2008). A relationship between the increase in QT interval and the rise in the plasma adrenaline concentration has been observed in some of these studies. Blockade of β-receptors can prevent QT lengthening during hypoglycaemia, suggesting that sympathoadrenal stimulation may contribute to these changes (Lee et al. 2005, Robinson et al. 2003a). Abnormalities of cardiac repolarisation such as prolonged QT interval particularly during hypoglycaemia could increase the likelihood of experiencing a fatal ventricular arrhythmia.

Lee et al. (2004) compared QTc interval prolongation between patients with overt or subclinical autonomic dysfunction and those with a normal autonomic response when exposed to induced hypoglycaemia. Their findings demonstrated a greater prolongation of the QTc interval in patients with normal or mild autonomic dysfunction compared with those with more severe dysfunction. The sympathetic response to hypoglycaemia is known to be impaired in diabetic patients with autonomic neuropathy. Lee’s group described a positive correlation between the adrenaline level and the QTc prolongation. The study demonstrated a possible protective effect of a reduced sympathetic response in cases with more advanced disease.

In his review of this topic, Bell (2006) hypothesised that the ‘dead in bed syndrome’ occurs as a result of a ventricular arrhythmia due to an acute prolongation of the QTc interval caused by hypoglycaemia-induced sympathetic overactivity in the young type 1 diabetic patient who is predisposed to developing ventricular arrhythmias because of an underlying cardiac dysautonomia associated with an underlying cardiac lesion, e.g., a mitral valve prolapse.
Heller (2008) has stated that the majority of sudden, unexplained deaths in young patients with diabetes may have resulted from an increase in the plasma adrenaline concentration and a fall in potassium accompanying hypoglycaemia, producing a prolongation of the QT interval on a background of early, subclinical autonomic neuropathy in an individual who has inherited polymorphisms in the LQT genes which make him/her susceptible to an exaggerated QT lengthening during sympathoadrenal activation.

Tu et al. (2010) sought to identify myocardial cellular changes and genetic influences that could contribute to the pathogenesis of the dead in bed syndrome. Post-mortem reports between 1994 and 2006 from the 2 largest Departments of Forensic Medicine in Australia were reviewed for dead in bed syndrome cases. Post-mortem heart sections were immunohistochemically stained for collagen types I and III and connective tissue growth factor (CTGF). Genomic DNA was prepared from post-mortem samples, and genetic analysis was performed focusing on the genetic factors associated with cardiac ion channels, cardiac fibrosis, glucose regulation, and autonomic nervous system function (cardiac voltage-gated sodium channel (SCN5A), glucose-6-phosphatase catalytic subunit (G6PC), paired-like homeobox 2b (PHOX2B), and CTGF genes). Twenty-two dead in bed syndrome cases were identified and staining of heart sections for collagen I and III, and CTGF detected no differences between dead in bed syndrome cases and controls. Genetic screening of SCN5A revealed 3 silent polymorphisms A29A, E1061E, and D1819D and 1 protein-changing variant H558R. No genetic variants were found in G6PC, PHOX2B, and CTGF, and the dead in bed syndrome cases were not associated with the presence of the G-945C CTGF promoter polymorphism.
3 Aims of the study

The aims of the present study were to investigate the cardiovascular autonomic control and the cardiac repolarisation during hypoglycaemia in patients with type 1 diabetes.

The more specific aims of the individual studies were:

1. To evaluate the cardiovascular autonomic control in type 1 diabetic patients and healthy controls with HRV analysis methods during insulin-induced hypoglycaemia (clamp study).
2. To evaluate cardiac repolarisation in type 1 diabetic patients and healthy controls with QT interval measurements and vector loop analysis during insulin-induced hypoglycaemia (clamp study).
3. To study the cardiovascular autonomic control in type 1 diabetic patients with HRV analysis methods during spontaneous, nocturnal hypoglycaemia.
4. To evaluate cardiac repolarisation in type 1 diabetic patients with QT interval measurements and vector loop analysis during spontaneous, nocturnal hypoglycaemia.
4 Subjects and methods

4.1 Subjects

This study was carried out in the Department of Internal Medicine of the Oulu University Hospital and in the Department of Exercise and Medical Physiology in Verve Research. The studies were carried out according to the principles of the Declaration of Helsinki. The study protocol was approved by the local ethical committee of the Oulu University Hospital. All patients and control subjects gave their informed consent before their inclusion into the study. Patients were selected from the medical records of the Oulu University Hospital and the Oulu Health Center. Patients over 50 years of age and with extremely poor glycaemic control (HbA1c > 11.0%) were excluded. None of the patients or the healthy controls had a previous history of cardiovascular disease or clinical evidence of heart disease and all had normal ECGs. There were no signs of diabetic complications, with the exception of nonproliferative and treated proliferative retinopathy. Microalbuminuria tests and clinical status were normal without signs of neuropathy.

In studies I and II, sixteen patients (7 males, 9 females), mean age 32 ± 8 yr (range 18–46 yr), with type 1 diabetes and eight healthy subjects (3 males, 5 females), mean age 34 ± 10 yr (range 22–48 yr), participated in the study. The known duration of diabetes was 13 yr (range 2–29 yr), and glycaemic control was fairly strict (mean HbA1c 7.0%, range 5.8–8.7%). The diabetic patients and healthy subjects were all of normal weight (BMI 23.2 ± 1.7 vs. 24.1 ± 2.3 kg/m² respectively). There was no clinical evidence of neuropathy, and the results of all bedside autonomic tests (Valsalva, deep breathing and orthostatic test) were within the normal range (Ewing et al. 1985, Piha 1991).

The clinical characteristics of the patients and healthy controls in the study I and II are presented in Table 2.
Table 2. The clinical characteristics of the patients with type 1 diabetes and healthy controls in the clamp study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Diabetic patients (n = 16)</th>
<th>Healthy subjects (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32±8</td>
<td>34±10</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>9/7</td>
<td>5/3</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>13±9</td>
<td>-</td>
</tr>
<tr>
<td>GHbA1c (%)</td>
<td>7.0±0.9</td>
<td>-</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2±1.7</td>
<td>24.1±2.3</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>126±12</td>
<td>124±22</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>77±8</td>
<td>76±15</td>
</tr>
<tr>
<td>BP response to standing (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>16±18</td>
<td>28±20</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>23±15</td>
<td>32±14</td>
</tr>
<tr>
<td>Valsalva ratio</td>
<td>2.0±0.43</td>
<td>1.9±0.25</td>
</tr>
<tr>
<td>Mean DBD (beats/min)</td>
<td>26±10</td>
<td>29±12</td>
</tr>
</tbody>
</table>

Data are mean ± SD. DBD, deep breathing difference.

In study III, 37 adults (15 men and 22 women) with type 1 diabetes were recruited for studies into spontaneous, nocturnal hypoglycaemia. There was no evidence of any diabetic complications apart from retinopathy. The subjects did not have any cardiovascular diseases nor were they taking any drugs affecting the cardiovascular system. Their 12-lead electrocardiograms (ECGs) were normal. Their mean age was 28±6 (mean±SD) years (range 19–41 years). The known duration of diabetes was 13 years (range 1–30 years) and mean HbA1c was 8.0% (range 5.2–10.6%). As a part of study III, an additional experiment was performed to determine the effect of sympathetic activation on the autonomic cardiac regulation. Ten of those 37 individuals with type 1 diabetes (5 females, 32 ± 7 years, HbA1c 7.1 ± 0.7%, means ± SDs) participated in this supplemental study.

For study IV, 11 subjects (7 females) with type 1 diabetes and with ECG recordings using Oxford Medilog System were selected from study III. The mean age of the participants was 28 ± 8 (mean ± SD) years (range 19–41 years) and the duration of diabetes ranged from 1 to 30 years (15 ± 11, mean ± SD, years). Their glycaemic control was reasonable (mean HbA1c 7.6%, range from 6.1 to 9.8%).
4.2 Methods

4.2.1 Clamp procedure

Both euglycaemic and hypoglycaemic clamp procedures were performed with an interval of at least one week in a random order. The study subjects arrived in the laboratory in the morning after an overnight fast. The diabetic patients had taken their last subcutaneous insulin injection before bedtime and did not take their morning insulin dose on the day of the study. They were instructed to avoid hypoglycaemia (symptoms or blood glucose < 3.0 mmol/l) during the preceding 24 hours and to eat a light snack if their bedtime glucose was < 6.0 mmol/l. On the morning of the study day, a cannula was inserted into an antecubital vein of the left arm for infusion of glucose and insulin. A second cannula was placed into a large vein on the right forearm for blood sampling. The hand and forearm were placed in a heated box (45 °C) to ensure arterialisation of venous blood. The RR-interval was measured continuously with a real-time microprocessor-based QRS detection system (Polar Electro OY, Kempele, Finland) (Tulppo et al. 2005). The RR-interval recorder was connected to the patient via two dermal electrodes. At every glucose level, a 12-lead ECG was recorded. Blood pressure (BP) was measured from a finger of the left hand using the Finapress finger-cuff method. Blood samples were taken for analyses of blood glucose, serum potassium and counterregulatory hormones. Hypoglycaemic symptoms were registered using a symptom questionnaire, which listed 11 different symptoms: tremor, heart pounding, nervousness, sweating, tingling, hunger, tiredness, faintness, dizziness, difficulty in concentration and blurry vision. The subjects rated the intensity of each symptom from 0 (none) to 10 (severe).

A primed continuous infusion of 80 mU/m²/min of regular human insulin was started, and the glucose clamp technique was used to adjust the blood glucose concentration (DeFronzo et al. 1979). Blood glucose was measured at every 2.5–5 min. The study subjects were blinded to the blood glucose concentration throughout the study. The glucose concentration was initially stabilized between 4.5 and 5.5 mmol/l. During the hypoglycaemic clamp, blood glucose was permitted to slowly decrease to the first target level of 3.0–3.5 mmol/l, (mild hypoglycaemia), and then to the second target level of 2.0–2.5 mmol/l (moderate hypoglycaemia). Thereafter, the glucose concentration was slowly increased and the study was continued during euglycaemia. During the euglycaemic clamp, the
blood glucose level was kept in the range 4.5–5.5 mmol/l throughout the study, but the measurements were performed four times (as during the hypoglycaemic clamp) at regular intervals. All the measurements were performed only after clamping the blood glucose level in the desired range.

Blood glucose concentrations were analysed at the bedside by the glucose dehydrogenase method (HemoCue B-Glucose Analyzer, HemoCue AB, Ängelholm, Sweden). HbA1c (Pharmacia, Uppsala, Sweden), plasma adrenaline and noradrenaline (Bio-Rad Acclaim, Diagnostic Group, CA, U.S.A) were determined by high-performance liquid chromatography. The glucagon (Diagnostic Products Corporation, Los Angeles, CA, U.S.A) growth hormone (GH) (Pharmacia, Uppsala, Sweden), intact adrenocorticotropic hormone (ACTH) (Nichols Institute Diagnostics, San Juan Capistrano, CA, U.S.A.) and cortisol (Orion Diagnostica, Oulunsalo, Finland) concentrations were determined by radioimmunoassay as recommended by the manufacturers. Serum potassium was measured by an ion-selective electrode.

**4.2.2 Continuous glucose and R-R interval monitoring**

In study III, with the first 26 participants, RR-interval was measured continuously via two dermal electrodes with a real-time microprocessor-based QRS detection (Polar Electro, Kempele, Finland). The last 11 patients underwent ECG recording using an Oxford Medilog System (Medilog AR12, Oxford Instruments). At the same time, they were attached to a continuous glucose monitoring system (CGMS) measuring the glucose level (range 2.2–22.0 mmol/l) via a subcutaneous MMT-7002 sensor (Medtronic Diabetes, Northridge, California, USA). The participants calibrated the CGMS in a standardized, recommended way during monitoring. They were asked to keep a diary detailing their meal times, insulin injections, exercise, hypoglycaemic symptoms and bed times. The participants were provided with the recorders overnight and returned them to the laboratory on the next day. Each patient underwent this procedure three times.

**4.2.3 Heart rate variability analysis**

The analysis of HRV was accomplished with a special software package (Hearts7, Heart Signal Co., Kempele, Finland). The spectral analysis was accomplished as described by the Task Force of European Society of Cardiology and the North
American Society of Pacing and Electrophysiology (Task Force 1996). The high-frequency (HF, 0.15–0.40 Hz) and low-frequency (LF, 0.04–0.15 Hz) components and the LF/HF ratio were calculated. For dynamic measures of HRV, the quantitative analysis of Poincaré plot was used as described in detail previously (Tulppo et al. 1996). Of the quantitative parameters, beat-to-beat R-R interval variability (SD1) and long-term HRV (SD2) were calculated. The standard deviation of N-N intervals (SDNN) of the Poincaré plot was used as a time domain measure of HRV.

In study I, at the steady state of every glucose level, a 15-minute period when no other tests were performed was selected for the analysis of HRV. In study III, nocturnal hypoglycaemia was defined as a glucose level below 3.5 mmol/l during sleep for a minimum duration of 20 minutes. The location and duration of hypoglycaemic episodes were determined. Those periods of hypoglycaemia which had a control, nonhypoglycaemic (glucose > 3.9 mmol/l) period of equal duration and time of night were selected for the analysis of HRV.

4.2.4 Muscle sympathetic nervous activation study

Before the start of the muscle sympathetic nervous activation (MSNA) study, the subjects were instructed to avoid hypoglycaemia and keep their blood glucose concentration > 5.0 mmol/l. The participants lay in a supine position in a quiet room for at least 15 minutes prior to data collection and became accustomed to breathing at a constant metronome-guided rate of 0.25 Hz for the duration of the experiments. The cold hand and handgrip tests were performed in a randomised order. The cold hand test was performed by immersing the subject's hand into ice water (0–1 °C) for 3 min. The handgrip test lasted 5 min at an intensity of 30% of maximal voluntary contraction. The recovery between the interventions was 15 min. ECG was recorded by standard methods (Nihon Kohden TEC-7700). BP was recorded on a beat-by-beat basis (Nexfin, BMEYE, Amsterdam, The Netherlands). BP was also measured with an automatic BP recorder at every 2 min throughout the protocol (Tango; Sun-Tech, Raleigh, NC, USA). Multifiber recordings of MSNA were obtained with a tungsten microelectrode inserted into the peroneal nerve. A reference electrode was placed subcutaneously at 2–3 cm from the recording electrode. The recording electrode was adjusted until a site was found in which muscle sympathetic bursts were clearly identified, using previously established criteria (Vallbo et al. 1979). The nerve signal was amplified (50 000 times), passed through a band-pass filter with a bandwidth of 700–2000 Hz, and
integrated with a time constant of 0.1 s. The nerve signal was also routed through an oscilloscope and a loudspeaker for monitoring throughout the study. Analog signals were recorded at a sampling frequency of 1000 Hz using the PowerLab data acquisition system (PowerLab/16SP, ADInstruments, Australia).

Burst frequency was analyzed as bursts/min and as bursts/100 heart beats and the area under the curve as described previously (Shoemaker et al. 2001, Tulppo et al. 2005). The power spectral analyses of R-R intervals and systolic BP variability were calculated with customized software (Tiinanen et al. 2008) using an autoregressive model (Burg's algorithm). The analysis was performed during the last 2 min in both interventions.

4.2.5 QT interval and QT dispersion measurements

The duration of the QT interval was measured manually from lead V2 in the first, clamp study and from lead V5 in the second study using the tangent method (fig 1). The heart rate adjusted QT intervals were calculated according to Bazett’s formula (QTc) (Bazett 1920), Fridericia’s cubic root formula (QTFc) (Fridericia 1920) and the nomogram method (QTNc) (Karjalainen et al. 1994). The QT interval was also measured in every lead of the surface ECG, and QT dispersion was calculated using the difference between the maximum and minimum of the QT interval in any of the leads.
4.2.6 Vector loop analysis of repolarisation

In study II, the ECGs were scanned and digitized by using UN-SCAN-IT Graph Digitizing System Version 6.0 (Silk Scientific, Orem, Utah, USA) (15,23). For each ECG, the digital signal of 1 beat of each lead was used.

In study IV, nocturnal hypoglycaemia was defined as a glucose level below 3.5 mmol/l for a minimum duration of 20 minutes during sleep. Ten consecutive heart beats in the middle of the deepest hypoglycaemia were selected for the analysis of T-wave morphology. A control period was a nonhypoglycaemic (glucose ≥ 5.0 mmol/l) time period of ten heart beats during the same recording either before or at least 30 minutes after the period of hypoglycaemia.

Several descriptors of T-wave and QRS complex morphology were automatically calculated from the 12-lead ECGs, using a custom-made software application (Linna et al. 2006, Perkiömäki et al. 2006). The maximum amplitude
of the T-wave (Tmax) was defined as the difference between the peak of the T-wave and the baseline and it may have also negative values. The area of the T-wave was the sum of the voltages deviating from the baseline. The software automatically created T-wave and QRS loops in the Frank’s 3-dimensional space using matrix modification and the singular value decomposition technique. The software calculated the plane where the loop had the maximum first and second dimensions. The loop was rotated until its longest axis was parallel to the x-axis. The longest axis of the loop defined its width, and the second longest axis perpendicular to the longest axis defined its height. The height/width ratio described the shape of the loop. The calculated E parameter illustrated the roughness of the plane, i.e. how well the loop could be adjusted in the plane. Subsequently, a rectangle was adjusted around the loop and divided into 100 subdivisions. T-wave loop dispersion (TWLD) and QRS loop dispersion (QRSLD) were defined as the number of subrectangles traversed by the borderline of the corresponding loop. The TCRT was the co-sine of the angle between the main vectors of the T-wave loop and the QRS loop in 3-dimensional space. This measured the vector deviation between the depolarisation and repolarisation fronts. In this parameter, small values corresponded to large differences in the orientation of the two loops.

4.2.7 Statistical analysis

In these studies, standard statistical methods were used for the calculation of means and standard deviations. In the studies of cardiac autonomic regulation, because of the skewed distributions, a logarithmic transformation to the natural base was made for the values of HRV.

In the clamp study, the statistical significances of the changes in the variables at the different glucose levels were examined using analysis of variance for repeated measurements. In the part of the study concerning autonomic regulation, the differences between groups at each glucose level were further analysed by independent-samples T-test. The nonparametric Friedman test was used on the absolute values of spectral analysis of HRV. The results are presented as means ± SD and Spearman’s correlation coefficients (r). In the part of the study concerning repolarisation, the statistical significances of the contrasts between the values of parameters at baseline and at hypoglycaemia (or between the first and third phase of the experiment during the euglycaemic clamp) for the parameters
that differed statistically significantly in the repeated measures analysis was subsequently analysed. The group of diabetic patients and the healthy controls were combined in the assessment of the statistical significance of changes in the unified model and the evaluation of the significance of differences of the changes between the groups. The group of diabetic patients and the healthy controls were also combined in the statistical analysis of correlations. The results are presented as means ± SD and Pearson’s bivariate correlation coefficients (r).

In the study of spontaneous, nocturnal hypoglycaemia, the differences of logarithmic values between hypoglycaemic and nonhypoglycaemic periods were analysed by paired-samples T-test. The nonparametric Wilcoxon test was used in the analysis of the absolute values of spectral analysis of HRV and the novel parameters of T-wave loop morphology. The results are presented as means ± SD and Spearman’s bivariate correlation coefficients (r).
5 Results

5.1 Cardiovascular autonomic regulation during hyperinsulinaemic clamp

5.1.1 Changes in heart rate, heart rate variability and blood pressure

At every glucose level, HR was higher and all other HRV indices, except LF/HF ratio, were lower in the diabetic patients than the healthy controls (Table 3). During hypoglycaemia, there was a similar increase of supine HR in both groups (Table 3). Mean BP showed a non-significant declining trend at hypoglycaemia with the change being similar in both groups.

Total HRV (SDNN15) did not change during hypoglycaemia among the diabetic patients (Table 3). However, the HF spectral component of HRV decreased significantly during the hypoglycaemic clamp, and a non-significant trend towards a decrease of the LF component was also observed (Table 3, Fig. 2). There was also a non-significant trend towards an increasing LF/HF ratio. SD1 also decreased at hypoglycaemic glucose levels, but long-term HRV (SD2) remained unchanged (Table 3). The HF spectral component and SD1 did not decrease significantly during mild hypoglycaemia, but a pronounced and significant decrease in these parameters was observed during moderate hypoglycaemia (Fig. 3).

Among the non-diabetic subjects, the changes in the values of HRV during hypoglycaemia were similar to those observed in the diabetic patients (Table 3). The HF spectral component and SD1 decreased significantly, and the LF/HF ratio showed a nonsignificant increasing trend. No significant differences were detected in the HR or HRV responses between the groups during the entire clamp procedure.

At euglycaemia, no significant changes were observed in the HR or the HF and LF spectral components or SD1 among all the study subjects. Only a modest increase in the LF/HF ratio occurred at the last euglycaemic step in the diabetic patients (p < 0.05). During the euglycaemic clamp, the HF and LF power of HRV and SD1 were significantly lower among the diabetic patients compared to the healthy controls (p < 0.01 for all).
Table 3. Mean HR, BP and HR variability in diabetic patients and healthy controls at each glycaemic plateau during a hypoglycaemic clamp and after returning to euglycaemia.

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>4.5-5.5</th>
<th>3.0-3.5</th>
<th>2.0-2.5</th>
<th>4.5-5.5</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>72±9†</td>
<td>76±11†</td>
<td>80±11†</td>
<td>73±9‡</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Control subjects</td>
<td>59±5</td>
<td>63±5</td>
<td>65±5</td>
<td>58±5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>81±7</td>
<td>79±8</td>
<td>78±10</td>
<td>82±9</td>
<td>ns</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>85±16</td>
<td>84±8</td>
<td>76±16</td>
<td>83±14</td>
<td>ns</td>
</tr>
<tr>
<td>SDNN₁ (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>65.2±26.6†</td>
<td>63.6±26.5†</td>
<td>61.1±27.2†</td>
<td>72.7±29.0‡</td>
<td>ns</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>122.3±35.1</td>
<td>111.8±42.3</td>
<td>98.3±35.3</td>
<td>129.6±30.0</td>
<td>ns</td>
</tr>
<tr>
<td>HF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic subjects Ln (ms²)</td>
<td>5.53±1.34†</td>
<td>5.22±1.56+</td>
<td>4.77±1.32+</td>
<td>5.54±1.26+</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Healthy controls Ln (ms²)</td>
<td>7.18±0.96</td>
<td>6.52±0.87</td>
<td>6.08±0.85</td>
<td>7.00±1.19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic subjects Ln (ms²)</td>
<td>6.49±0.82†</td>
<td>6.42±1.05+</td>
<td>6.03±1.07+</td>
<td>6.64±0.88+</td>
<td>ns</td>
</tr>
<tr>
<td>Healthy controls Ln (ms²)</td>
<td>7.56±0.54</td>
<td>7.37±0.85</td>
<td>7.05±0.69</td>
<td>7.51±0.59</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LF:HF ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>3.7±2.9*</td>
<td>4.6±3.7</td>
<td>4.8±3.7</td>
<td>4.0±2.8</td>
<td>ns</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>1.7±0.8</td>
<td>2.5±1.0</td>
<td>2.8±1.1</td>
<td>2.1±1.5</td>
<td>ns</td>
</tr>
<tr>
<td>SD1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>3.01±0.62†</td>
<td>2.90±0.72+</td>
<td>2.70±0.71+</td>
<td>3.05±0.57+</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>3.76±0.46</td>
<td>3.48±0.44</td>
<td>3.30±0.40</td>
<td>3.72±0.54</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic subjects</td>
<td>85.8±33.7†</td>
<td>85.9±36.6†</td>
<td>82.6±37.8</td>
<td>100.9±40.2‡</td>
<td>ns</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>165.7±45.6</td>
<td>153.5±57.9</td>
<td>135.2±50.1</td>
<td>176.7±38.5</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR = heart rate, BP = blood pressure, SDNN = standard deviation of RR intervals, HF = high-frequency power of HRV, LF = low-frequency power of HRV, SD1 = standard deviation of instantaneous beat-to-beat R-R interval variability, SD2 = standard deviation of continuous beat-to-beat R-R interval variability.

The symbols indicate the difference between the diabetic patients and healthy controls as assessed by independent samples T-test (†p < 0.05, †p < 0.01, ‡p < 0.001).
Fig. 2. Individual values of the high-frequency spectral component at euglycaemic and hypoglycaemic glucose levels of nondiabetic control subjects (A) (n = 8) and diabetic patients (B) (n = 16), respectively.

Fig. 3. Examples of power spectra and Poincaré plots from a diabetic patient during euglycaemia and during hypoglycaemia.
5.1.2 The response of counterregulation hormones during the hyperinsulinaemic clamp

In both groups, the serum concentrations of potassium decreased during the hypoglycaemic and euglycaemic clamp (Table 4). As expected, the diabetic patients displayed an impaired response of glucagon to hypoglycaemia compared to the healthy subjects (Table 4). The elevation in the adrenaline levels was also blunted in the diabetic patients (from 0.2 ± 0.1 nmol/l to 1.7 ± 1.6 nmol/l in the diabetic group and from 0.2 ± 0.1 nmol/l to 3.1 ± 1.4 nmol/l in the control group) (Table 4). The change in the level of noradrenaline tended to be attenuated in the diabetic group (from 1.3 ± 0.3 nmol/l to 1.8 ± 0.6 nmol/l) compared to the control group (from 1.3 ± 0.3 nmol/l to 2.2 ± 0.4 nmol/l). The increases in the concentrations of cortisol, ACTH and GH were significant among the healthy controls at hypoglycaemia. The response of these counterregulation hormones was blunted in the diabetic patients. During the euglycaemic clamp there were no significant increments in the concentrations of glucagon, adrenaline or noradrenaline or other hormones in either group (Table 4).

The rise in the plasma adrenaline or noradrenaline levels exhibited no significant correlation with the changes in any of the HRV measures either in the diabetic patients or the healthy controls.

As expected, the healthy subjects experienced more hypoglycaemic symptoms at the lowest glucose level than the diabetic patients (31 ± 25 points vs. 18 ± 10 points, p < 0.01).
Table 4. Serum potassium, cortisol, and GH concentrations and plasma glucagon, adrenaline and noradrenaline, and ACTH concentrations at each glycaemic plateau during a hypoglycaemic and euglycaemic clamp.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Hypoglycaemic clamp, blood glucose (mmol/l)</th>
<th>Euglycaemic clamp, blood glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.5-5.5</td>
<td>3.0-3.5</td>
</tr>
<tr>
<td>Potassium (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>3.7±0.2</td>
<td>3.7±0.2</td>
</tr>
<tr>
<td>Control subjects</td>
<td>3.7±0.3</td>
<td>3.6±0.3</td>
</tr>
<tr>
<td>Glucagon (ng/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>15.6±4.6</td>
<td>14.8±3.9</td>
</tr>
<tr>
<td>Control subjects</td>
<td>19.5±8.3</td>
<td>24.5±8.0</td>
</tr>
<tr>
<td>Adrenaline (nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>0.2±0.1</td>
<td>0.4±0.3</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.2±0.1</td>
<td>0.3±0.2</td>
</tr>
<tr>
<td>Noradrenaline (nmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>1.3±0.3</td>
<td>1.5±0.3</td>
</tr>
<tr>
<td>Control subjects</td>
<td>1.4±0.3</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>Cortisol (μmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>0.4±0.2</td>
<td>0.4±0.3*</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.2±0.1</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>ACTH (pmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>6.8±11.9</td>
<td>4.8±3.1</td>
</tr>
<tr>
<td>Control subjects</td>
<td>4.2±1.7</td>
<td>4.1±1.2</td>
</tr>
<tr>
<td>GH (μmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic patients</td>
<td>3.4±5.7</td>
<td>4.9±9.1</td>
</tr>
<tr>
<td>Control subjects</td>
<td>0.5±0.6</td>
<td>0.3±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SD. ACTH = adrenocorticotropin hormone, GH = growth hormone.

The symbols indicate the difference between the diabetic patients and healthy controls in independent samples T-test (* p < 0.05, †p < 0.01, ‡p < 0.001).
5.2 Cardiovascular autonomic regulation during spontaneous, nocturnal hypoglycaemia

Altogether, 12 of 37 patients experienced 18 periods of nocturnal hypoglycaemia which lasted at least 20 minutes and for which there was an acceptable control recording. The duration of hypoglycaemia-control pairs ranged from 20 to 190 min (mean 71 min). However, this may be underestimating the incidence of hypoglycaemia because some of the participants experienced extremely long nocturnal, hypoglycaemic episodes (up to 480 minutes) which made it difficult to locate appropriate control periods.

5.2.1 Changes in heart rate and heart rate variability

Spontaneous hypoglycaemia did not have any effect on HR (Table 5). During hypoglycaemia, total HRV (SDNN\(_{15}\)) showed a non-significant decreasing trend.

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>&gt; 3.9</th>
<th>&lt; 3.5</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>62±7</td>
<td>63±9</td>
<td>0.30</td>
</tr>
<tr>
<td>SDNN(_{15}) (ms)</td>
<td>93.4±38.7</td>
<td>79.0±41.7</td>
<td>0.079</td>
</tr>
<tr>
<td>HF (ms(^2))</td>
<td>2002±1965</td>
<td>1336±1506</td>
<td>0.26</td>
</tr>
<tr>
<td>HFln (ms(^2))</td>
<td>6.79±1.60</td>
<td>6.37±1.51</td>
<td>0.12</td>
</tr>
<tr>
<td>LF (ms(^2))</td>
<td>2134±1635</td>
<td>1169±1029</td>
<td>0.006</td>
</tr>
<tr>
<td>LFln (ms(^2))</td>
<td>7.23±1.14</td>
<td>6.70±0.91</td>
<td>0.011</td>
</tr>
<tr>
<td>VLF (ms(^2))</td>
<td>2938±2616</td>
<td>2132±1964</td>
<td>0.088</td>
</tr>
<tr>
<td>VLFln (ms(^2))</td>
<td>7.64±0.87</td>
<td>7.37±0.75</td>
<td>0.089</td>
</tr>
<tr>
<td>LF:HF ratio</td>
<td>1.8±1.1</td>
<td>1.8±1.3</td>
<td>0.61</td>
</tr>
<tr>
<td>LFln:HFln ratio</td>
<td>1.09±0.12</td>
<td>1.08±0.15</td>
<td>0.76</td>
</tr>
<tr>
<td>SD1 (ms)</td>
<td>45.8±29.0</td>
<td>35.5±23.6</td>
<td>0.090</td>
</tr>
<tr>
<td>SD1ln (ms)</td>
<td>3.57±0.80</td>
<td>3.32±0.76</td>
<td>0.093</td>
</tr>
<tr>
<td>SD2 (ms)</td>
<td>122.9±48.9</td>
<td>104.6±55.0</td>
<td>0.098</td>
</tr>
<tr>
<td>SD2ln (ms)</td>
<td>4.72±0.47</td>
<td>4.53±0.48</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR = heart rate, SDNN\(_{15}\) = standard deviation of RR intervals, ln = natural logarithm, HF = high-frequency power of HRV, LF = low-frequency power of HRV, SD1 = standard deviation of instantaneous beat-to-beat R-R interval variability, SD2 = standard deviation of continuous beat-to-beat R-R interval variability.
The LF component of HRV decreased significantly (Fig. 4), and a non-significant trend toward a decrease of the VLF component was also observed (Table 5). The subtle decrease in the HF component of HRV did not reach statistical significance. Nevertheless, there was no change in the LF/HF-ratio during hypoglycaemia. A non-significant decreasing trend was seen in SD1 and also in SD2. The decline in the glucose concentration exhibited a significant, positive correlation with the decrease of the LF component of HRV ($r = 0.48$, $p < 0.05$).

Fig. 4. Individual values of low-frequency spectral component (LFln) at different glucose levels in diabetic subjects.

5.2.2 Results of the muscle sympathetic nervous activation study

HR increased during the handgrip test ($p < 0.001$) but did not change significantly during the cold pressor test. LF or HF powers of R-R intervals did not change significantly during the handgrip or during the cold pressor test. MSNA increased in all cases during both interventions e.g. from $9 \pm 3$ to $26 \pm 16$ bursts/min ($p = 0.082$) during the handgrip and from $9 \pm 5$ to $28 \pm 6$ bursts/min ($p = 0.004$) during the cold pressor test but these changes did not reach statistical significance during the handgrip test due to the low number of cases.
The correlation between the change in LF power of the R-R intervals and the change in the other variables was further studied across both stimulations. The change in LF power of R-R interval was negatively correlated with the change in MSNA ($r = -0.70, p = 0.050$), i.e. high sympathetic activity as documented by the increase in MSNA bursts was associated with decreased LF power of the R-R intervals (Fig. 5). For example, those two subjects who showed a decrease in the LF power spectral components during sympathetic intervention displayed the highest increase in the MSNA bursts. The typical change in the sympathovagal outflow is seen in figure 6 where extreme sympathetic activation (cold hand immersion) resulted in a saturation and a decrease in LF power and a paradoxical vagal activation as indicated by the lower HR and the higher HF power compared with the baseline.

Fig. 5. Correlation between the change in LF power of R-R intervals and the change in MSNA from baseline to sympathetic stimulation. Open circles are during the cold pressor test and closed circles during the handgrip test.
Fig. 6. Raw MSNA signal (30 sec recording for all) and corresponding spectral analysis of R-R interval (2 min recording for all) at baseline (upper panel), during the handgrip (middle panel) and during the cold pressor tests (lower panel).
5.3 Cardiac repolarisation during hyperinsulinaemic clamp

5.3.1 The effects of the hyperinsulinaemic clamp on heart rate, QT intervals, QT dispersion and T-wave morphology

At every glucose level, HR was higher in the diabetic patients than in the healthy controls (Table 6 and 7). During hypoglycaemia, there was a similar increase in the supine HR in both groups (Table 6). The QTc interval increased significantly during hypoglycaemia. The subtle increase in QTcF and QTcN did not reach statistical significance. In fact, QTcN was significantly longer at euglycaemia at the end of the clamp protocol (Table 6). The QT dispersion did not change statistically significantly during hypoglycaemia in either group. There was no statistically significant increase in the QT interval during the euglycaemic clamp procedure irrespective of, which heart rate correction formula was used (Table 7).

The shape of the T-wave changed considerably during hypoglycaemia. The area and maximum amplitude of T-wave decreased significantly, particularly in precordial chest leads (Table 6). Flattened T-waves were also observed during euglycaemia but this change was less marked (Table 7).

Table 6. Effects of hypoglycaemia on QT intervals, QT dispersion and T-wave in a 12-lead electrocardiogram.

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>4.5-5.5</th>
<th>3.0-3.5</th>
<th>2.0-2.5</th>
<th>p-valuea</th>
<th>4.5-5.5</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HR (bpm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5-5.5</td>
<td>72±9</td>
<td>76±11</td>
<td>80±11</td>
<td>0.006</td>
<td>73±9</td>
<td>0.003</td>
</tr>
<tr>
<td>Controls</td>
<td>59±5</td>
<td>63±5</td>
<td>65±5</td>
<td>0.001</td>
<td>58±5</td>
<td>0.008</td>
</tr>
<tr>
<td>D + C</td>
<td>68±10</td>
<td>71±11</td>
<td>75±12</td>
<td>0.001</td>
<td>68±10</td>
<td>&lt;0.001 (&lt;0.001)</td>
</tr>
<tr>
<td>QTc (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5-5.5</td>
<td>406±23</td>
<td>409±31</td>
<td>419±35</td>
<td>0.04</td>
<td>425±33</td>
<td>0.004</td>
</tr>
<tr>
<td>Controls</td>
<td>416±33</td>
<td>406±31</td>
<td>448±38</td>
<td>0.062</td>
<td>435±29</td>
<td>0.006</td>
</tr>
<tr>
<td>D + C</td>
<td>409±27</td>
<td>408±31</td>
<td>429±38</td>
<td>428±32</td>
<td>&lt;0.001 (0.34)</td>
<td></td>
</tr>
<tr>
<td>QTcF (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5-5.5</td>
<td>398±27</td>
<td>399±33</td>
<td>403±28</td>
<td>0.10</td>
<td>411±30</td>
<td>0.10</td>
</tr>
<tr>
<td>Controls</td>
<td>418±29</td>
<td>411±27</td>
<td>440±43</td>
<td>0.20</td>
<td>443±31</td>
<td>0.046</td>
</tr>
<tr>
<td>D + C</td>
<td>404±28</td>
<td>403±31</td>
<td>415±38</td>
<td>421±33</td>
<td>0.002 (0.04)</td>
<td></td>
</tr>
<tr>
<td>QTnc (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5-5.5</td>
<td>400±24</td>
<td>405±34</td>
<td>408±24</td>
<td>0.18</td>
<td>417±28</td>
<td>0.007</td>
</tr>
<tr>
<td>Controls</td>
<td>417±29</td>
<td>422±25</td>
<td>440±48</td>
<td>0.20</td>
<td>453±25</td>
<td>0.053</td>
</tr>
<tr>
<td>D + C</td>
<td>406±26</td>
<td>411±32</td>
<td>419±36</td>
<td>429±32</td>
<td>&lt;0.001 (0.04)</td>
<td></td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>4.5-5.5</td>
<td>3.0-3.5</td>
<td>2.0-2.5</td>
<td>p-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5-5.5</td>
<td>p-value&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>-----------------</td>
<td>---------</td>
<td>-----------------</td>
</tr>
<tr>
<td>QTD (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>41±15</td>
<td>41±16</td>
<td>41±15</td>
<td>39±17</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>45±27</td>
<td>43±18</td>
<td>51±20</td>
<td>40±19</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>42±19</td>
<td>41±16</td>
<td>44±17</td>
<td>40±17</td>
<td>0.61 (0.30)</td>
<td></td>
</tr>
<tr>
<td>Tmax(V&lt;sub&gt;2&lt;/sub&gt;) (mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.51±0.27</td>
<td>0.49±0.28</td>
<td>0.40±0.32</td>
<td>0.45±0.29</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.48±0.22</td>
<td>0.50±0.12</td>
<td>0.23±0.36</td>
<td>0.46±0.19</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.50±0.25</td>
<td>0.49±0.24</td>
<td>0.34±0.33</td>
<td>0.45±0.26</td>
<td>&lt;0.001 (0.80)</td>
<td></td>
</tr>
<tr>
<td>Tmax(V&lt;sub&gt;5&lt;/sub&gt;) (mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.30±0.17</td>
<td>0.26±0.15</td>
<td>0.15±0.18</td>
<td>0.22±0.16</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.37±0.16</td>
<td>0.34±0.15</td>
<td>0.10±0.28</td>
<td>0.36±0.14</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.32±0.17</td>
<td>0.29±0.15</td>
<td>0.13±0.21</td>
<td>0.27±0.16</td>
<td>&lt;0.001 (0.29)</td>
<td></td>
</tr>
<tr>
<td>Tarea(V&lt;sub&gt;2&lt;/sub&gt;) (s*mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.50±0.27</td>
<td>0.49±0.28</td>
<td>0.38±0.32</td>
<td>0.44±0.29</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.47±0.23</td>
<td>0.49±0.13</td>
<td>0.21±0.35</td>
<td>0.45±0.19</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.49±0.26</td>
<td>0.49±0.24</td>
<td>0.33±0.34</td>
<td>0.45±0.26</td>
<td>&lt;0.001 (0.83)</td>
<td></td>
</tr>
<tr>
<td>Tarea(V&lt;sub&gt;5&lt;/sub&gt;) (s*mV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.29±0.18</td>
<td>0.25±0.16</td>
<td>0.13±0.16</td>
<td>0.20±0.16</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.36±0.17</td>
<td>0.34±0.15</td>
<td>0.10±0.28</td>
<td>0.36±0.15</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.31±0.17</td>
<td>0.27±0.16</td>
<td>0.12±0.20</td>
<td>0.26±0.17</td>
<td>&lt;0.001 (0.21)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. BG = blood glucose, D + C = diabetics and controls, HR = heart rate, QTc = QT/RR1/2 proposed by Bazett, QTdc = QT/RR1/3 proposed by Fridericia, QTNc = QT + correction number i.e. nomogram method, QTD = QT dispersion, TmaxV<sub>2</sub> = maximum amplitude of T-wave in lead V2, TmaxV<sub>5</sub> = maximum amplitude of T-wave in lead V5, TareaV<sub>2</sub> = area of T-wave in lead V2, TareaV<sub>5</sub> = area of T-wave in lead V5. The p-value<sup>a</sup> corresponds to the statistical significance of the contrasts between the first and third measurements. The p-value<sup>b</sup> indicates the statistical significance of the change in the analysis of variance for repeated measurements and the p-value in parenthesis indicates the statistical significance of the difference in the behaviour of the parameter between the diabetic patients and healthy controls.

Table 7. QT intervals, QT dispersion and parameters of T-wave in a 12-lead electrocardiogram during euglycaemic clamp.

<table>
<thead>
<tr>
<th>HR (beats/min)</th>
<th>4.5-5.5</th>
<th>4.5-5.5</th>
<th>4.5-5.5</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4.5-5.5</th>
<th>p-value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetics</td>
<td>73±9</td>
<td>73±9</td>
<td>72±9</td>
<td>74±9</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>59±6</td>
<td>58±6</td>
<td>59±6</td>
<td>61±8</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>69±10</td>
<td>68±11</td>
<td>68±10</td>
<td>70±10</td>
<td>0.02 (0.003)</td>
<td></td>
</tr>
</tbody>
</table>

77
<table>
<thead>
<tr>
<th>BG (mmol/l)</th>
<th>4.5-5.5</th>
<th>4.5-5.5</th>
<th>4.5-5.5</th>
<th>p-value⁵</th>
<th>4.5-5.5</th>
<th>p-value⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTc (ms)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>402±21</td>
<td>405±28</td>
<td>410±31</td>
<td>409±29</td>
<td>0.37</td>
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<tr>
<td>Controls</td>
<td>418±19</td>
<td>418±37</td>
<td>428±34</td>
<td>419±26</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>407±22</td>
<td>409±31</td>
<td>416±32</td>
<td>412±27</td>
<td>0.34 (0.20)</td>
<td></td>
</tr>
<tr>
<td>QTc( ) (ms) Diabetics</td>
<td>393±23</td>
<td>395±27</td>
<td>397±28</td>
<td>396±23</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>415±21</td>
<td>423±38</td>
<td>426±36</td>
<td>420±29</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>401±24</td>
<td>404±33</td>
<td>407±33</td>
<td>404±27</td>
<td>0.43 (0.03)</td>
<td></td>
</tr>
<tr>
<td>QTc( ) (ms) Diabetics</td>
<td>401±24</td>
<td>402±24</td>
<td>405±23</td>
<td>402±18</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>419±24</td>
<td>435±43</td>
<td>427±33</td>
<td>422±25</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>407±25</td>
<td>413±35</td>
<td>412±28</td>
<td>409±23</td>
<td>0.19 (0.03)</td>
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</tr>
<tr>
<td>QTc( ) (ms) Diabetics</td>
<td>33±14</td>
<td>34±14</td>
<td>34±18</td>
<td>33±10</td>
<td>0.97</td>
<td></td>
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<tr>
<td>Controls</td>
<td>35±24</td>
<td>54±32</td>
<td>46±27</td>
<td>54±30</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>34±18</td>
<td>41±23</td>
<td>38±22</td>
<td>40±21</td>
<td>0.02 (0.09)</td>
<td></td>
</tr>
<tr>
<td>Tmax(V2) (mV) Diabetics</td>
<td>0.51±0.29</td>
<td>0.50±0.29</td>
<td>0.50±0.33</td>
<td>0.49±0.31</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.46±0.21</td>
<td>0.41±0.18</td>
<td>0.41±0.17</td>
<td>0.38±0.27</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.49±0.26</td>
<td>0.46±0.25</td>
<td>0.47±0.28</td>
<td>0.45±0.29</td>
<td>0.047 (0.48)</td>
<td></td>
</tr>
<tr>
<td>Tmax(V5) (mV) Diabetics</td>
<td>0.28±0.12</td>
<td>0.27±0.11</td>
<td>0.23±0.15</td>
<td>0.24±0.10</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.35±0.10</td>
<td>0.31±0.13</td>
<td>0.29±0.13</td>
<td>0.30±0.12</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.30±0.12</td>
<td>0.28±0.11</td>
<td>0.25±0.14</td>
<td>0.26±0.11</td>
<td>0.002 (0.27)</td>
<td></td>
</tr>
<tr>
<td>Tarea(V2) (s*mV) Diabetics</td>
<td>0.51±0.30</td>
<td>0.49±0.29</td>
<td>0.49±0.34</td>
<td>0.48±0.32</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.46±0.21</td>
<td>0.40±0.18</td>
<td>0.40±0.19</td>
<td>0.39±0.25</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.49±0.26</td>
<td>0.46±0.25</td>
<td>0.46±0.29</td>
<td>0.44±0.29</td>
<td>0.04 (0.52)</td>
<td></td>
</tr>
<tr>
<td>Tarea(V5) (s*mV) Diabetics</td>
<td>0.27±0.13</td>
<td>0.24±0.11</td>
<td>0.22±0.15</td>
<td>0.03</td>
<td>0.22±0.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Controls</td>
<td>0.35±0.10</td>
<td>0.30±0.13</td>
<td>0.29±0.13</td>
<td>0.02</td>
<td>0.29±0.12</td>
<td>0.02</td>
</tr>
<tr>
<td>D + C</td>
<td>0.30±0.12</td>
<td>0.26±0.12</td>
<td>0.24±0.14</td>
<td>0.24±0.11</td>
<td>0.001 (0.19)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. BG = blood glucose, D + C = diabetics and controls, HR = heart rate, QTc = QT/RR1/2 proposed by Bazett, QTc² = QT/RR1/3 proposed by Fridericia, QTnc = QT + correction number i.e. nomogram method, QTc = QT dispersion, TmaxV2 = maximum amplitude of T-wave in lead V2, TmaxV5 = maximum amplitude of T-wave in lead V5, TareaV2 = area of T-wave in lead V2, TareaV5 = area of T-wave in lead V5. The p-value⁵ corresponds to the statistical significance of the contrasts between the first and third measurements. The p-value⁶ indicates the statistical significance of the change in the analysis of variance for repeated measurements and the p-value in parenthesis indicates the statistical significance of the difference in the behaviour of the parameter between the diabetic patients and healthy controls.

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5.3.2 Changes in T-wave loop and QRS loop morphology

At hypoglycaemia, T-W decreased significantly both in controls and patients with diabetes (Table 8). In the diabetic subjects, there was a decreasing trend in T-H during hypoglycaemia. There were no statistically significant changes in the T-H/T-W ratio, the roughness of the T-wave loop or the morphology of the QRS loop in either of the study groups during the hypoglycaemic clamp. In the diabetic patients, there was a non-significant trend towards a decrease in TWLD but in the healthy controls TWLD remained relatively stable. During hypoglycaemia, the most remarkable change observed was the significant decrease noted in TCRT in the diabetic subjects (Table 8, Figure 7). Changes in TCRT in the healthy controls were not statistically significant but there was a clear trend in the significant differences of the behaviour of TCRT between the patients with diabetes and the controls during the hypoglycaemic clamp. During the euglycaemic clamp, a small decline in T-W was seen in the diabetic subjects, however the change was not statistically significant between the first and third phases of the experiment. The other T-wave loop/QRS loop parameters did not change statistically significantly during the euglycaemic clamp in either group.

Table 8. Effects of hypoglycaemia on T-wave loop and QRS loop morphology

<table>
<thead>
<tr>
<th>BG (mmol/l)</th>
<th>4.5-5.5</th>
<th>3.0-3.5</th>
<th>2.0-2.5</th>
<th>p-valuea</th>
<th>4.5-5.5</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-H</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.12±0.05</td>
<td>0.11±0.05</td>
<td>0.10±0.05</td>
<td>0.089</td>
<td>0.09±0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Controls</td>
<td>0.11±0.04</td>
<td>0.12±0.04</td>
<td>0.11±0.04</td>
<td>0.11±0.04</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.12±0.05</td>
<td>0.12±0.05</td>
<td>0.10±0.05</td>
<td>0.10±0.05</td>
<td>0.22 (0.89)</td>
<td></td>
</tr>
<tr>
<td>T-W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.34±0.15</td>
<td>0.31±0.14</td>
<td>0.25±0.14</td>
<td>0.002</td>
<td>0.27±0.14</td>
<td>0.002</td>
</tr>
<tr>
<td>Controls</td>
<td>0.38±0.10</td>
<td>0.34±0.10</td>
<td>0.28±0.11</td>
<td>0.004</td>
<td>0.35±0.10</td>
<td>0.003</td>
</tr>
<tr>
<td>D + C</td>
<td>0.35±0.13</td>
<td>0.32±0.12</td>
<td>0.26±0.13</td>
<td>0.29±0.13</td>
<td>&lt;0.001 (0.45)</td>
<td></td>
</tr>
<tr>
<td>T-H/T-W</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.40±0.18</td>
<td>0.39±0.18</td>
<td>0.42±0.21</td>
<td>0.34±0.20</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.29±0.07</td>
<td>0.38±0.16</td>
<td>0.40±0.12</td>
<td>0.34±0.14</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.37±0.16</td>
<td>0.39±0.17</td>
<td>0.42±0.18</td>
<td>0.34±0.18</td>
<td>0.43 (0.39)</td>
<td></td>
</tr>
<tr>
<td>T-E</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.023±0.010</td>
<td>0.021±0.009</td>
<td>0.020±0.013</td>
<td>0.016±0.007</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.026±0.012</td>
<td>0.022±0.010</td>
<td>0.031±0.015</td>
<td>0.017±0.005</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.024±0.011</td>
<td>0.021±0.009</td>
<td>0.024±0.014</td>
<td>0.016±0.006</td>
<td>0.02 (0.14)</td>
<td></td>
</tr>
<tr>
<td>BG (mmol/l)</td>
<td>4.5-5.5</td>
<td>3.0-3.5</td>
<td>2.0-2.5</td>
<td>p-value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5-5.5</td>
<td>p-value&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>----------------</td>
<td>---------</td>
<td>----------------</td>
</tr>
<tr>
<td><strong>TWLD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>41.7±9.0</td>
<td>38.8±5.7</td>
<td>33.4±9.5</td>
<td>37.9±12.2</td>
<td>0.055</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>39.4±4.4</td>
<td>36.7±5.0</td>
<td>37.9±2.8</td>
<td>38.3±3.1</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>41.0±7.8</td>
<td>38.2±5.5</td>
<td>34.9±8.1</td>
<td>38.0±10.0</td>
<td>0.24 (0.82)</td>
<td></td>
</tr>
<tr>
<td><strong>QRS-H</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>1.02±0.36</td>
<td>1.01±0.29</td>
<td>0.92±0.39</td>
<td>0.95±0.40</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.85±0.24</td>
<td>0.97±0.32</td>
<td>0.97±0.18</td>
<td>1.11±0.23</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.97±0.33</td>
<td>1.00±0.29</td>
<td>0.93±0.33</td>
<td>1.00±0.35</td>
<td>0.71 (0.92)</td>
<td></td>
</tr>
<tr>
<td><strong>QRS-W</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>1.75±0.40</td>
<td>1.74±0.41</td>
<td>1.68±0.61</td>
<td>1.69±0.61</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.79±0.29</td>
<td>1.89±0.48</td>
<td>1.89±0.43</td>
<td>1.91±0.35</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>1.76±0.36</td>
<td>1.78±0.43</td>
<td>1.75±0.56</td>
<td>1.77±0.54</td>
<td>0.60 (0.84)</td>
<td></td>
</tr>
<tr>
<td><strong>QRS-H/QRS-W</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.58±0.16</td>
<td>0.59±0.16</td>
<td>0.52±0.22</td>
<td>0.54±0.22</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.50±0.19</td>
<td>0.54±0.22</td>
<td>0.54±0.15</td>
<td>0.59±0.15</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.55±0.17</td>
<td>0.58±0.18</td>
<td>0.53±0.19</td>
<td>0.56±0.20</td>
<td>0.61 (0.86)</td>
<td></td>
</tr>
<tr>
<td><strong>QRS-E</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.21±0.11</td>
<td>0.20±0.12</td>
<td>0.22±0.16</td>
<td>0.20±0.16</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>0.19±0.12</td>
<td>0.17±0.09</td>
<td>0.24±0.10</td>
<td>0.20±0.08</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.20±0.11</td>
<td>0.19±0.11</td>
<td>0.23±0.14</td>
<td>0.20±0.14</td>
<td>0.25 (0.90)</td>
<td></td>
</tr>
<tr>
<td><strong>QRSLED</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>34.1±3.3</td>
<td>33.4±1.7</td>
<td>31.5±8.8</td>
<td>31.9±8.8</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>33.9±1.2</td>
<td>32.7±1.7</td>
<td>33.4±1.7</td>
<td>33.0±2.4</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>34.0±2.8</td>
<td>33.2±1.7</td>
<td>32.1±7.2</td>
<td>32.3±7.3</td>
<td>0.55 (0.88)</td>
<td></td>
</tr>
<tr>
<td><strong>TCRT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>0.41±0.41</td>
<td>0.38±0.41</td>
<td>0.17±0.44</td>
<td>0.03</td>
<td>0.34±0.40</td>
<td>0.04</td>
</tr>
<tr>
<td>Controls</td>
<td>0.67±0.22</td>
<td>0.61±0.16</td>
<td>0.43±0.45</td>
<td>0.71±0.10</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>D + C</td>
<td>0.49±0.38</td>
<td>0.45±0.36</td>
<td>0.26±0.45</td>
<td>0.46±0.37</td>
<td>0.15 (0.07)</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. BG = blood glucose, D + C = diabetics and controls, H = height of loop, W = width of loop, H/W = ratio of H and W of loop, E = roughness of loop, TWLD = T-wave loop dispersion, QRSLD = QRS loop dispersion, TCRT = total cosine R to T. The p-value<sup>a</sup> corresponds the statistical significance of the contrasts between the first and third measurements. The p-value<sup>b</sup> indicates the statistical significance of the change in the analysis of variance for repeated measurements and the p-value in the parenthesis indicates the statistical significance of the difference in behaviour of the parameter between the diabetic patients and healthy controls.
5.3.3 The relationship between the repolarisation variables and the biochemical and heart rate variability parameters during the hyperinsulinaemic clamp

In both study groups, the serum concentrations of potassium decreased during the hypoglycaemic and euglycaemic clamp. As expected, the response of the counter-regulatory hormones (glucagon, adrenaline, noradrenaline, cortisol, ACTH, GH)
was blunted in the diabetic patients at hypoglycaemia as mentioned above. During the euglycaemic clamp, there were no significant increments in concentrations of glucagon, adrenaline, noradrenaline or other hormones in either of the groups. Measures of HR variability reflecting cardiac vagal outflow also decreased significantly during hypoglycaemia as reported above.

The change in the serum potassium level at hypoglycaemia exhibited a significant relationship with the change in the maximum amplitude of T-wave (V5) but was not significantly associated with the change in QT<sub>No</sub>, T-W, T-H or TCRT (Table 9). There were no significant associations between the changes in the catecholamine levels or HRV and the changes seen in the repolarisation parameters (Table 9).

Table 9. The correlations between the repolarisation variables and serum potassium, catecholamines and the parameters of HRV

<table>
<thead>
<tr>
<th>Repolarisation variables</th>
<th>∆potassium (mmol/l)</th>
<th>∆adrenaline (nmol/l)</th>
<th>∆noradrenaline (nmol/l)</th>
<th>∆lnHF (ms&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>∆lnSD1 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>∆QT&lt;sub&gt;No&lt;/sub&gt; (ms)</td>
<td>-0.15</td>
<td>0.34</td>
<td>0.11</td>
<td>-0.14</td>
<td>-0.26</td>
</tr>
<tr>
<td>∆Tmax(V5) (mV)</td>
<td>0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.35</td>
<td>-0.16</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>∆T-W</td>
<td>0.03</td>
<td>-0.24</td>
<td>-0.00</td>
<td>0.03</td>
<td>-0.03</td>
</tr>
<tr>
<td>∆T-H</td>
<td>-0.33</td>
<td>-0.02</td>
<td>0.11</td>
<td>0.33</td>
<td>0.21</td>
</tr>
<tr>
<td>∆TCRT</td>
<td>0.11</td>
<td>0.02</td>
<td>0.07</td>
<td>-0.08</td>
<td>-0.07</td>
</tr>
</tbody>
</table>

Values are Pearson’s correlation coefficients. ∆ = changes in a parameter between the third and first measurements, QT<sub>No</sub> = QT + correction number i.e. the nomogram method, TmaxV5 = maximum amplitude of T wave in lead V5, T-W = width of T-wave loop, T-H = height of T-wave loop, TCRT = total co-sine R to T, SD1 = standard deviation of instantaneous beat-to-beat R-R interval variability, HF = high-frequency spectral component. The symbols indicate the significance of correlation (*p < 0.01).

5.4 Cardiac repolarisation during spontaneous, nocturnal hypoglycaemia

Six of 11 patients experienced ten at least 20-min long nocturnal hypoglycaemic episodes for which there was an acceptable control period. The duration of hypoglycaemia during sleep ranged from 20 to 205 min (mean 93 min). However, this actually underestimates the true incidence of hypoglycaemia because some of the participants experienced extremely long (up to 435 min) or recurrent hypoglycaemic episodes which led to difficulties in finding appropriate control periods between the hypoglycaemic episodes, and these hypoglycaemic periods could not be included in the analysis.
5.4.1 The effects of the spontaneous hypoglycaemia on heart rate, QT intervals, QT dispersion and T-wave morphology

HR increased significantly during spontaneous nocturnal hypoglycaemia (Table 10). Both the QTc and the QT_{Nc} interval shortened during hypoglycaemic episodes, but the QT dispersion did not change significantly. There was a decreasing trend in the area and maximum amplitude of the T-wave during hypoglycaemia, particularly in precordial chest leads (Table 10). However, this change did not achieve statistical significance.

Table 10. Effects of spontaneous hypoglycaemia on QT intervals, QT dispersion and T-wave in a 12-lead electrocardiogram

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>≥ 5.0 mmol/l</th>
<th>&lt; 3.5 mmol/l</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>65±12</td>
<td>85±9</td>
<td>0.028</td>
</tr>
<tr>
<td>QT_{V5}(ms)</td>
<td>439±5</td>
<td>373±5</td>
<td>0.025</td>
</tr>
<tr>
<td>QT_{NcV5} (ms)</td>
<td>435±4</td>
<td>367±5</td>
<td>0.017</td>
</tr>
<tr>
<td>QTD (ms)</td>
<td>39±9</td>
<td>37±13</td>
<td>0.69</td>
</tr>
<tr>
<td>Tmax(V2) (mV)</td>
<td>0.25±0.28</td>
<td>0.19±0.21</td>
<td>0.069</td>
</tr>
<tr>
<td>Tarea(V2) (s*mV)</td>
<td>0.26±0.25</td>
<td>0.19±0.16</td>
<td>0.082</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR = heart rate, QTc = QT/RR\textsuperscript{1/2} proposed by Bazett, QT_{Nc} = QT + correction number i.e. nomogram method, QTD = QT dispersion, Tmax_{V2} = maximum amplitude of T-wave in lead V2, Tarea_{V2} = area of T-wave in lead V2.

5.4.2 The changes in T-wave loop and QRS loop morphology during spontaneous hypoglycaemia

At hypoglycaemia, neither the height nor the width of the T-wave loop or their ratio changed significantly (Table 11). Hypoglycaemia did not have any effect on the dispersion of the T-wave loop. However, the roughness of the T-wave loop increased significantly. In addition, the angle between the main vectors of the T-wave loop and the QRS loop increased (Fig 8) i.e. there was a significant decline in the TCRT during hypoglycaemia (Fig 9). The parameters describing QRS loop did not change significantly during hypoglycaemia.
Table 11. Effects of hypoglycaemia on T-wave loop and QRS loop morphology

<table>
<thead>
<tr>
<th>Blood glucose (mmol/l)</th>
<th>≥ 5.0</th>
<th>&lt; 3.5</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-H</td>
<td>0.10±0.06</td>
<td>0.10±0.05</td>
<td>0.80</td>
</tr>
<tr>
<td>T-W</td>
<td>0.41±0.13</td>
<td>0.41±0.19</td>
<td>0.58</td>
</tr>
<tr>
<td>T-H/T-W</td>
<td>0.26±0.13</td>
<td>0.30±0.18</td>
<td>0.58</td>
</tr>
<tr>
<td>T-E</td>
<td>0.007±0.003</td>
<td>0.011±0.006</td>
<td>0.037</td>
</tr>
<tr>
<td>TWLD</td>
<td>36.5±1.9</td>
<td>36.6±2.5</td>
<td>0.80</td>
</tr>
<tr>
<td>QRS-H</td>
<td>0.73±0.37</td>
<td>0.76±0.35</td>
<td>0.96</td>
</tr>
<tr>
<td>QRS-W</td>
<td>2.55±0.94</td>
<td>2.69±0.92</td>
<td>0.51</td>
</tr>
<tr>
<td>QRS-H/QRS-W</td>
<td>0.34±0.24</td>
<td>0.34±0.24</td>
<td>0.65</td>
</tr>
<tr>
<td>QRS-E</td>
<td>0.04±0.026</td>
<td>0.05±0.043</td>
<td>0.80</td>
</tr>
<tr>
<td>QRSDL</td>
<td>34.7±1.8</td>
<td>35.5±1.4</td>
<td>0.26</td>
</tr>
<tr>
<td>TCRT</td>
<td>0.816±0.206</td>
<td>0.746±0.241</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Values are means ± SD. H = height of loop, W = width of loop, H/W = ratio of H and W of loop, E = roughness of loop, TWLD = T-wave loop dispersion, QRSDL = QRS loop dispersion, TCRT = total cosine R to T.

Fig. 8. An example of QRS complex (larger) and T-wave (smaller) loops during hypoglycaemia (grey line) and during a control period (black line). The straight lines represent main vectors of 2 loops. The angle between the QRS vector loop and the T-wave loop increases during the period of spontaneous hypoglycaemia.
Fig. 9. Individual values of the co-sine of the angle between the main vectors of the T-wave loop and the QRS loop (TCRT) at different glucose levels in 10 hypoglycaemia-control pairs. TCRT decreases during hypoglycaemia. TCRT = total co-sine R to T.
6 Discussion

6.1 General aspects

In 1991, Tattersall and Gill, introduced the term ‘dead in bed syndrome’ (Tattersall & Gill 1991), i.e., the sudden and unexpected death of young people with type 1 diabetes. The pathophysiologic background of nocturnal sudden death has remained something of an enigma. While nocturnal hypoglycaemia may play an important role, it is still not well understood how hypoglycaemia itself affects the cardiac electrical properties and autonomic regulation predisposing the individual to sudden arrhythmic death.

The present study was designed to assess and compare changes in HR, HRV and cardiac repolarisation during a hyperinsulinaemic hypoglycaemic and euglycaemic clamp among type 1 diabetic patients and age-matched non-diabetic subjects. Since the measurement of the QT interval and dispersion may include many methodological inaccuracies and does not provide any information on the morphology of the T-wave, new descriptors of cardiac repolarisation were used. Symptoms at the time of hypoglycaemia induced by the clamp technique in the awake state may contribute to autonomic responses, and subsequently on cardiac repolarisation patterns. Therefore, it was decided to assess the effects of spontaneous hypoglycaemia on HRV and these novel repolarisation parameters during sleep in type 1 diabetic subjects.

6.2 Changes in cardiac autonomic regulation during controlled hypoglycaemia

The main finding of this study was that cardiac vagal activity, as assessed by the HF component and SD1, decreased progressively during hypoglycaemia. The changes were similar in the diabetic patients and the non-diabetic subjects. These data show that experimental hypoglycaemia is associated with decreased cardiac vagal outflow simultaneously with adrenomedullar neurohumoral activation.

The average HR was higher and all HRV indexes were lower at all glucose levels during the euglycaemic and hypoglycaemic clamps among the diabetic patients in comparison to the controls, suggesting that they had impaired cardiovascular autonomic function, although the routine tests of the autonomic nervous system were within normal limits (Ewing et al. 1984, Ewing et al. 1991,
Despite the impaired autonomic function, the responses of cardiac autonomic regulation to hypoglycaemia were similar among the diabetic patients and the non-diabetic controls.

The HF spectral component of HRV and SD1 analyzed from Poincare plots have been previously proven to reflect the cardiac vagal outflow (Katona & Jih 1975, Penttilä et al. 2003, Tulppo et al. 1996, Tulppo et al. 2001). Both HF oscillations of HR and short-term HR oscillations (SD1) measured from return plots are almost absent after vagal blockade (Penttilä et al. 2003, Tulppo et al. 2001). The latter index (SD1) was used in this study in addition to spectral analysis, because it is less sensitive to trends in HR itself compared to the analysis of the HF power spectral component (Tulppo et al. 1996). The increase of HR itself, regardless of its origin, may also result in a reduction of these HRV indexes. In the present study, the increase of HR and the decrease of HF power and SD1 were not related to an increase of adrenaline or noradrenaline. This indicates that the adrenomedullary sympathoexcitation was not the primary cause of altered HRV, but that these changes were most likely a result of reduced cardiac vagal outflow caused by hypoglycaemia.

Previous studies have provided conflicting results regarding the effect of hypoglycaemia on HR and HRV. Premature ventricular beats, severe nodal bradycardia and an increase in HR have been observed (Lindström et al. 1992, Shimada et al. 1984). Russell III et al. (2001) described an increase in HR in non-diabetic and intensively treated type 1 diabetic patients both during hypoglycaemic and euglycaemic hyperinsulinaemia without there being any statistically significant difference between the euglycaemic and hypoglycaemic conditions.

When one considers the effect of hypoglycaemia on HR and HRV in healthy subjects, the present results differ from the findings of two earlier studies (Laitinen et al. 2003, Schächinger et al. 2004). Laitinen et al. (2003) did not observe any responses in cardiac parasympathetic regulation during a hyperinsulinaemic hypoglycaemic clamp in healthy, non-diabetic subjects. Schächinger et al. (2004) did not detect any significant changes in HR during a hyperinsulinaemic hypoglycaemic clamp but they did observe a small increase in the HF spectral component.

There are salient differences between the previous studies and the present one, which probably explain the divergent results. Laitinen et al. (2003) used a single-
day study protocol where hypoglycaemia followed euglycaemia, which differed from the present protocol. They also targeted for higher blood glucose values (3.0 mmol/l) with weaker stimulation of the autonomic nervous system. Schächinger et al. (2004) used a two-day single-blinded crossover design similar to that used in the present experiments, but they also targeted for a higher glucose level (2.7 mmol/l) than in these present work. In both studies, a shorter period (5 minutes) was used for the analysis of changes in HR and HRV compared to the present study (15 minutes). Together, these observations support the view that mild, short-term experimental hypoglycaemia does not result in any significant changes in either the HR or HRV, but more marked and prolonged hypoglycaemia clearly increases the HR and decreases the HF oscillations in HR.

In the present study, no significant changes occurred in any of the HRV indexes during the hyperinsulinaemic euglycaemic clamp, clearly suggesting that the observed changes in vagal indexes during the hypoglycaemic clamp were due to hypoglycaemia itself but not due to hyperinsulinaemia. Furthermore, the last measurements of HRV were assessed after reaching euglycaemia during the hypoglycaemic clamp, and these measurements revealed the restoration of HF and LF power to the level preceding hypoglycaemia. However, a small increase of the LF/HF ratio was observed in the euglycaemic, hyperinsulinaemic state in the diabetic subjects, suggesting that insulin itself may have some effects on the sympatho-vagal balance. Airaksinen et al. (1985) did not observe any changes in the average HR during a hyperinsulinaemic euglycaemic clamp. Bellavere et al. (1996) documented marked reductions in the HF bands and an increase in the LF/HF ratio after high- and low-rate insulin infusions among healthy women during euglycaemia.

When considering the clinical implications of the present study, it should be taken into consideration that the responses to hypoglycaemia were assessed during a hyperinsulinaemic clamp. The experimental conditions were artificial and differed from real life, where the rate and depth of hypoglycaemia are not controlled. There is also evidence that supraphysiological concentrations of insulin per se have effects on neuroendocrine responses. At comparable levels of hypoglycaemia, the responses in the concentrations of adrenaline, noradrenaline, cortisol and GH appear to increase in conjunction with greater hyperinsulinaemia (Galassetti & Davis 2000).
6.3 Effects of controlled hypoglycaemia on cardiac repolarisation

The present study demonstrated that controlled hypoglycaemia evokes profound changes in cardiac repolarisation but it has no influence on depolarisation. There were three clearly evident changes, 1) a decrease of TCRT, which reflects the heterogeneity of global cardiac repolarisation, 2) decreases in the height and width of T wave loop, and 3) an attenuation of T-wave amplitude in precordial leads. These changes tended to be even more evident in the diabetic subjects compared to those occurring in their healthy counterparts. The changes in TCRT were not associated with changes in the autonomic regulation of heart rate, sympatho-adrenal activation, or changes in the potassium level, suggesting that hypoglycaemia per se has direct effects on cardiac repolarisation patterns. Only the change in plasma potassium level exhibited a moderate correlation with the T-wave amplitude measured from the precordial leads.

In several studies, insulin-induced hypoglycaemia has evoked QTc interval prolongation (Due-Andersen et al. 2008a, Landstedt-Hallin et al. 1999, Lee et al. 2004, Lee et al. 2005, Marques et al. 1997, Rothenbuhler et al. 2008) and an increase in QT dispersion (Landstedt-Hallin et al. 1999). According to earlier studies (Karjalainen et al. 1994), the most extensively used Bazett’s formula usually overadjusts the QT interval at high heart rates and undercompensates at low heart rates. Fridericia’s equation is superior to Bazzett’s formula when the heart rate is in the 40 to 120 beats/min range but it fails at high heart rates underestimating the QT interval. The nomogram method has been shown to be most accurate in estimating the QT interval at all heart rates (Karjalainen et al. 1994). Therefore, all three methods were used since it was anticipated that there would be significant changes in heart rate during hypoglycaemia. By using the nomogram method and the Fridericia’s equation, only non-significant trends towards prolongation of repolarisation were observed. Furthermore, no significant changes were observed in the QT dispersion during hypoglycaemia.

These conflicting results with respect to the effects of hypoglycaemia on the QT interval and QT dispersion compared to previous studies may be due to methodological problems in the accurate measurement of the end of the T-wave, particularly during hypoglycaemia. The shape of the T-wave changed markedly particularly in the precordial chest leads during hypoglycaemia. The flattened T-waves made the manual measurement of QT interval frequently virtually impossible. Consequently, the QT interval seems to be an inaccurate variable with
poor reproducibility in hypoglycaemia and it is clearly not an ideal index for depicting changes in repolarisation caused by hypoglycaemia. The flattening of the T-wave during hypoglycaemia in healthy subjects has been well documented in several earlier studies (Eckert & Agardh 1998, Laitinen et al. 2008, Meinhold et al. 1998). Nonetheless, recent studies concerning individuals with diabetes have focused on the QT interval and QT dispersion and only a few studies have focussed on changes in T-wave morphology during hypoglycaemia (Lindström et al. 1992, Skyrme-Jones & Gribbin 2001).

Both QTc and QTnc were prolonged during the euglycaemic phase after the hypoglycaemic clamp. This may again be partly due to the more straightforward identification of the end of the T-wave at the time when T-waves were no longer flattened. It is also possible that the influence of hypoglycaemia on the QT interval becomes more evident after a certain time delay due to the cardiac memory effect (Patberg et al. 2005). The existence of this phenomenon will require further experimental evaluation.

TCRT describes the heterogeneity in global cardiac repolarisation. This index is not sensitive to subjective definition of the end of the T-wave, and is therefore a reproducible and reliable index of cardiac repolarisation. TCRT has recently been shown to represent a risk marker of mortality in several populations (Anttonen et al. 2009, Huang et al. 2009, Kardys et al. 2003, Pavri et al. 2008, Peng et al. 1995, Perkiömäki et al. 2006, Priori et al. 1997, Rautaharju et al. 2006, Zabel et al. 2000, Zabel et al. 2002). Abnormal (smaller) values i.e. a greater angle between the main vectors of the T-wave loop and the QRS loop are predictive of a poorer prognosis. TCRT has been observed to predict mortality and arrhythmic events in postinfarction patients (Perkiömäki et al. 2006, Zabel et al. 2000). An increased planar QRS-T angle has also been associated with a risk of death or arrhythmic events in patients with nonischemic cardiomyopathy (Pavri et al. 2008). Low TCRT values have been shown to predict total mortality in the follow-up study conducted in United States veterans (Zabel et al. 2002). The spatial QRS-T angle has been found to be a predictor of cardiac mortality in the general population (Kardys et al. 2003) and a predictor of incident congestive heart failure and all-cause mortality in postmenopausal women (Rautaharju et al. 2006). TCRT has also been shown to have prognostic value in patients with systolic heart failure (Huang et al. 2009). TCRT values tended to be more abnormal (smaller) in diabetic patients even when glucose levels were normal and the abnormality became more marked during hypoglycaemia, suggesting that hypoglycaemia further increases the heterogeneity in global repolarisation. The
basic mechanisms of TCRT and its relation to arrhythmia vulnerability are still under investigation.

6.4 Changes in cardiac autonomic regulation during nocturnal, spontaneous hypoglycaemia

Earlier clamp studies provide conflicting results regarding the effect of hypoglycaemia on HRV in diabetic and healthy subjects. The above-mentioned methodological differences between the clamp studies may explain the divergent results. In the present study, cardiac vagal activity, as assessed by the HF component and SD1, decreased progressively during controlled hypoglycaemia. The changes were similar in both diabetic patients and non-diabetics, and were not observed during euglycaemic clamp. However, the measurements in these studies have been performed when there was a supraphysiological insulin concentration under experimental conditions which may have had some influence on the results. This means that these previous results could not be directly extrapolated to real life and studies during spontaneous hypoglycaemia were needed.

It was observed that during spontaneous hypoglycaemia, the LF component of HRV decreased significantly and that this change correlated positively with the change in the glucose concentration. In addition, total HRV, other measured components of spectral analysis and the parameters of Poincaré plot displayed a decreasing but statistically non-significant trend. Since abrupt changes in HR are more closely related to cardiac vagal outflow and the average HR did not increase during hypoglycaemia, it can be hypothesized that the reduction in the LF spectral component of HRV might have resulted mainly from pure sympathetic activation without any concomitant vagal withdrawal. This was confirmed in the MSNA experiment, where a negative correlation was observed between sympathetic activation and the LF component of HRV. Accordingly, an increase in sympathetic activation without any concomitant vagal withdrawal can lead to a decrease in LF power. Hence, the reduction in LF power during spontaneous hypoglycaemia may reflect the hypoglycaemia induced activation of sympathetic nervous system.

Analysis of HRV is regarded a valid way to assess in a non-invasive manner the sympathovagal balance in the heart. The interpretation of the genesis of the LF component of HRV is somewhat controversial. It can be considered as a
marker of sympathetic modulation or as a parameter including both sympathetic and vagal influences.

Previously, a paradoxal decrease of the LF component of HRV and a reduction in the total power of spectral analysis have been observed in patients with advanced cardiac failure (Mortara et al. 1994). This condition is characterized by marked sympathetic activation during which the sinus node seems to exhibit diminished responsiveness to neural inputs. A subgroup of patients with high sympathetic activation and advanced cardiac failure has been reported to be at major risk of suffering adverse events (Galinier et al. 2000, La Rovere et al. 2003, Mortara et al. 1994, Ponikowski et al. 1997). Observational follow-up studies have indicated that the reduced LF spectral component after myocardial infarction is associated with worse outcome (Bigger Jr. et al. 1992), such as fatal or near-fatal arrhythmic events (Huikuri et al. 2009), but reduced HF spectral component has not been shown to be a risk marker of mortality. In this respect, the reduced LF spectral component observed here during spontaneous hypoglycaemia may also indicate an increased risk, while reduced HF spectral component observed previously during controlled hypoglycaemia may not be marker of untoward events. A similar reduction in LF power has also been detected during high intensity exercise at the time of sympathoexcitation (Perini et al. 1990). Recently, Tulppo et al. (2011) have observed significantly reduced LF power and a lower LF/HF ratio after exercise during sympathetic activation as compared to baseline values in healthy subjects. In the present MSNA experiment, there was a negative correlation between sympathetic activity and the LF power of HRV. In addition, during extreme sympathetic activation, a paradoxal increase in the vagal outflow was seen as was apparent in the MSNA experiment.

6.5 Effects of spontaneous, nocturnal hypoglycaemia on cardiac repolarisation

It is believed that this is the first study examine the effect of spontaneous hypoglycaemia on the novel descriptors of repolarisation. The roughness of the T-wave loop increased whereas the value of TCRT decreased. Hypoglycaemia had also some influence on the T-wave morphology. There was a decreasing trend in the T-wave amplitude and area though this change was not statistically significant. Flattened T-waves were observed particularly in the precordial leads. There did not seem to be any prolongation of the QT interval or any increase in the QT
dispersion during spontaneous hypoglycaemia. Hypoglycaemia had no effect on the parameters depicting depolarisation.

Clearly, the main finding in this study was that spontaneous hypoglycaemia induced significant changes in T-wave loop morphology. First, hypoglycaemia produced a similar, significant diminution in the TCRT as in the clamp study, indicating that also spontaneous hypoglycaemia can increase the heterogeneity in global repolarisation. The increasing heterogeneity of repolarisation may also contribute to the increased vulnerability to arrhythmias during spontaneous hypoglycaemia. However, the basic mechanisms of TCRT and its relation to arrhythmia vulnerability still needs to be clarified and more work will be needed to establish its potential usefulness in measuring cardiac repolarisation.

Secondly, the roughness of the T-wave loop increased during the period of nocturnal hypoglycaemia. Previous observations showing that the non-planarity of T-wave loop is superior in separating healthy subjects from those subjects with myocardial infarction have emphasized the role of T-wave loop roughness as an index of repolarisation heterogeneity (Karsikas et al. 2009). This hypothesis and these present findings support the concept that spontaneous hypoglycaemia results in non-homogeneity of repolarisation.

In addition, similar changes in the T-wave morphology during spontaneous hypoglycaemia were detected as in the clamp study. The area and the amplitude of the T-wave tended to decrease, particularly in the precordial leads. Consequently, the changes in the shape of T-wave during hypoglycaemia converted the QT interval into an inaccurate variable with poor reproducibility.

Interestingly, in this present study, the QTc and QTnc intervals shortened during hypoglycaemia. Additionally, no significant changes were observed in the QT dispersion during spontaneous hypoglycaemia. The effects of spontaneous hypoglycaemia on cardiac repolarisation have been assessed in four studies (Christensen et al. 2010, Gill et al. 2009, Murphy et al. 2004, Robinson et al. 2004). In the studies of Gill et al. (2009), Murphy et al. (2004), Robinson et al. (2004), lengthening of QTc interval during spontaneous hypoglycaemia was observed. In the latest study (Christensen et al. 2010), Bazett’s formula, Fridericia’s formula, the nomogram method and a linear subject-specific method were used for correction of the QT interval. QT intervals corrected with formulas other than Bazett’s were not associated with any significant change which confirms the present findings in this clamp study in which the QT interval corrected for heart rate by the Bazett’s formula lengthened in hypoglycaemia
unlike the situation when the QT interval was corrected for heart rate by the nomogram method. Due to the profound methodological differences and the diverse study populations, it is problematic to compare the results of this present investigation with those of previous studies.

6.6 Subject selection and methodological aspects

The present study has some limitations. Though we tried to select otherwise healthy diabetic subjects, some of them had retinopathy because of the long duration of disease. This increased their risk to have other microangiopathic complications which could not be detected by using conventional methods. This was clearly observed in the clamp study where all HRV indexes were lower among the diabetic patients in comparison to the controls, suggesting that they had impaired cardiovascular autonomic function, although the routine tests of the autonomic nervous system were within normal limits. Though we detected extensive individual differences in cardiac autonomic regulation during hypoglycaemia, no subgroup analysis was possible because of the small size of the study population. Especially, in study IV the patient number and the number of hypoglycaemic episodes were relatively small.

Exploring autonomic nervous system is challenging because experimental conditions may have an effect on this complex system. In the clamp study, hyperinsulinaemia and arterialisation of venous blood by using a heated box might have affected the results. In a real-life situation, HRV analysis is almost the only suitable method to observe changes in cardiac autonomic regulation. In studies III and IV, a time period during sleep at night was selected to standardize the conditions and to minimize the effect of diurnal rhythm and physical strain, for example. In study IV, control periods were selected either before or after the hypoglycaemic period due to the long-lasting hypoglycaemic episodes during the recordings which might have affected the results. Continuous MSNA measurements cannot be performed during long-term spontaneous conditions and due to these practical reasons, the MSNA measurements were done under controlled conditions. In addition, there may be some inaccuracies in measuring glucose by CGMS as compared with direct plasma measurements, though the system has been validated at low glucose levels (Hoi-Hansen et al. 2005).
7 Conclusions

The findings of the present study indicate that in type 1 diabetes hypoglycaemia has major impacts on cardiac autonomic regulation and repolarisation which may partly explain the vulnerability of these individuals to life-threatening cardiac arrhythmias and may have some clinical importance in contributing to the occurrence of ‘dead-in-bed’ syndrome. The main conclusions are:

5. Moderate, prolonged hypoglycaemia results in reduced cardiac vagal outflow and affects the cardioprotective role of vagal activity in both diabetic patients and non-diabetic subjects in experimental conditions during controlled hypoglycaemia.

6. Insulin-induced, controlled hypoglycaemia causes marked changes in T-wave loop morphology (the height and the width of the T-wave loop) and TCRT which tend to be even more evident among diabetic patients than in healthy controls during hypoglycaemia. These changes in the electrical properties of cardiac tissue may partly explain the vulnerability of these individuals to life-threatening cardiac arrhythmias.

7. Spontaneous hypoglycaemia decreases markedly the LF component of HRV. The results are similar with the findings encountered in severe heart failure. In all these conditions, there is an excessive sympathetic activation during which there is a change in the responsiveness of the sinus node to neural inputs. These abnormalities in the cardiac autonomic regulation have been associated with cardiac adverse events and poor prognosis.

8. Spontaneous hypoglycaemia evokes alterations in the T-wave loop morphology (the roughness of the T-wave loop) and in TCRT values indicative of profound changes in global repolarisation. The increase of heterogeneity in repolarisation may contribute to the vulnerability to experience arrhythmic events.
References


Ewing DJ, Neilson JM, Shapiro CM, Stewart JA & Reid W (1991) Twenty four hour heart rate variability: effects of posture, sleep, and time of day in healthy controls and comparison with bedside tests of autonomic function in diabetic patients. Br Heart J 65(5): 239–244.


Rana BS, Lim PO, Naas AAO, Ogston SA, Newton RW, Jung RT, Morris AD & Struthers AD (2005) 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. Heart 91(1): 44–50.


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