T.C.
FATIH UNIVERSITY
INSTITUTE OF BIOMEDICAL ENGINEERING

EVALUATION OF HYPOGLYCEMIA SYNDROME ANALYZING RELATED ELECTROPHYSIOLOGICAL SIGNALS

GAMZE ÇELİK

MSc THESIS
BIOMEDICAL ENGINEERING PROGRAMME

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ASSIST. PROF. DR. ŞÜKRÜ OKKESİM

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T.C.
FATİH ÜNİVESİTESİ
BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

HİPOGLSEMİ SENDROMUNUN İLGİLİ ELEKTROFİZYOLOJİK SİNYALLERİN ANALİZİ İLE DEĞERLENDİRİLMESİ

GAMZE ÇELİK

YÜKSEK LİSANS
BİYOMEDİKAL MÜHENDİSLİĞİ PROGRAMI

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Gamze Çelik, a **MSc** student of Fatih University **Institute of Biomedical Engineering** student ID 520112011, successfully defended the **thesis** entitled "**Evaluation of Hypoglycemia Syndrome Analyzing Related Electrophysiological Signals**", which he/she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Director

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To my Family,

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JUNE 2014 Gamze ÇELİK

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LIST OF SYMBOLS

μ	Mean
σ	Standard Deviation
σ_2	Variance
fs	Sampling Frequency
P	Power Spectral Density
W	Hann Function

ABBREVIATIONS

ADA: American Diabetes Association

ANS: Autonomic Nervous System

CGMS: Continuous Glucose Measuring System

CNS: Central Nervous System

DM: Diabetes Mellitus

ECG: Electrocardiography

EDA: Ectodermal Activity

FFA: Free Fatty Acids

GSR: Galvanic Skin Response

HRV: Heart Rate Variability

IDF: International Diabetes Federation

IIHT: Insuline Induced Hypoglycemia Test

LED: Light-Emitting Diode

PNS: ParaSympathetic Nervous System

PPG: Photo- Plethysmogrpahy

PRV: Pulse Rate Variability

PSD: Power Spectral Density

RMSSD: Root Mean Square Differences of Successive

RT: Reaction Time

SD: Standart Deviation

SNS: Symaphetic Nervous System

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SUMMARY

EVALUATION OF HYPOGLYCEMIA SYNDROME ANALYZING RELATED ELECTROPHYSIOLOGICAL SIGNALS

Gamze ÇELİK

Biomedical Engineering Programme
MSc Thesis

Advisor: Assist. Prof. Şükrü OKKESİM

Hypoglycemia is most important complications of diabetes mellitus (DM) treatment. The severe hypoglycemia occurs in elderly individuals, those having additional disease such as hypo-pituitarism, liver and kidney disorders, hypo-thyroidism, hypo-adrenalism, pregnant women and in children with Type 1 Diabetes (T1DM). The severe complications of hypoglycemia contain cardiovascular events, neurologic damage, trauma and death. In addition to, hypoglycemia occurring at night during sleep that is an uncommon case may result in death, because nocturnal hypoglycemia may cause cardiac arrhythmia. The latest researchs have demonstrated that hypoglycemia syndrome leads to QT interval prolongation, consequently it cause ventricular arrhythmias. As a result of this studies, insulin-induced hypoglycaemia in diabetics is a liable risk factor for sudden death is concluded, although developed technology and new insulin analogs, the fear of hypoglycemia is still a significant problem for DM.

Hypoglycemia is medical emergency that necessitates quick diagnosis and treatment to preclude organ and brain injury. Early diagnosis for Hypoglycemia has vital significance. Therefore, the purpose of this study is to evaluate the Electrophysiological

signals associated with hypoglycemia, in order to develop systems for able to detect the occurrence of Hypoglycemia. The aim of this study to contribute to the studies that are the systems developed for the early detection of hypoglycaemia with results obtained. To this end, prior to Insuline-Induced Hypoglycemia Test (IHT), during and after testing Electrocardiography (ECG), Pulse Plethysmography (PPG), Galvanic Skin Response (GSR) and Reaction Time (RT) signals of 5 patients were recorded and features obtained from mentioned electrophysiological signals were evaluated.

Features extracted from ECG, PPG and GSR prove that the highest sympathetic activity is obtained in the during hypoglycemia. Consequently, the Sympathetic Nervous System is controlled by the presence of changes in the electrophysiological parameters is shown. Additionally, the results obtained with RT is shown that hypoglycaemia has negative effects on cognitive level and learning. The obtained features can be used in early detection of hypoglycemia.

Keywords: Electrocardiography; Galvanic Skin Response; PhotoPlethysmogrpaphy; Reaction Time; Hypoglycemia; Dead In Bed Syndrome

FATIH UNIVERSITY - INSTITUTE OF BIOMEDICAL ENGINEERING

ÖZET

HİPOGLSEMİ SENDROMUNUN İLGİLİ ELEKTROFİZYOLOJİK SİNYALLERİN ANALİZİ İLE DEĞERLENDİRİLMESİ

Gamze ÇELİK
Biyomedikal Mühendisliği Programı
Yüksek Lisans

Danışman: Yrd. Doç. Dr. Şükrü OKKESİM

Hipoglisemi diyabet tedavisinin en önemli komplikasyonlarından biridir. Şiddetli hipoglisemi riski yaşlı hastalarda, vasküler hastalıklar, böbrek rahatsızlıkları gibi ek hastalıklara sahip hamile kadınlar ve tip 1 diyabetli çocuklarda yüksektir. Hipogliseminin şiddetli komplikasyonları nörolojik hasarları, travmaları, kardiyovasküler olayları hatta ölümleri içerebilir.

Bununla beraber gece boyunca uykuda oluşan hipoglisemi kardiyak aritme sebep olduğu için, nadirde olsa ölümle sonuçlanabilir. Son yapılan çalışmalarda hipogliseminin QT aralığını uzattığını ve böylece ventriküler aritmiye neden olduğu gösterilmiştir. Bu çalışmalarla insülin indüklü hipogliseminin diyabetiklerde ani ölüm için olası bir risk faktörü olduğu sonucuna varılmıştır. Yeni teknolojiler ve insülin analogları geliştirilmesine rağmen, hipoglisemi korkusu diyabetikler için hala önemli bir problemdir.

Hipoglisemi beyin ve organ zararlarını önlemek için acil tanı ve tedavi gerektiren tibbi acil bir durumdur ve bu nedenle hipogliseminin önceden belirlenebilmesi hayati öneme sahiptir. Bu çalışmanın amacı hipoglisemi gelişen hastalarda, hipogliseminin önceden

oluşumunu tespit edebilmeye yönelik sistemlerin geliştirilebilmesi amacıyla hipogliseminin ilgili elektrofizyolojik sinyallerle değerlendirilmesidir. Bu çalışmadan elde edilecek sonuçlar ile hipogliseminin önceden tespit edilebilmesine yönelik çalışmalara katkı sağlanacaktır. Bu amaçla insülin hipoglisemi testi öncesi, test esnası ve sonrasında 5 hastadan elektrokardiyografi, pulse- piletismografi, deri iletkenliği ve reaksiyon süresi sinyalleri kaydedilecek ve bu sinyallerden elde edilen öznitelikler ile hipoglisemi değerlendirilmiştir.

EKG, PPG VE GSR sinyallerinden elde edilen öznitelikler, hipoglisemi boyunca sempatik aktivitenin yoğun olduğunu gösterir. Sonuç olarak sempatik aktivitedeki artış elektrofizyolojik sinyallere yansır ve değişikliklere yol açar. Aynı zamanda RT ile elde edilen sonuçlar, hipogliseminin algılamada gecikme ve öğrenme üzerindeki olumsuz etkilerini gösterir. Elde edilen öznitelikler hipogliseminin önceden tespit edilebilmesinde kullanılabilir.

Keywords: Elektrokardiyografi; Deri iletkenliği cevabı; Pulse Pletismografi; Reaksiyon süresi; Hipoglisemi; Dead in Bed Sendromu

FATİH ÜNİVERSİTESİ -BİYOMEDİKAL MÜHENDİSLİK ENSTİTÜSÜ

CHAPTER 1

INTRODUCTION

1.1 Purpose of the Thesis

Hypoglycemia, is a widespread and serious side effect of insulin therapy in individuals with Diabetes Mellitus (DM) as mentioned above. There are risks of hypoglycaemia imposes several restrictions in the daily life of individuals with DM. For example, irregular nourishment, alcohol, exercise, and wrong timing in insulin injections are all frequent leads to hypoglycaemia in Type 1 Diabetes Mellitus (T1DM) [1]. Studies demonstrated that individuals with T1DM have intensive insulin therapy, decreasing glycemia levels frequently do not react counter-regulatory responses at euglycemia levels, allowing glycemia levels to drop to precariously low values [2]. In this way, deficiency to notice warning symptoms of hypoglycemia owing to decreased autonomic response during sleep may also increase the occurrence of hypoglycemia. All of these physiological changes cause to impaired glucose counterregulation and increase experience rate of severe hypoglycemia [3].

Hypoglycemia induces a series of metabolic, neural and clinical responses. Insulin hormone secretion reduces whereas glucagon, epinefrine, norepinefrine, cortisol and growth hormon rises via autonomic nervous system (ANS) are activated in defense to hypoglycemia [3]. Symptoms of hypoglycemia occurs from the activation of the ANS and it is called autonomic symptom. Another Symptoms of hypoglycemia occurs from decreased cerebral glucose depletion and it ic called neuroglycopenic symptoms [2]. The autonomic clinical features of hypoglycemia are tremor, palpitations, anxiety, diaphoresis, hunger, parasthesias and neuronal clinical features are fatigue, weakness, dizziness, cognitive and behavioral symptoms.

The ‘dead in bed’ syndrome is the name of the sudden unexplained deaths of healthy young patients with T1DM who are found dead in an undisturbed bed. This situation may be formed by hypoglycemia-induced cardiac arrhythmia and it supported with QT interval prolongation. The rate of experience severe hypoglycemia increases at night,

with at least 50% of all severe episodes occur of during that time and it is especially risky due to autonomic counter-regulatory responses reduces at sleep [2]. Therefore, dead-in-bed syndrome is still a concerned with condition by diabetic patients.

Several researches demonstrated that induced and spontaneous hypoglycaemia effects to cardiovascular function and ECG signal. The most considerable finding showed that hypoglycaemia altered cardiac repolarisation as a lengthening QT interval in this researches. Therefore, changes in the QT interval during hypoglycemia is investigated too.

Figure 1.1 A. Portable ECG and **B**. Holter monitoring devices [4].

There is various type of non-invasive glycemia monitoring systems, but these systems have some disadvantage in the way of functioning, cost, reliability and obtrusiveness. To give an example for this system, GlucoWatch measures the glycemia levels and it has several disadvantage like expensive, uneconomic disposable components, sweating might lead to skipped readings, and in the measurement has a time delay. This system is no longer usable due to these limitations. Intensive research has been dedicated to develop a hypoglycemia alarm. In this research different princibles are used as skin conductance and glucose sensors. However, none of these have proved sufficiently not reliable.

Real-time continuous glucose monitoring systems (CGMS) shown in Figure 1.2 are usable to measurement real-time glycemia levels, but it can not be used due to deficiency of sensitivity. It has been confirmed that the CGMS has not sensor accuracy

in detecting of hypoglycemia. Consequently, the manufacturers are to say that CGMS do not use as an alarm for hypoglycemia [5].

Hung T. Nguyen et al. have designed bayesian neural network algorithms to detect hypoglycemia using some physiological parameters heart rate, corrected QT interval of the ECG signal and skin impedance [2].

As shown in Figure 1.2, they have designed a continuous hypoglycemia monitor also known as HypoMon and it measures continuously the aforementioned parameters to detect of hypoglycemia [2]. The system occurs bio-sensor electrodes, a wireless handheld receiver computer and an alarm system.

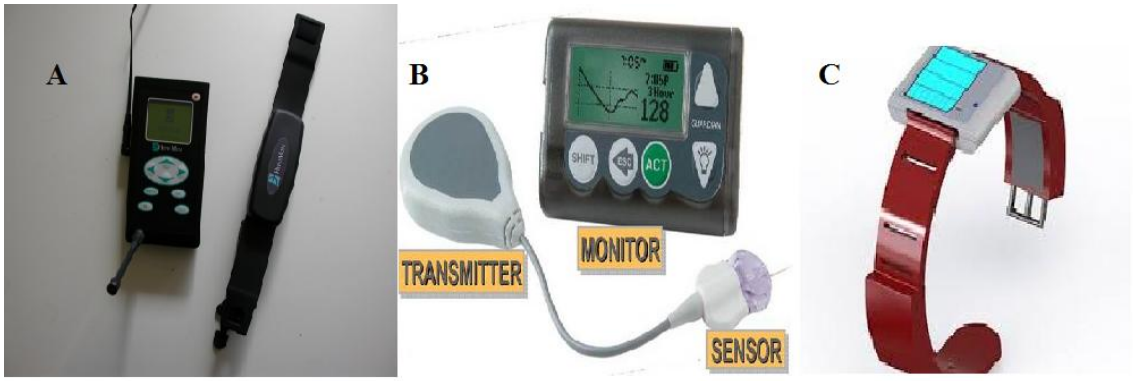


Figure 1.2 A. HypoMon sleep-time hypoglycaemic monitor, **C**. Hypo-Sense alert watch for nocturnal hypoglycemia. **B**. CGMS [6].

As shown in Figure 1.2, Hypo-Sense, a wrist watch like unit with an array of non-invasive sensors. The unit monitors patient's physiological parameters during sleep time. The sensor unit detects breathing and heart pulse, sweating, skin temperature, motion & tremor [7], and then it processes the signals via means of machine learning and decides if the signal reports a hypoglycemic event, on the detection of an event an alert is activated. Therfore, aforementioned parameters are still important to be used effectively for early detection of nocturnal hypoglycemia.

Sympathetic nervous system activation during hypoglycemia, causes some physiological changes. SNS activation leads to changes in the physiological signals and parameters regulated by the SNS.

To this end, the relationship between both the degree prolongation of QTc and HRV signals with low blood glucose levels have been investigated in this study. As is known, HRV is measured as the series of instantaneous cycle intervals acquired from the ECG. The HF (0.15-0.40 Hz) and LF (0.04-0.15 Hz) components of the HRV and PRV signal are regulated by Parasympathetic and Sympathetic nervous system [8]. The LF/HF ratio also known as "sympathovagal balance" provides an information autonomic balance between sympathetic and parasympathetic activities.

Photoplethysmography (PPG) is a simple and low-cost measurement technique for detecting blood volume variation in arms and legs [9]. Portable ECG and Holter monitoring devices shown in Figure 1.1 are difficult to use in clinical practice [10]. Therefore, the decision algorithms in the literature, is formed by using some of the physiological parameters. For these reasons, we investigated the estimation of variation in heart rate from a PPG signal is called Pulse Rate Variability (PRV) in this study. PRV is assessed accuracy as an estimate of HRV in hypoglycemia. The recordings were obtained at three phases: "prior to testing (IHT)," "during the test," and "after the test" . The obtained signals were then processed for feature extraction. And then, extracted features were analyzed.

ECG and HRV has some disadvantages like cost of wireless ECG device and motion artifacts effect on ECG signal. Therefore, usefulness of PhotoPlethysmography and Pulse Rate Variablity signals to detect hypoglycmia was analyezd in the thesis. In our study, as physiological signals, we have recorded Electrocardiography (ECG), PhotoPlethysmogrpaphy (PPG), Galvanic Skin Response (GSR) and Reaction Time (RT) from 5 patients who will be Insuline Hypoglycemia Test.

Additionally, the activity of sweat glands is regulated by SNS. Therefore, variation the skin conductivity returns the variation in the stimulation level of SNS [11].

Hypoglycemia cause confusion, brain damage, seizures, coma and even death, based on its severity and duration [12]. Damaged cognitive function can have injurious and cumulative long-term effects on mental function, particularly in individuals. Reaction time is a stimulus-reaction circumstance, where indiviuals hear a stimulus (the starter's pistol) and react to it in some way (response) [13]. Therefore, in our study, relationship between hypoglycemia and Reaction time has been evaluated.

1.2 Arrangement of the Thesis

This thesis is set up as follows:

- In the next chapter, information about the Diabetes, Hypoglycaemia, Physiology of Glucose Counterregulatory, Physiological effects of Hypoglycemia, Dead in bed syndrome, ANS and Hypoglycemia is presented.
- In the third chapter, ECG and HRV, PPG and PRV, GSR and its relationship with Hypoglycemia, Reaction time signals definition is presented, in addition analyzing methods are implemented as described in literature.
- In the fourth chapter, obtained results using the implemented methods are discussed.

CHAPTER 2

LITERATURE REVIEW

IDF regions and global projections of the number of people with diabtes (20-79 years) 2013 and 2035

IDF REGION	2013 MILLIONS	2035 MILLIONS	INCREASE %
Africa	19.8	41.4	109%
Middle East and North Africa	34.6	67.9	96%
South-East Asia	72.1	123	71%
South and Central America	24.1	38.5	60%
Western Pacific	138.2	201.8	46%
North America and Caribbean	36.7	50.4	37%
Europe	56.3	68.9	22%
World	**381.8**	**591.9**	**55%**

Figure 2. IDF (International Diabetes Federation) regions and global projections of the number of individuals with DM (20-79 years) 2013 and 2035 [14].

DM is a global disease. Today, there are 382 million individuals living with DM. By 2030, 592 million individuals are expected to have the disease. By the end of 2013, DM have been induced 5.1 million deaths and cost USD 548 billion in healthcare spending. In 2013, the number of children with type 1 diabetes are more than 79,000. The number of live births by DM during pregnancy are more than 21 million [14].

2.1 Diabetes

Diabetes Mellitus (DM) is a chronic condition qualified by elevated glycemia levels that it means hyperglycemia. DM occurs due to defects in insulin secretion, insulin action or both and the late development of vascular and neuropathic complication. Insulin hormone is occured by the β cells of the pancreas that provides the uptake of glucose in liver, muscle and fat tissue. The effect of insufficient insulin causes in elevated glycemia levels or hyperglycaemia, which is the cardinal symptom of DM . Figure 2.1.1 demonstrates the impacts of insulin insufficiency on body fuel metabolism. Insufficiency of insulin causes mobilization of substrates for gluconeogenesis and ketogenesis in muscle and adipose tissue. Thus production of glucose and ketones expedites by the liver, and impairs removal of endogenous and exogenous fuels by insulin-responsive tissues [1].

In the long term hyperglycemia is related with harm to the body and failure of various organs and tissues. It leads to heart disease, stroke, foot ulcers, renal failure, retinopathy, and neuropathy, atherosclerosis, disrupted autoregulation of blood flow, inflammatory response, oxidative stress, and hypercoagulable case [15].

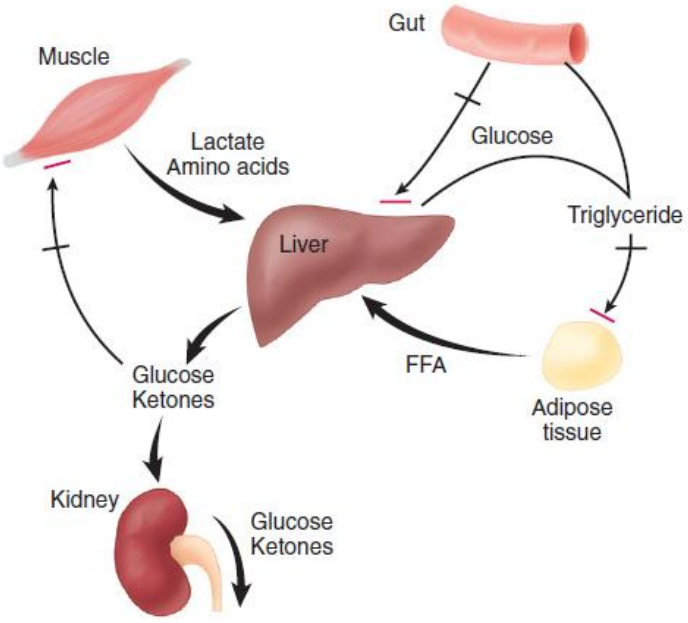


Figure 2.1.1 Summarizes the impacts of insulin deficiency on metabolism, FFA = free fatty acids [16].

DM can be classified into four several types by the American Diabetes Association (ADA). In this thesis will be provided information about the most common type 1 and type 2 diabetes.

Firstly, T1DM occurs with impairment insulin- manufacturing β-cells in the pancreatic islets of Langerhans and this case results with insulin insufficiency, but the pathogenesis of T1DM is still largely unknown. DM begins with juvenile onset and it usually occurs with insulin injection treatment [15]. Daily insulin injections are required for individuals who have this form of DM so as to control the levels of glycemia. Without insulin injection patient with T1DM can die due to ketoacidosis [17].

T2DM, the most common type of DM. It is hallmarked by insulin resistance in the liver and skeletal muscle. [18]. The reason of T2DM is a defect in the reactivity of body tissues to insulin as a result of unknown mechanisms. In contrast to T1DM, individuals with T2DM retain a significantly production of insulin although deficiency to maintain the glycemia within normal range. T2DM is a lifestyle -related disease such as excess calorie intake and physical inactivity [15]. Treatment of T2DM starts with oral medication and it increases insulin responsivity. If the disease progresses, insulin injections may be needed [17].

2.2 Hypoglycaemia (Glycemia level < 70 mg/dl, <3.9 mmol/L)

Hypoglycemia have described as “it is a syndrome that involves an abnormally a decrease content of glucose in the blood and also it low blood sugar, insulin reaction or insulin shock “by both the American Diabetes Association and the European Medicines Agency [19].

Glycemia are normally maintained between 4-8 mmol/L (72 and 144 mg/dL). Whipple’s triad adapted for DM is shown in Table 2.1.2, more accurate for the definition of hypoglycaemia [20].

Table 2.1.2 Whippe's triad adapted for DM from Watkins et al [20].

Criteria of Hypoglycemia
1.Symptoms or signs compatible with low glycemia
2.Glycemia < 3.5 mmol/ L
3.Relief of symptoms and signs by restoration of circulating glycemia levels

In principle, to diagnose of hypoglycaemia, all entire criteria from Table 2.1.2 required be fulfilled but in reality, it can be hard to insure fulfilment of all criteria in a clinical setting since symptoms and signs are subjective and several in subjects. For instance, it develops on different factors such as duration of DM, glycaemic control [21]. When evaluating the severity of hypoglycaemia are often divided into the three categories as seen in Figure 2.2.2.

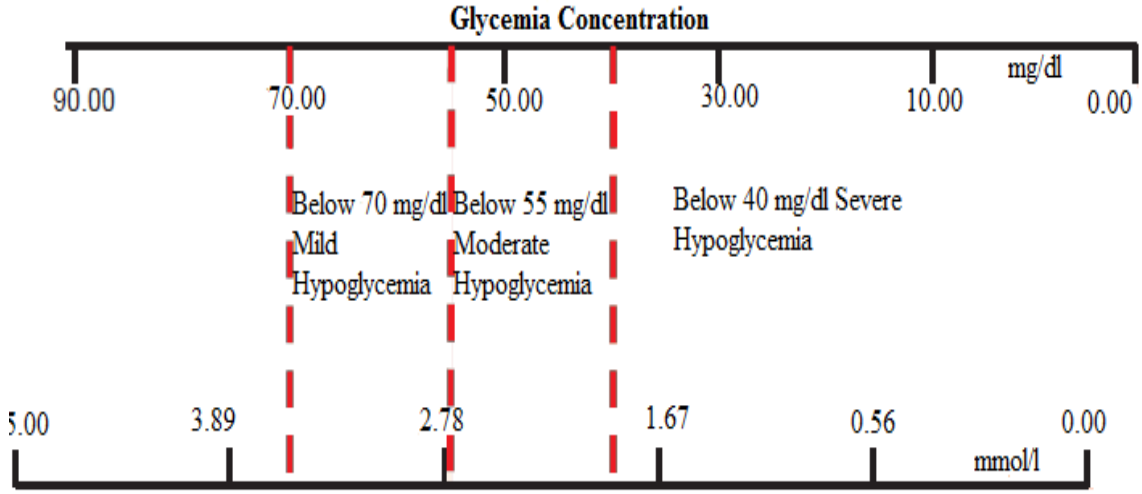


Figure 2.2.2 Classification of hypoglycemia.

Hypoglycemia is a serious problem, that is the most common and significant side effect of glucose-lowering, especially insulin therapies. The incidence of hypoglycemia is widely less common in T2DM than T1DM, in T2DM individuals treated with oral medications only 2-3% live severe hypoglycaemia while the number for insulin treated T2DM individuals is 11%, in contrast with, 65% of densely treated T1DM individuals live severe hypoglycaemia [22].

2.2.1 Causes and Symptoms of Hypoglycemia

Low glycemia levels lead to an array of symptoms by signaling central nervous system (CNS)–mediated ANS responses and by limiting neuronal metabolism.

All three efferent components of the ANS;

-Adrenomedullary,

-Sympathetic neural,

-Parasympathetic neural

are triggered by hypoglycemia. Autonomic or Neurogenic symptoms occurs by the Central nerveous system –mediated sympathoadrenal induction triggered by low glycemia levels. Adrenergic symptoms occurs as a result by catecholamines released from sympathic postganglionic neurons, the adrenal medullae [22, 23]. These symptoms are include trembling, palpitations, sweating, anxiety, hunger, nausea and tingling.

Neuroglycopenic symptoms occur as a result of CNS glucose deficiency and contain hardship concentrating, confusion, weakness, drowsiness, vision changes, hardship speaking, headache, dizziness and tiredness [24].

At the same time, hypoglycemia may develop from reasons such as endocrine disease, pancreatic or non-islet cell tumors, inborn failures of metabolism, autoimmune conditions, organ failure, cortisol and growth hormone deficiencies, endogenous hyperinsulinism [25].

Therefore hypoglycemia is a problem for individuals with DM that has not been solved. Generally, insufficient food depletion was the main reason described for severe hypoglycemia 43% in T1DM and 47% in T2DM.

Other reasons contained physical exercise, insulin dose miscalculation, stressful situations, oscillating glycemia levels, impaired hypoglycemia awareness in T1DM and T2DM, respectively [26]. Table 2.2.3 demonstrates the causes of hypoglycemia in individuals with DM [27].

Table 2.2.3 Causes of Hypoglycemia [27].

Cause	Examples
Incorrect insulin administration	Insulin taken in excess or at the wrong time relative to food intake and/or physical activity; incorrect type of insulin taken
Insufficient exogenous carbohydrate	Delayed or missed meals or overnight fast
Decreased endogenous glucose production	Excess alcohol consumption
Increased utilization of carbohydrate/depletion of hepatic glycogen stores	Exercise or weight loss
Increased insulin sensitivity	During the night, exercise, weight loss
Delayed gastric emptying	Condition such as gastroparesis
Decreased insulin clearance	Condition such as progressive renal failure

2.2.2 Physiology of Glucose Counterregulatory Factors

Glycemia is maintained with high precision, and is prevented hypoglycemia or hyperglycemia by coordinated regulation. Hormones, neurotransmitters, and substrate effects are important glucoregulatory factors. However, the main hormones are insulin, glucagon, and epinephrine [28].

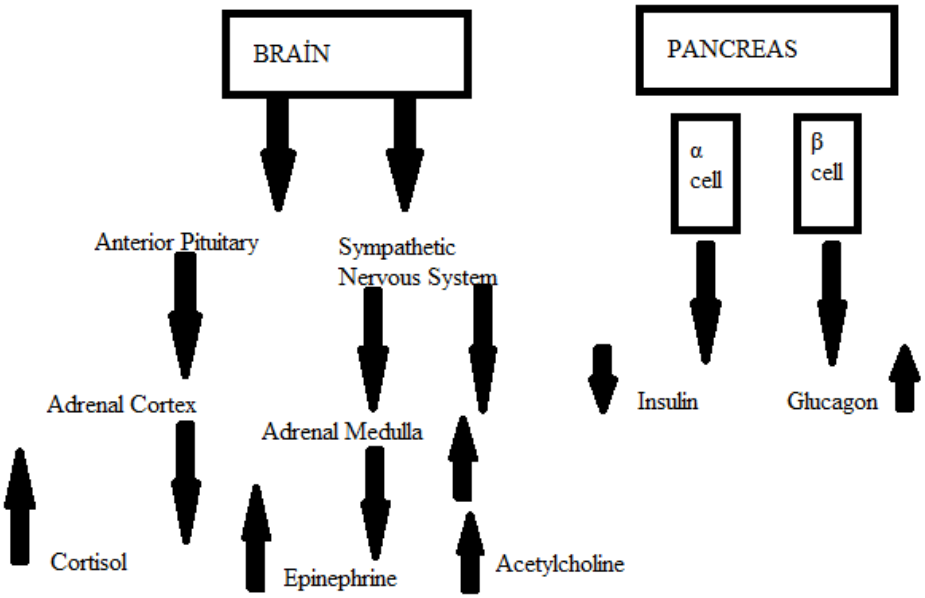


Figure 2.2.3 Glucose Sensing and the Physiological Response to Hypoglycemia [29]

Insulin is a peptide hormone, the half-life of circulating insulin is nearly 5 minutes and degredation of insulin occurs in the liver and kidneys [30]. Insulin prevents hepatic and nephritic glucose production and it causes glucose utilization by insulin-responsive tissues such as muscle and fat as well as the liver and the kidneys.

For instance, insulin prevents hepatic or renal glucose production, glucagon secretion, levels of nonesterified fatty acid and gluconeogenic precursor such as amino acids, glycerol levels [31]. Therefore, both glycogenolysis (is the breakdown of glycogen) and gluconeogenesis (is the generation of glucose) are decreased. It causes glucose utilization in insulin- responsive tissues both by containing glucose transfer directly and indirectly by suppressing FFA levels.

Glucagon is secreted from α-cells of pancreas against to hypoglycemia and it increases glycemia levels by inducing hepatic glucose production [32]. Both glycogenolysis and gluconeogenesis are increased, but the direct impact of glucagon on glycogenolysis is temporal.

Epinephrine is an adrenomedullary hormone and secreted in defense to hypoglycemia. It raises glycemia levels with inducing hepatic glucose production and limiting glucose utilization in insulin-responsive tissues that are mediated via both β2-adrenergic and α2-adrenergic receptors. [33, 34].

Indirect glycemic actions of epinephrine contain; β2-adrenergic excitation of lipolysis that both provides glucose production and restricts glucose utilization, glycolysis that ensures precursors for gluconeogenesis such as lactate and alanine and glucagon release α2-adrenergic restriction of insulin secretion. Restriction of insulin secretion is significant. [35].

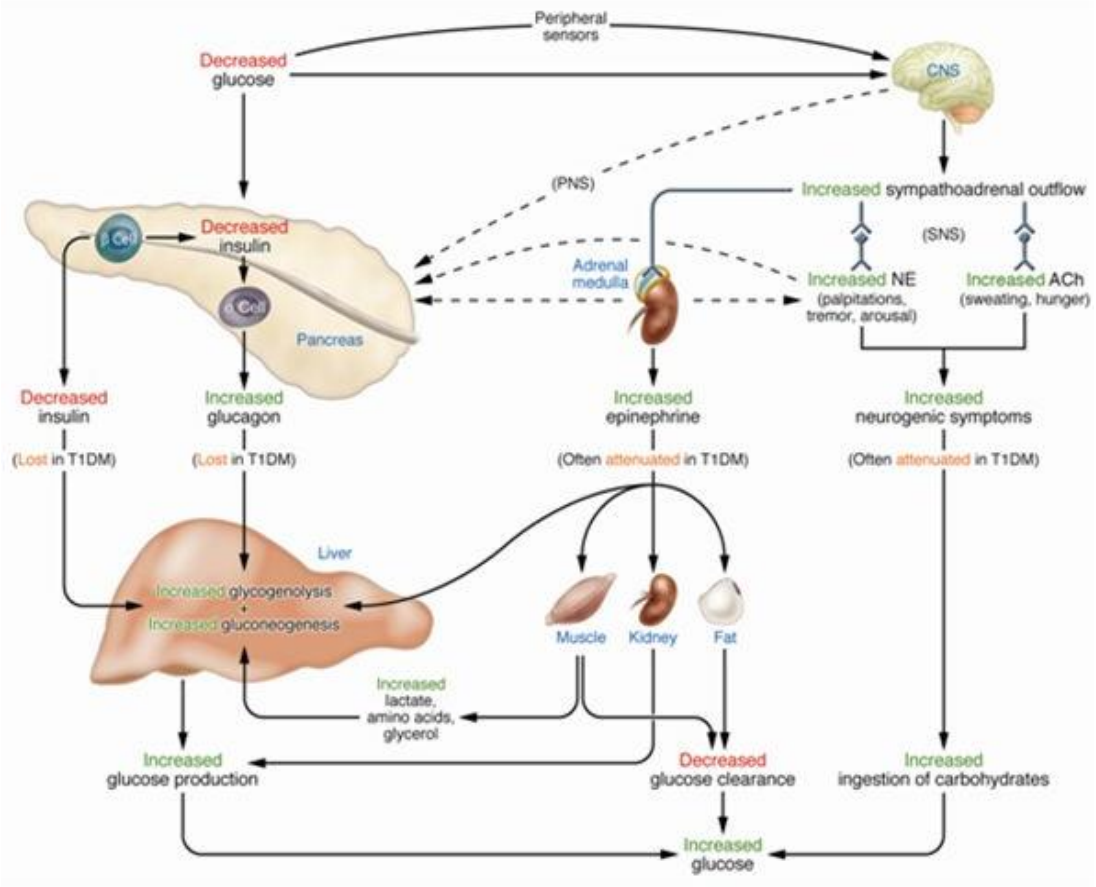


Figure 2.2.4 Physiological defenses against hypoglycemia. [36].

Glucagon and epinephrine more quickly increases glycemia levels in comparison with the pituitary growth hormone and adrenocortical cortisol. Growth hormone and cortisol restrict glucose usage, also promote glucose production [37].

Additionally, many other hormones are secreted in response to hypoglycemia. As shown in Figures 2.2.4, especially significant glucose counterregulatory neurotransmitters contain parasympathetic neural acetylcholine and sympathetic neural norepinephrine and along with sympathetic activation, norepinephrine increases glycemia levels via mechanisms similar to those of epinephrine [38].

Acetylcholine secreted from induced parasympathetic neurons and it reduces hepatic glucose production, an impact provable in people only in the lack of glucagon secrete [39]. Moreover, it is advisable that some of the array of neuropeptides secreted along hypoglycemia might influence glucose metabolism.

2.2.3 Counterregulation to hypoglycaemia

As Falling glycemia below a certain level a sequence of physiological responses develop to elevate glycemia to normal levels, these responses are known as counterregulation to hypoglycaemia. In Figure 2.2.5 the several responses of counterregulation are demonstrated during the glucose level at which they are initiated.

Table 2.2.4 Glycemia thresholds for counterregulatory responses including secrete of hormones and onset of warning symptoms and cognitive impairment [40].

Arterialised venous BG concentration (mmol/l)	**Counterregulatory Responses**
4.6 mmol/l	Inhibition of endogeneous insulin secretion
3.8 mmol/l	Counterregulatory hormone release -Glucagon -Adrenaline
3.2 – 2.8 mmol/l	Onset of symptoms -Autonomic -Neuroglycopenic
3.0 – 2.4 mmol/l	Neuorophysiological dysfunction -Evoked Responses
2.8 mmol/l	Cognitive dysfunction -Inability to perform complex tasks
2 mmol/l	Onset of EEG changes
< 1.5 mmol/l	Severe Neuroglycopenia -Reduced conscious level -Convulsion -Coma

- As shown in table 2.2.6, the first counterregulatory response in non-diabetic individuals is a decrease in insulin secretion, this response absent in T1DM due to absence of endogenous insulin production. The physiological postabsorptive glycemia level range is 72–108 mg/dl (4.0–6.0 mmol/l) while the about glycemic threshold for a reduce in insulin is 81 mg/dl (4.5 mmol/l).
- The second counterregulatory mechanism is the secrete of glucagon. Glucagon is antagonistic to insulin and it induces glucogenolysis and gluconeogenesis while preventing glucogenesis. In individuals with T1DM, the glucagon release defense to low glycemic value is lost within a few years of beginning of the disease.
- The third counterregulatory mechanism is the epinephrine, cortisol and growth hormone secretion. The glycemic threshold is 65–70 mg/dl (3.6–3.9 mmol/l) [41]. Alone epinephrine has an impact on acute hypoglycaemia for increasing the glycemia level and producing warning symptoms. Commonly, in individuals with T1DM an reduced epinephrine defense remains as the only counterregulatory defense before glycemia falls to a level where cognitive function is influenced. Finally, hepatic autoregulation starts and o cerebral blood increases when glycemia falls below 2 mmol/l [41].

2.2.4 Physiological Effects of Hypoglycemia

Hypoglycemia leads to remarkable physiological responses, this physiological responses as an outcome of autonomic activation, mainly of the sympatho-adrenal system. Concequently secretion of adrenaline. This autonomic stimulus leads to hemodynamic changes, the hemodynamic responses developing against to hypoglycemia is as net vasodilation with increments in heart rate and cardiac output and widening of pulse pressure, have been attributed to increased epinefrine secretion [42].

Outcomes of hemodynamic responses is important that are to sustain the provide of glucose to the brain and trigger of the hepatic production of glucose. To this end, blood flow is raised towards myocardium, the splanchnic circulation that is to provide initiators of gluconeogenesis to the liver, and the brain.

The hemodynamic changes involved with hypoglycemia contain;

-A raise in heart rate and peripheral systolic blood pressure,

-A decline in central blood pressure,

-Decreased peripheral arterial resistance (resulting a widening of pulse pressure),

-Raised myocardial contractility, stroke volume, and cardiac output [42].

As a result, the cardiac workload is transiently but significantly increased during hypoglycemia. This case does not lead to critical functional situation in healthy young individuals. However, in some special cases may result in risky as in many older individuals with DM, particularly people with T2DM, many of whom have coronary heart disease [42].

Hypoglycemia has been known lead to some changes on the ECG, as shown in figure 2.2.5 such as resulting ST wave changes with prolongation of the QT interval and cardiac repolarization [43, 44]. ECG represents the electrical activity and it is an helpful "picture" of heart activity. If there are interruptions of the electrical signal generation or transmission, some changes can seen on the ECG signal and these changes can be profitable in diagnosing [45].

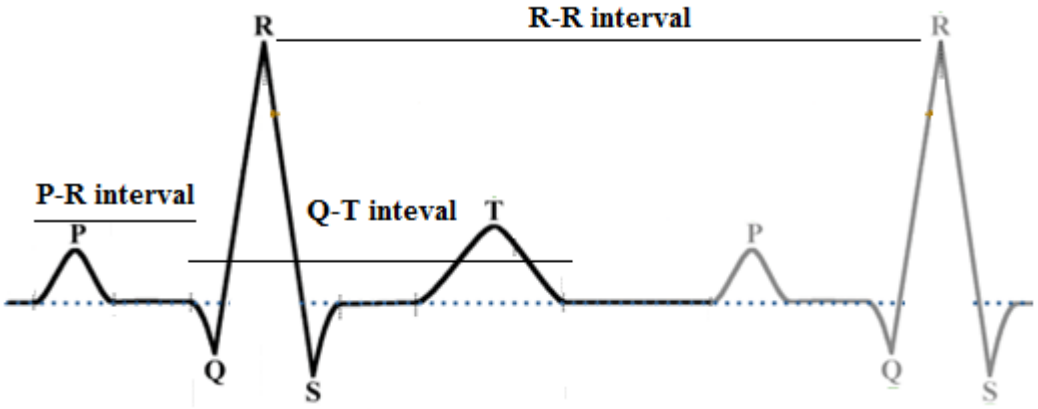


Figure 2.2.5 Components of the ECG & Electrical and mechanical events of the cardiac cycle [45].

ECG are commonly recorded as a pattern of a baseline or isoelectric line and it is separated by a P wave, a QRS complex, and a T wave and also the wave components of the ECG, as shown in Table 2.2.5 there are intervals and segments [45]. Both spontaneous and induced hypoglycemic episodes extends cardiac repolarization, the physical process whereby the heart is regulated for coordinated contraction along diastole and where abnormalities in other cases can raise the risk of cardiac arrhythmias.

Table 2.2.5 Components of the ECG [45].

ECG Component		**Represent**
WAVES	**P**	Depolarization of the right and left atria
	QRS complex	Depolarization of the right and left ventricles.
	T	Repolarization of the right and left ventricles.
INTERVALS	**P-R**	Time from the onset of atrial depolarization to the onset of ventricular depolarization
	Q-T	Time from onset of ventricular depolarization to the end of ventricular repolarization. This interval reflects the refractory period of the ventricles.
	R-R	Time between two successive ventricular depolarizations
SEGMENTS	**P-R**	Time of impulse conduction from the AV node to the ventricular myocardium
	S-T	Period of time reflecting the initial part of ventricular repolarization through which ventricles are more or less uniformly excited.
	T-P	Time from the end of ventricular repolarization to the onset of atrial depolarization.

These changes are result as shown in Figure 2.2.6 in the T wave of the ECG. Hypoglycemia causes declination in T wave amplitude with flattening and lengthening of the T wave, that is quantified by measuring the prolong of the QTc interval that is mathematically corrected according to heart rate [QTc] [46]. Prolongation of QTc, especially may induce a high risk of tachycardia, fibrillation and sudden cardiac death. Sudden death has been reported in individuals with T1DM, possibly due to a notable cardiac arrhythmia induced by nocturnal hypoglycemia [47] are discussed in detail and in this case occurring electrophysiological variations are involved hypokalemia, that is a effect of the abundant release of catecholamines.

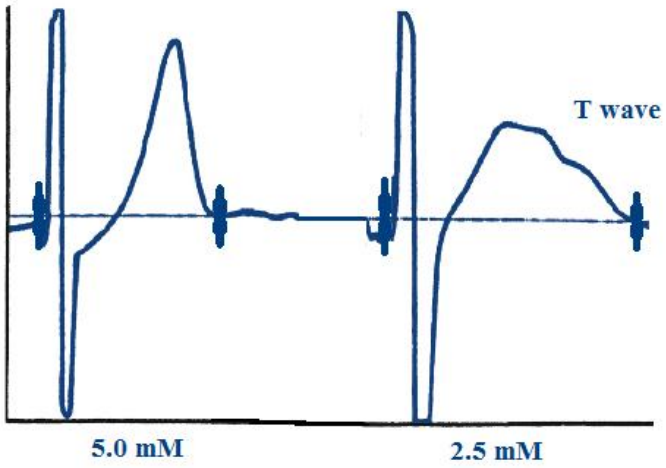


Figure 2.2.6 QT measurement during euglycemia in left panel, screening a plainly defined T wave. Hypoglycemia in right panel, screening extended repolarization and a obvious U wave [41].

Glucose is an imperative metabolic substrates for brain and visceral organ function and so glucose homeostasis is regulated with high precision by hormones and ANS activities, because the brain cannot produce or store glucose more than a few minutes', the brain requires a continuous supply from the circulation. If the arterial plasma glucose level drops below the specific range, blood-to brain glucose transport becomes restricting to brain glucose metabolism and thus brain function and finally, survival. Except for the potentially destructive effects of hypoglycemia on the brain, the glycemic management of diabetes may be rather straightforward [22].

ANS regulate the metabolic circumstance of liver, muscle and fat tissue, additionally the secretory activity the endocrine pancreas. Adequate insulin, or in order to as well as effective drug, to lower glycemia levels to or below the physiological range would remove the symptoms of hyperglycemia. At the same time restrain the acute hyperglycemic complications as ketoacidosis, the long-term microvascular complications, and almost likely decrease macrovascular risk.

However the impact of hypoglycaemia on the brain is a reality and so the glycemic administration of DM is complicated and commonly only relatively successful [22].

Severe hypoglycemic episodes in elder individuals with DM have been demonstrated to be involved with a raised risk of dementia [48], headache, seizure, stroke-like episodes, cognitive dysfunction, and coma [49] and cerebellar ataxia [50], and at the same time hypoglycemia may impact cognitive performance and learning. There are reports and experimental studies connecting various cognitive disturbances with hypoglycemia but, the connection between repeated hypoglycemia and long period development of dementia is still being studied [51].

In defense to IIH, epinephrine induces heat production and sweating with mediated sympathetic cholinergic nerve fibers in the skin and it induces evaporative heat loss in consequence of a fall in in skin or body core temperature [52]. A transition position from sympathetic vasoconstrictor to sudomotor activity is likewise causing in raised skin blood flow, which further contributes to support heat loss and changes in skin sympathetic activity in response to IIH, causing to reduced body temperature may be an further defense mechanism with possibly neuroprotective effects [53]. In brief, hypoglycemia-induced symptoms develops as a cause of the perception of physiologic changes due to sympathetic cholinergic, adrenomedullary and sympathetic noradrenergic activation, CNS.

2.2.5 The QT interval

As shown in Figure 2.2.7, the QT interval can be measured using ECG signal and it gives the time between the onset of the Q and end of the T wave. The QT interval consists of the QRS interval and the T wave that are depolarisation of the ventricles and repolarisation of the ventricles, respectively. The QT interval is the time from the beginning of ventricular depolarization to the ending of repolarization and the QT interval changes with the heart rate. For example, longer with bradycardia and shorter with tachycardia, standardization with the Bazzett formula is used to calculate the QTc.

The interval from the peak of one R to the peak of the next as demonstared on ECG and the average RR interval over a number of beats is used for heart rate correction. The most commonly used formula in ECG studies research variations in the QT interval is the square root formula by Bazett [54].

$$QT_C = \frac{QT}{\sqrt{RR}} \qquad [54]$$

Lengthening of the QT interval has been identified as a risk factor for cardiac death in individuals with;

-Heart failure,

-Myocardial infarction,

-T1DM and T2DM

-In the general population.

Hypoglycaemia leads to changes cardiac repolarization severely, with raises QTc interval and QT dispersion (QTd) on the ECG and these changes are associated to the counterregulatory sympatho-adrenal response [55].

Some research in literature proved that there are two conditions which can be responsible for QT prolongation:

- The sympathoadrenal activation to hypoglycaemia causing a release of catecholamines (epinefrine or norepinefrine) as part of the glucose counterregulation
- Hypokalemia (lowered potassium concentration) caused by elevated levels of adrenaline and insulin during hypoglycaemia [55].

The QT interval is generally measured manually using lead II, V5 or V6 in the 12-lead ECG and manual assesments of QT interval are commonly performed. Nowadays computerised methods are started to use.

2.2.6 Hypoglycemia as a Potential Risk Factor for Sudden Death in DM

Mortality due to iatrogenic hypoglycemia reflects a major fear for insulin-treated DM individuals and their families. In studies are demonstrated that 6–10% of deaths in young individuals with T1DM are associated with hypoglycemia [12]. The multiple event reports of cardiac arrhythmias induced by spontaneous hypoglycemia accentuate the clinical relevance of the association, especially since ethical considerations restrict experimental studies in this area [42].

Clinical and experimental findings have demonstrated that hypoglycemia may lead to abnormal electrical activity of heart and it can induce sudden death [12]. Clinical episodes of hypoglycemia have been demonstrated lead to QT prolongation, measured with ambulatory ECG monitoring and simultaneous measurement of glycemia (by either intermittent venous sampling or CGM) and these changes is induced by activation of the sympathoadrenal system.

Hypoglycemia per se may have a impact by immediately inhibiting cardiac ion channels that are liable for potassium (K+) efflux along cardiac repolarization and so, it leads to a drop in plasma (K+) through sympathoadrenal activation and a direct impact of insulin [56]. QTc prolongation reflects scattering of ventricle depolarization and can cause to increased risk of fatal cardiac arrhythmias. The insulin-induced decrement in K+ concentrations and the counterregulatory-induced raise in catecholamine levels have been theorized to contribute to arrhythmias [57].

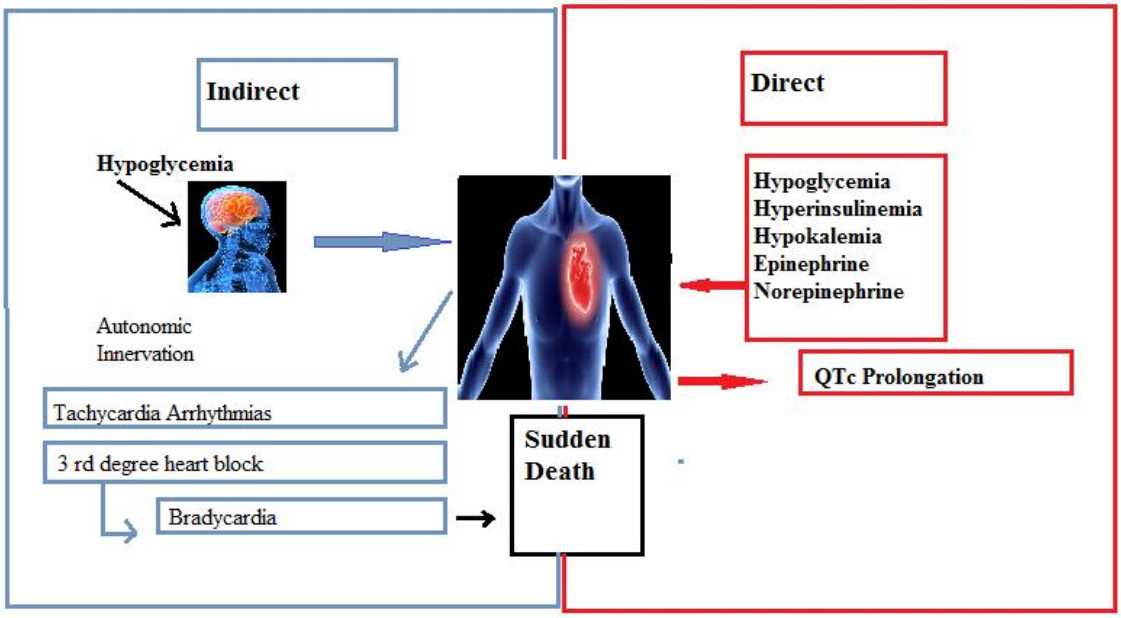


Figure 2.2.8 Mechanisms of Sudden Cardiac Death. Red panel contains circulating glucose, electrolytes, and hormones; blue panel contains autonomic innervations [12].

As shown in Figure 2.2.8, hypoglycemia excited with insulin, changed levels of glucose, potassium, and hormones sach as insulin, epinephrine, and norepinephrine may have a immediate arrhythmogenic impact on the heart.

Moreover, since hypoglycemia is appointed at the level of the brain, indirect (CNS) impacts of hypoglycemia likewise support to fatal cardiac arrhythmias through the efferent nervous system and local secrete of norepinephrine at nerve terminals within the heart.

At the same time, in studies are demonstrated that hypokalemia is likewise support to cardiac arrhythmias. Hypoglycemia-excited sudden cardiac death depending on containing ischemic or depolarization/repolarization changes causes from increased adrenergic signaling at the level of the heart that results to sinus tachycardia.

However, tachycardia is followed by third-degree heart block that concludes into a fatal bradycardic rhythm causing cardiorespiratory defectiveness and sudden death [12].

2.2.7 Dead in bed syndrome

A relationship among hypoglycemia and sudden death was increased in the 1960s, however the first elaborated explanation showed in 1991 after analysis of a series of deaths of young individuals with T1DM. In 1991, The British Diabetic Association demonstrated a study for unexplained deaths in adult people (<50 years) with T1DM [58], individuals were all found dead in unimpaired bedcovers without sign of sweating or struggle, the previous day, while they were good and the autopsy was negative in all cases. And their were documented autonomic neuropathy, but the majority had a case of important nocturnal hypoglycaemia. Thus, the term ‘dead in bed syndrome’ took place in the diabetes literature [59].

One hypothesis to clarify the ‘dead in bed syndrome’ is that autonomic neuropathy. Autonomic neuropathy is a risk factor for sudden death in people with DM. In people with autonomic neuropathy appears decrease in parasympathetic activity and this effect leading to increase in sympathetic activity. However, there are flaws in this hypothesis [60].

Other hypothesis is explain that hypoglycaemia directly leads to defects in cardiac electrophysiology. Abnormalities of cardiac repolarization have been demonstrated to increase risk of sudden death from ventricular dysrhythmias.

The long QT syndromes caused by genetic or acquired abnormalities of the potassium or sodium channels of the myocardiocyte and it occured majority by drugs that bind to cardiac ion channels, affecting their function [61] and these causes leads to repolarization abnormalities. Recently, there is a new evidence for this phenomena. As shown in Figure 2.2.11, Insulin-induced hypoglycaemia causes an acquired form of long QT syndrome [56].

The hypoglycemia-induced prolongation of QT interval has been detected with Holter monitoring in researchs and it has been hypothesized that these unexpected deaths are because of a cardiac arrhythmia occured [59].

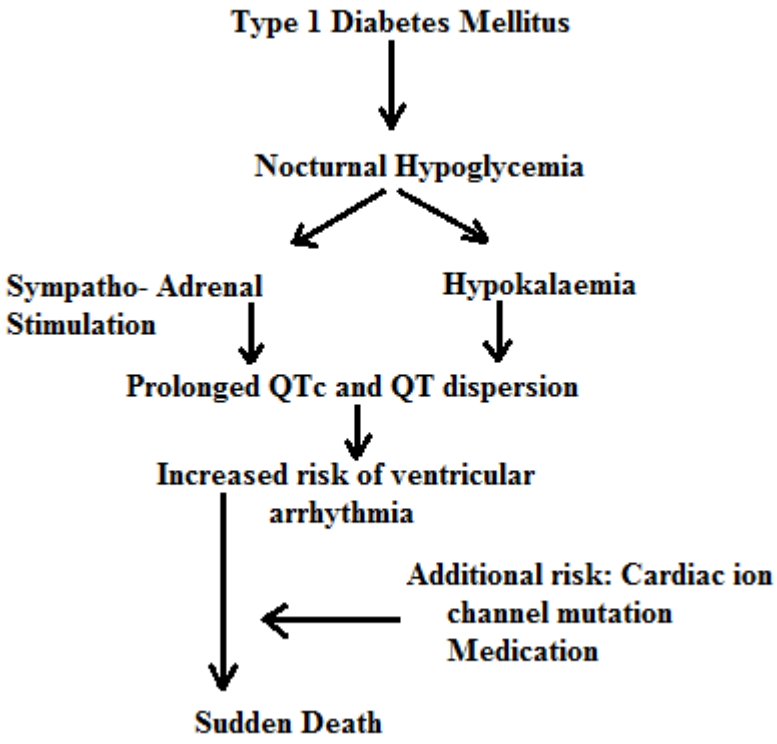


Figure 2.2.9 Hypothesis for ‘dead in bed syndrome’ in individuals T1DM

Additionally to hypoglycemia-induced prolongation QTc, QT dispersion increases. This situation has been demonstred in other conditions to increase risk of sudden death. Sudden deaths of adult people with DM are a rare emergence, whereas hypoglycaemia is very common. As shown in Figure 2.2.8, if changes in hypoglycemia-induced cardiac repolarization are related in the mechanism of sudden death, then perhaps such as mutations in cardiac ion channels or medications are related [62].

CHAPTER 3

MATERIALS AND METHODS

3.1 Subjects and Hardware

ECG, PPS, GSR and RT signals were recorded of totally 5 patients consisting from 2 males and 3 females (Mean age = 49.2, standard deviation: 9.09). Insulin- Induced Hypoglycemia Test (IIHT) was applied to all patients, in Bezmialem Vakıf University, department of Endocrine and Metabolic Diseases. The ECG, PPG, GSR and RT recordings were done at 3 stages as prior to IIH testing, during and after IIH testing. 5 minute of baseline Electrophysiological signals were recorded 30 minutes prior to testing. As shown in Figure 3.1.1, the patients in the supine position in their room, signals were recorded simultaneous with IIH test during this test and immediately after the test was terminated, the signals were recorded of the patients in their beds.

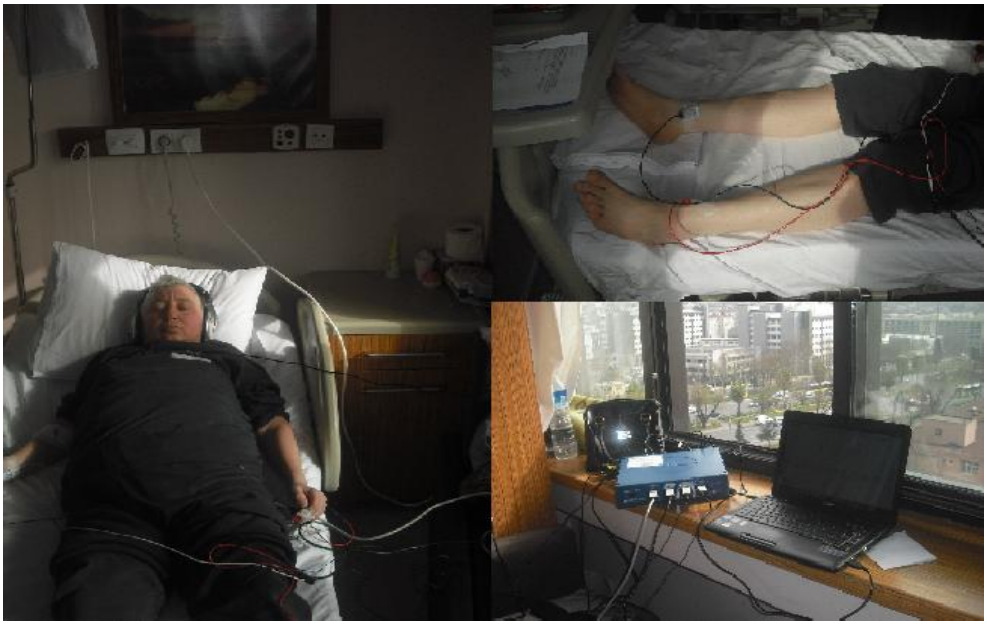


Figure 3.1.1 The electrophsiological signals recorded from a patient in the supine position in their room.

Clinics of Endocrinology and Metabolism Diseases, identify for Secondary Adrenal Insufficiency, they apply routinely Insulin- Induced Hypoglycemia Test and in this study were included patients who required testing with Insulin-Induced Hypoglycemia in clinical. Exclusion criteria for patients in this study are as follows.

- Using of drugs that can affect the heart rhythm and function
- Using of Alcohol, smoking and caffeine
- Doing extreme exrcise
- Being over the age of fifty

The Insulin-Induced Hypoglycemia Test (IIHT) is a physiological stres test and it is utilized to evaluate the response to physiological stress how the hypothalamus, pituitary and adrenal glands respond to physiological stres for diagnosis as adrenal insufficiency, growth hormone deficiency, and Cushing's syndrome and such. Hypoglycemia leads to a physiological stress response, with raises in ACTH and serum cortisol, growth hormone, and prolactin, and activation of the SNS [63]. As shown in Figure 3.1.2, the patient's was opened vascular access prior to the test and insulin dose of 0.15 U/kg (bodyweight) was administered to cause hypoglycemia. [64]. Glucose is transported from plasma to peripheral tissues while insulin is administered through IIH test, in addition insulin prevents glucose production in the liver, which further attends in a steep decline of plasma and brain glucose levels. During this emergency circumstance, the glucose-sensing ability of the CNS becomes activated [60].

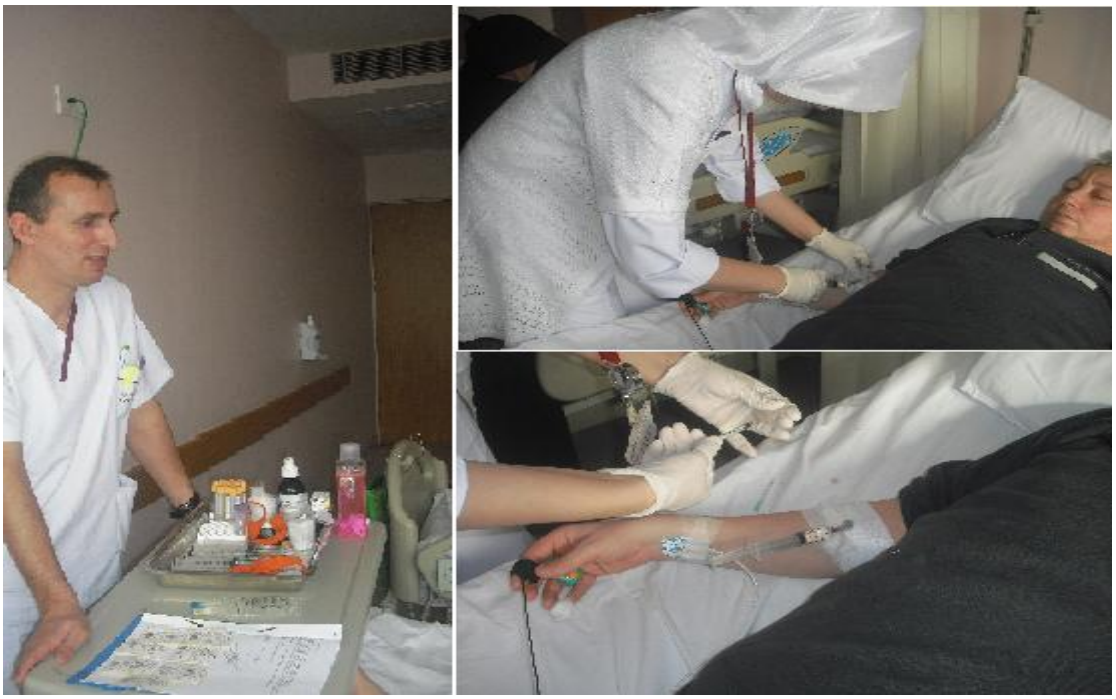


Figure 3.1.2 Insulin Administration

Glycemia was measured with glucometer 30 minutes prior to insulin injection and as shown in Figure 3.1.3 and each 10 minutes following the injection during this test. When glycemia reached 40mg/dl the test is terminated and intravenous dextrose was administered to restore the glycemia. And than, glycemia and cortisol levels are measured again 0, 30, 45 and 90 minutes after the insulin injection.

5 minute of baseline Electrophysiological signals was recorded prior to insulin injection and the sampling was continued until glycemia nadir, approximately 120- 180 minutes, and than signals was recorded after glycemia nadir, approximately 10-15 minutes. Additionally, continuous glucose measurement during the test were done with Continuous Glucose Monitoring System (CGMS). CGMS is attached patient's to the abdominal area or to left shoulder and more frequent glycemia measurement is provided. This device enables us to achieve more blood glucose data and thereby, it has been provided a better evaluation of the relationship between recorded electrophysiological signals and glycemia.

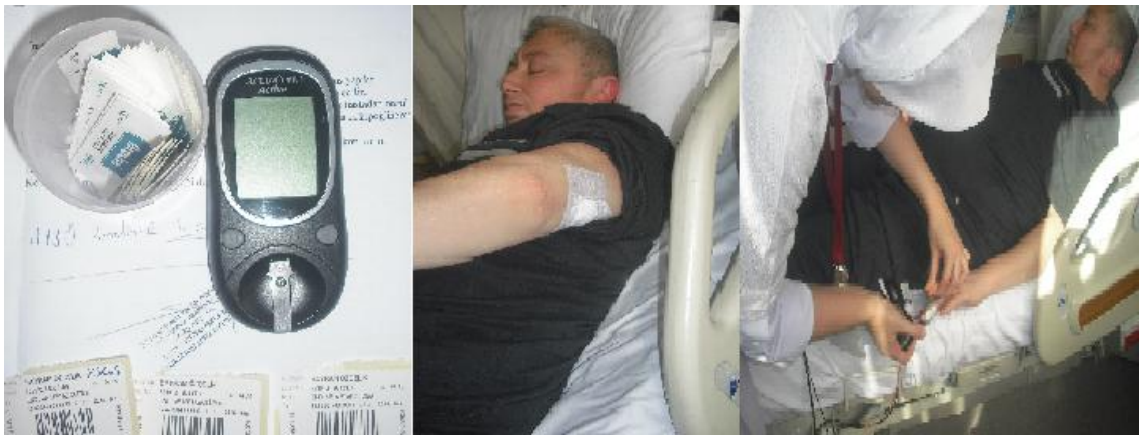


Figure 3.1.3 Measurement of blood sugar

When selecting the patients who participated to the study, has not been done limitations such as age, gender, etc. and detailed demographic information for the patient in Table 3.1 are given.

Table 3.1.1 The demographic data of the subjects.

NUMBER	5
AGE (mean±SD)	49,2 / 9,09
GENDER(Female/Male)	3/2

Reaction time was recorded prior to starting the IHH test andat the end of the test, the Electrophysiological signals were recorded with body surface electrodes ECG, Pulse plethysmography (PPG), Electrical Skin Conductivity (Galvanic Skin Response) and Reaction time was repeated after completion of this process. Electrophysiological signals were recorded with the MP36 electrophysiological data acquisition unit (Biopac Systems, Goleta, CA, USA). Entire the signals were sampled at 1000 Hz and digitized (A/D converted) at a resolution of 24 bits per sample with the MP36 unit. In Figure 3.1, is shown projects flow diagram.

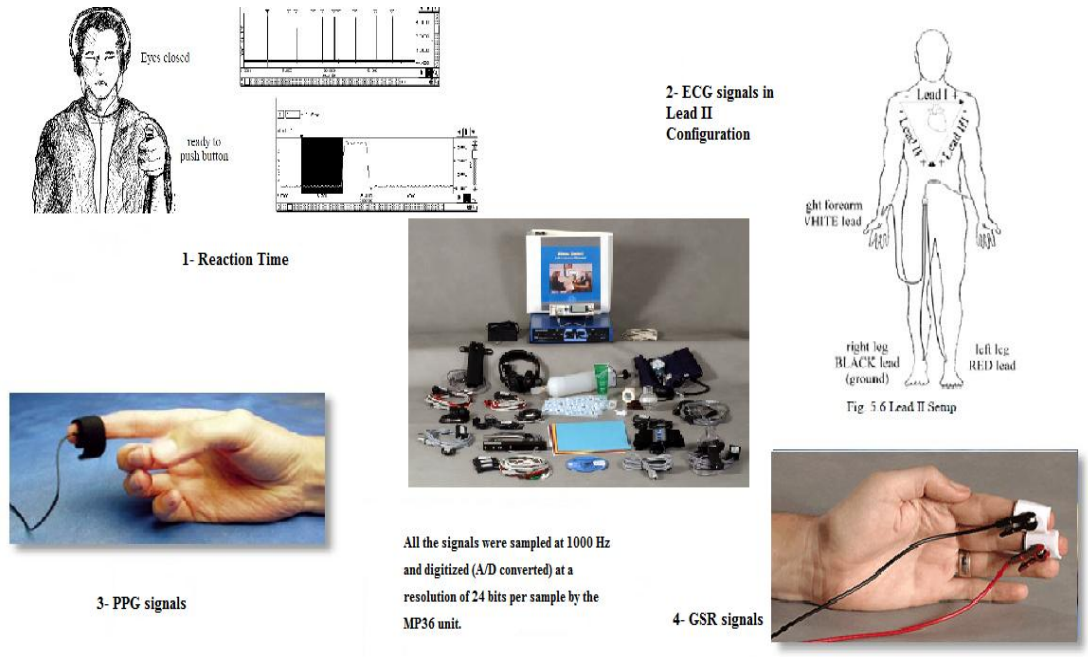


Figure 3.1.4 Flow diagram of Project.

The ECG is the basis for several studies containing Heart Rate Variability (HRV) analysis. In this study, relationship between the degree of the prolongation of QTc and HRV signals with low blood glucose level is analyezd. The QT and QTc intervals were computed from ECG signals.

The MP36 data acquisition unit was used to amplify and filter the ECG signal using with the 35 Hz Low-Pass (LP) filter, 0.05 Hz High-Pass (HP) filter, and 500 gain. Skin surface was abraded and electrode gel was used to decrease the electrode impedance.

PPG signals were recorded with MP36 data acquisition unit. The probe of the TSD200 module, that pruduces on 860 ± 60 nm wave length. It was attached on the right ring finger and the module filter settings were as 3 Hz Low Pass filter and 0.5 Hz High Pass filter.

GSR signals were recorded with MP36 data acquisition unit in DC mode. The 6 mm diameter Ag-AgCl electrodes were used with conductive gel and attached on the left middle finger and forefinger.

The SS10L pushbutton hand switch were used to assed for psychophysiological response tests. When data from the button were displayed on the screen, it normally reads 0 Volts, and when the button is pressed it reads +5 mV.

All the steps of medical part of the study have been done by Prof. Dr. Ertuğrul Taşan, Assoc. Prof. Dr. Özcan Karaman and Assoc. Prof. Dr. Mahmut Muzaffer İlhan in Bezmialem Vakıf University, Endocrinology and Metabolic Diseases department. Ethics approval has been taken for study from Bezmialem Vakıf University Clinical Research Ethics Committee- Decision No: 71306642/050-01-04/219. Figure 3.1.2 demonstrates raw signals obtained from subject during the Insulin-Induced Hypoglycemia Test.

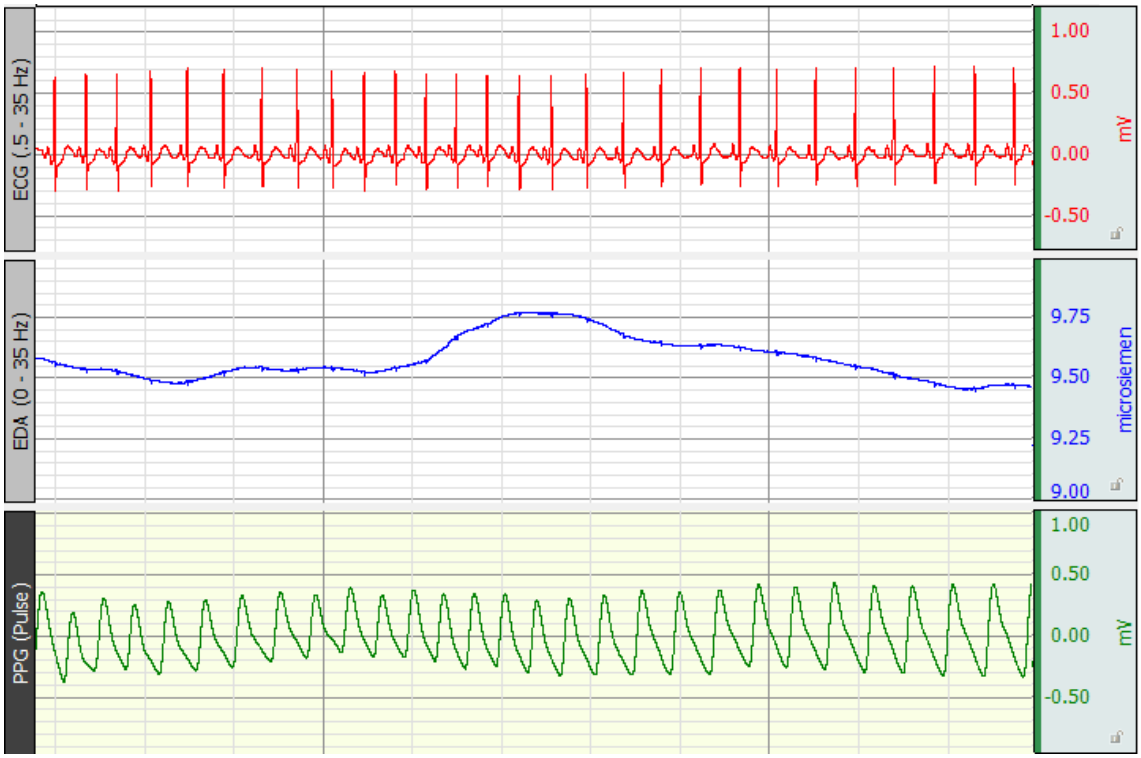


Figure 3.1.5 The raw ECG, PPG and GSR signals recorded from a patient during the Insulin-Induced Hypoglycemia Test.

3.2 Analysis of Physiological Signals

3.2.1 Calculation of Sympathovagal Balance and Power Spectral Density of Heart Rate Variability

Heart rate (HR) variability is a non-invasive technique to measue the effectiveness of the ANS, evaluate the balance between the SNS and PNS, therefore Heart rate variability (HRV) is an important test for the evaluation of any irregularities that may occur in heart rate and control procedures. HRV is regulated by arterial pressure and respiratory oscillations, parasympathetic and sympathetic outflow, humoral factors, and status of sinus node [65].

Variations in heart rate may be assessed by a number of methods but, methods generally used are for HRV analysis: time and frequency domain methods. The heart rate at any point in time between successive normal complexes are established with time domain methods, QRS complexes causing from sinus node depolarizations are appointed in a continuous ECG record, and the so-called normal-to-normal (NN) intervals, is specified [66]. In some time domain variables of mean heart rate, the difference between the longest and shortest NN interval etc. are analyzed with statistical methods and related parameters are calculated. The variety of time–domain measures of HRV is summarized in Table 3.2.1.

Table 3.2.1 Selected time-domain measures of HRV.

Variable	**Units**	**Description**
SDNN	ms	Standard deviation of all NN intervals.
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals.

In the frequency-domain methods, a power spectrum density (PSD) that ensures the basic information of how power distributes as a function of frequency estimate is calculated from the RR interval series. In HRV analysis, the PSD function calculating methods commonly used in the literature for performed using either FFT based methods or parametric AR modeling based methods. The advantage of FFT based methods is simplicity of the algorithms and thus can be applied in practice within a short time.

The AR spectrum yields improved resolution especially for short samples, the other most prominent feature of AR spectrum in HRV analysis is that it can be factorized into separate spectral components. In spite of that, the disadvantages of the AR spectrum are the complexity of model order selection and the contingency of negative components in the spectral factorization. Nevertheless, it may be advantageous to calculate the spectrum with both methods to have comparable results.

HRV is commonly used to assess the function of the ANS and balance between the sympathetic and parasympathetic activities that control heart rate and this assessment provides with the spectral analysis of HRV. As shown in Tablo 3.2.2 three frequency bands are commonly of interest: Very low (< 0.04 Hz, VLF), Low (0.04 – 0.15 Hz, LF) and High frequency band (0.15 – 0.4 Hz, HF).

Table 3.2.2 Selected frequency domain measures of HRV

Variable	**Unit**	**Description**	**Frequency Rnage**
LF	ms^2	Power in low frequency range	0·04–0·15 Hz
HF	ms^2	Power in high frequency range	0·15–0·4 Hz
LF/ HF ratio or Sympathovagal Balance		Ratio LF [ms^2]/HF [ms^2]	

The HF components reflects respiratory sinus arrhythmia and are more associated to parasympathetic control of the heart rate and LF primarily represents sympathetic activity to the Sinoatrial Node discharge, and LF/HF ratio may represent a cardiac sympatho-vagal balance. There has been an increase in sympathetic activity if this rate increased compared to the previous state. Similarly, there has been an increase in parasympathetic activity if this rate decrease compared to the previous state. The physiological explication of the components in the VLF band is undetermined [65]. As shown in Figure 3.2.2, Power component of this frequency regions can be calculated as absolute power from PSD graphs.

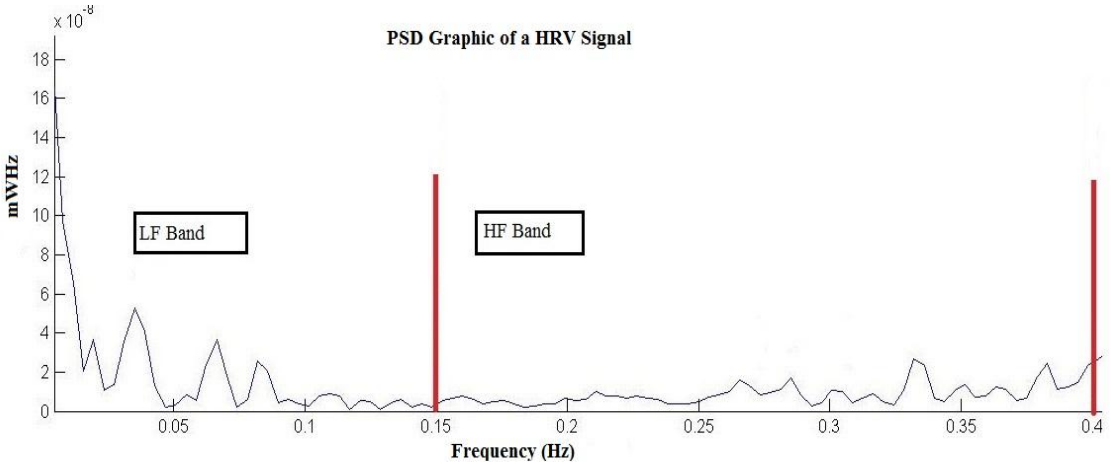


Figure 3.2.1 Power spectral density analysis of HRV signal of a patient during Insulin-Induced Hypoglycemia Test.

The frequency-domain evaluates elided from the PSD estimate for each frequency band contain absolute and relative powers of VLF, LF, and HF bands, LF and HF band powers in normalized units, the LF/HF power ratio, and peak frequencies for each band.

There is very little information about the variation in heart rate due to the hypoglycemia. Therefore, HRV analysis was performed in this study. Raw ECG recorded through lead II configuration was used for obtaining HRV and QT interval. The methodological guidelines listed in and to detect R peaks were used in HRV analysis. To this end, we used the algorithm recommended by Manriquez and Zhang [67, 68]. This algorithm flow chart is given in Figure 3.3.3.

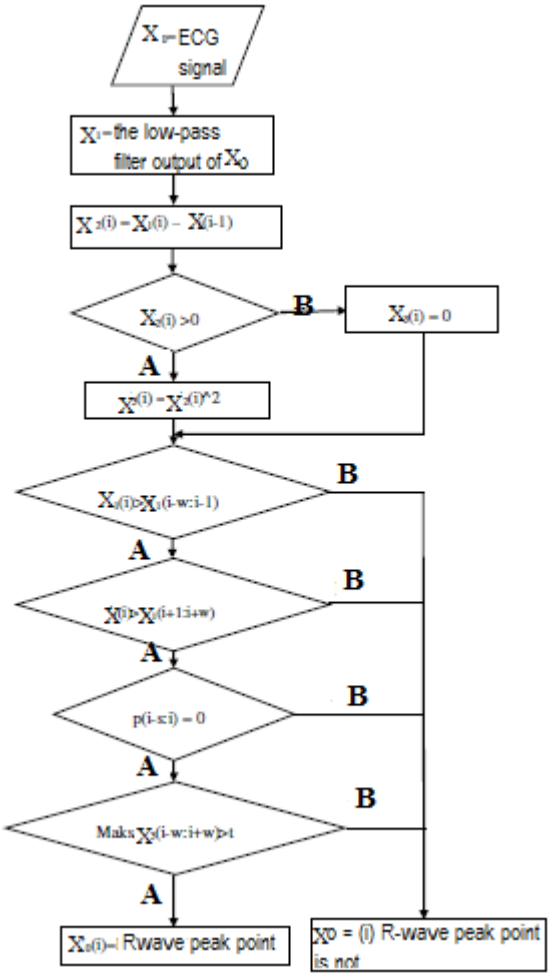


Figure 3.2.2 Flow diagram of the algorithm used to determine R-wave

And than, the durations between successive peak locations were evaluated to produce RR intervals and RR intervals with the length less than 0.33 second and more than 1.5 were deleted from time series. RR interval series is innately nonuniformly sampled series. Therefore, interpolation is essential to produce a uniformly sampled HRV time series out of an RR interval series. Therfore, the cubic interpolation frequency was selected as 4 Hz. After interpolation process, PSD analysis was implemented by use of Welch's method with Hann window.

The Welch method calculates an average over the modified periodograms that is described as a classical nonparametric method to calculate PSD. The important deficiency of the periodogram method is the effect of offside lobe leakage by the reason of finite data sets, therefore to decrease the effect of the offside lobe leakage, time series of the signal is segregated into overlapping sequences.

The later each data sequence is windowed in order to smooth the edges of the signals, therefore this improvement Welch method was used to evaluate the PSD of the interpolated HRV signal. [69, 70].

n= 0,1, … ,(L-1),

i= 0,1, … ,(K-1),

x_i (n) = x (n+ i x S) S is the size of the sequences and S ≠ L, (3.1)

The ith modified periodogram is:

$$\hat{P}_i(f) = \frac{1}{UxL}\left|\sum_{n=0}^{L-1} x_i(n)xw(n)xe^{(-jx^{2x\pi x}fxn)}\right|^2 \quad (3.2)$$

Where U is the normalizing constant.

$$U = \frac{1}{L}x\sum_{n=0}^{L-1} w^2(n) \quad (3.3)$$

The power spectral density is:

$$\hat{P}_\omega(f) = \frac{1}{k}x\sum_{j=0}^{k-1}\hat{P}_{i(f)} \quad (3.4)$$

L is the lenght of the interpolated Heart Rate Variability signal and W(n) is the Hann function and in this study the window size of 256 samples and 50% overlap were selected so that 0.0156 Hz spectral resolution was obtained, like so, as shown in Figure 3.2.2 power in LF and HF bands and, LF/HF ratio, were calculated for each individual [70].

3.2.2 Calculation of Features from Pulsepletismograf Signal

The vasomotor activity, regulating the wall width of the veins is controlled by SNS, for this reason the alterations in the PPG signal amplitude provides information about the stimulation of SNS.

For example, an increase in the PPG signal amplitude demonstrates that there is a decrease in SNS stimulation, which causes widening of vein walls and more blood circulation. The photo-plethysmograph is a noninvasive, cheap device and very suitable to use with the PPG device being applied to patients' fingers, for detecting blood volume fluctuations by optical means and it is commonly used to evaluate vascular compliance, blood oxygen saturation, heart rate and respiratory rate. [71, 72]. A Light Emitting Diode (LED is connected in series) attached at a fingertip sends an infrared light beam and a photo-detector (conected in paralel) placed next to the LED receives reflected light from the tissues underneath. Blood is pumped to the vascular system with each contraction of the heart so that the blood flow are become too much in vessels containing the capillaries under the LED , and consequently the amount of light reaching photo-detector changes, the resultant nearly periodic signal is called as the PPG signal [71].

The pulsatile feature of the PPG waveform, at the same time it is called the AC component and is synchronized with each heartbeat and its main frequency based on heart rate. The pulse waves occur from alterations of blood volume in arterial tissues by means of each heartbeat. It can be used to assess the variation of heart rate. PRV, at the same time it is called as Pulse-to-pulse variability and can evaluated from peak to peak time intervals of the PPG electrorphysiological signal [9].

In this study, we analyzed the accuracy of PRV measured from PPG as an estimate of HRV measured from ECG signal in Hypoglycemia. PPG signal amplitude of each patient to calculate, we first find local maximum and minimum points of the signal. In Figure 3.2.3, we demonstrate a sample PPG signal and the peaks detected on this signal. The series of pulse amplitude values are acquired as:

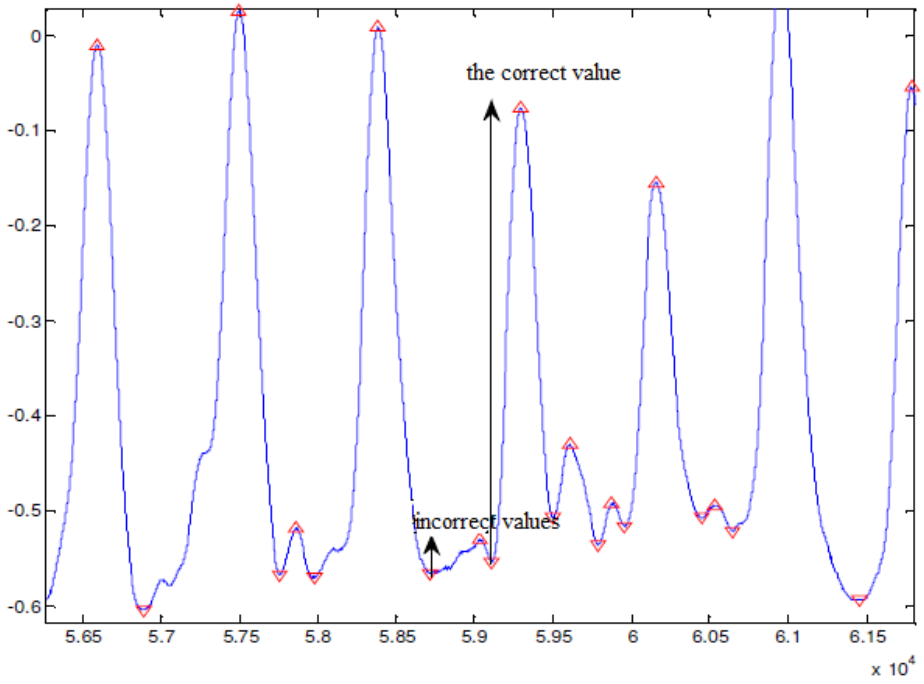


Figure 3.2.3 Local maximum and minimum points in the PPG signal

$$B_k = a_k \max - a_k min. \tag{3.5}$$

After finding local maximum and minimum points of each pulse wave, the time series of PP intervals in other words, between two successive pulse peaks is for each segment were calculated.

As processes applied to RR interval, the PP intervals with the length less than 0.33 second and more that 1.5 were deleted and by implementing even resampling of PP intervals at 4 Hz, the PRV signal was acquired. As with HRV, the power of the PRV in the LF and HF band was calculated through the PSD and to contrast the indices calculated for PRV and HRV signals, the commonly used time and frequency domain parameters were estimated pursuant to the SD of HRV parameters [66]

As shown in Table 3.2.1 and 3.2.2.2 in the time domain, for each overlapped segment of RR or PP intervals (NN interval), the mean NN interval, the SD of entire RR and PP intervals (SDNN) and the root mean square of the difference of successive RR or PP intervals (RMSSD) were estimated and additionally, the ratio of the LF/HF was determined in the frequency domain.

3.2.3 Calculation of Features from Galvanic Skin Response Signal

Electrodermal activity (EDA) also known as Galvanic Skin Response is the general term used for describing autonomic changes in the electrical properties of the skin. The most studied feature is the skin conductance, that may be measured by performing an electrical potential between two points of skin contingence and measuring the causing current flow between them.

During hypoglycemia, the activity of the sweat glands raise till the glands reach saturation and a rapid raise in the skin conductivity or GSR happens. Therefore, the the stimulation level of SNS are involved skin condtivity.

In literature GSR studies, in which signal recording is conducted during the sudden stimulus free period, the most commonly used feature is the mean amplitude value of the GSR signal and is called as skin conductance level [11].

In GSR analysis of this study, mean amplitude values and standart deviation of the GSR signals were calculated for each individual during Insulin- Induced Hypoglycemia Test. Additionally, GSR signal was divided into 10 second long segments. The later, mean values for each of these segments were also calculated:

$$seg\_mean(k) = \frac{1}{f_s} \times \sum_{i=t_{\min}(k)}^{t_{\max}(k)} GSR(i), \tag{3.6}$$

Here, t_{max} and t_{min} are given as: (3.7)

$$t\min(k) = f_S x(k-1) + 1$$

$$t\max(k) = f_s xk \tag{3.8}$$

f_S = sampling frequency,

N = lenght of the GSR signal, k= (1,2,3,…,N / f)

3.2.4 Evaluatin of Reaction Time

Reaction time is the delay between the stimulus presentation and the begininng of the response. This delay between stimulus and response is due to the time for the sensory signal to reach the brain, the time required for the brain to process the sensory information and generate a motor response, and the time for motor signals to reach the skeletal muscles. In this process with learning, reaction time typically decreases, at the same time, reaction time changes from subject to subject and from situation to situation, for example, most individuals have delayed reaction times late at night and early in the morning.

In order to compare the reaction times from the two types of situation,(prior to IHT testing and after IHT testing) we can summarize the results as statistics or measures of subjects. There are significant statistics. They are commonly reported for the results of a study: mean, range, variance, and standard deviation. Using the statistics of mean and distribution, we were compared the situation of prior to IHT testing and after IHT testing.

$$\text{Variance} = \frac{1}{n-1}\sum_{J=1}^{n}\left(x_J - \bar{x}\right)^2 \tag{3.9}$$

$$\text{Standart Deviation} = \sqrt{variance} \tag{3.10}$$

Where

N= Number of subjects

X_j = Mean reaction time for each subjects

$\bar{X}$ = Group mean (constant for entire subjects)

$\sum_{j=1}^{n}$ = Sum of entire subject data (3.11)

Entire the physiological data processing was carried out using in-house programs developed under MATLAB R20011b Software (MathWorks Inc., Natick MA, USA)

CHAPTER 4

RESULTS

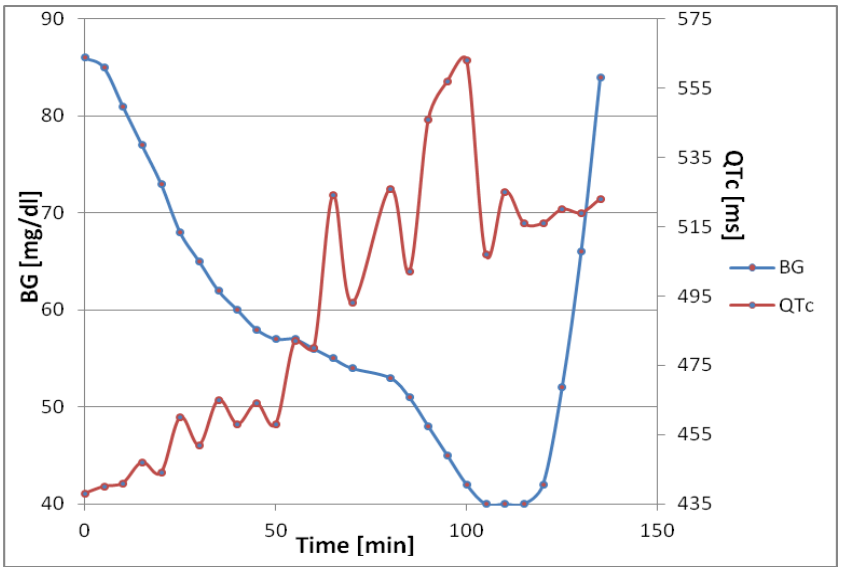
In our study, in order to evaluate the Electrophysiological reactions to Hypoglycemia that is resulted by IHT test, the ECG, PPG, GSR and RT signals were recorded at three stages of the test. Some features that could reflect the changes in the sympathetic nervous system were calculated and analyzed. The features that are obtained from these signals was investigated for three different phases as prior, during and after. To this end, insulin were administered to 5 patients participating in the study.

Computed Features;

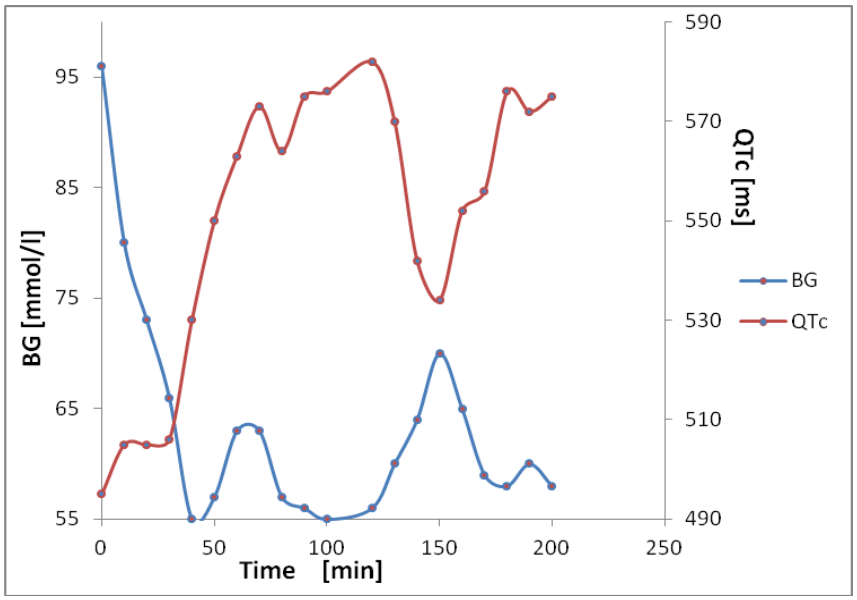
Electrophysiological Signals	Features
ECG	**I.** QT and QTc interval was calculated from ECG signal **II.** LF / HF ratio, was the calculated from values of the power spectral density of the HRV using ECG signal
PPG	**I.**LF / HF ratio, was the calculated from values of the power spectral density of the PRV using PPG signal
GSR	**I.** The mean amplitude value (skin conductance level)
RT	**I.** Mean value of Reaction Time

4.1 Results for QTc Interval Analysis

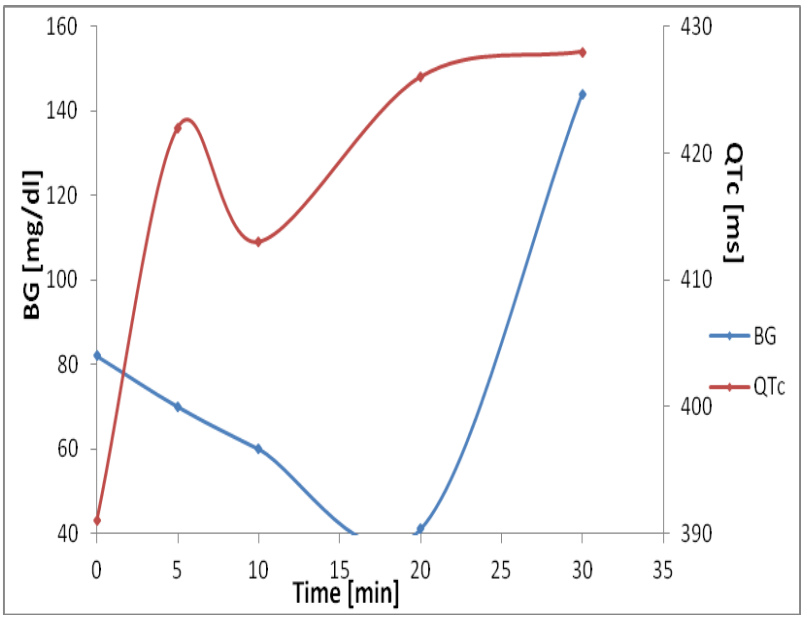
In the analysis of ECG signal, the QTc value was used as a feature**.** An increase in this value corresponds to an increase in the SNS. In contrast to, a decrease in this velue corresponds to an incrase in PNS. The relationship between BG and QTc are shown below in Figure 4.1.1



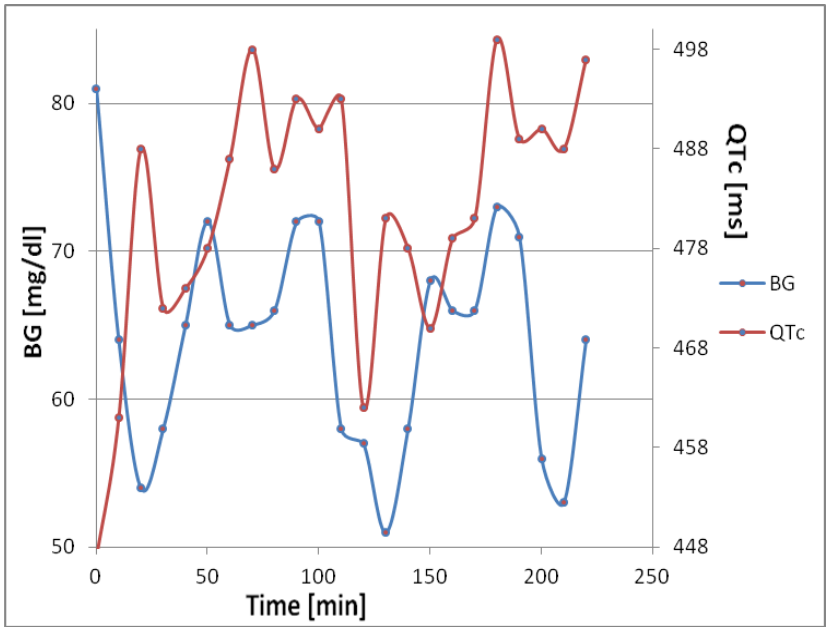
a. The first patient



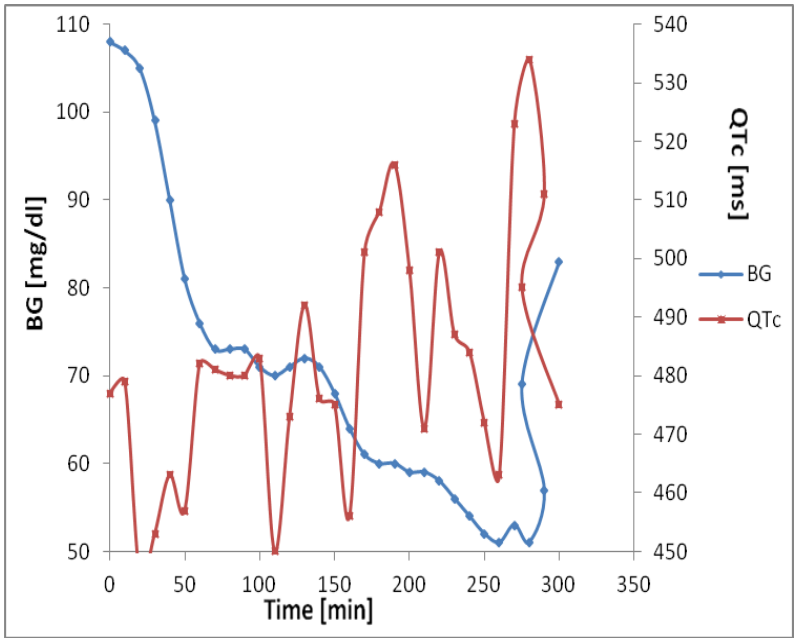
b.The second patient



c.The third petient



a.The fourth patient



b.The fifth patient

Figure 4.1.1 Graphics for the BG and QTc during IHT test.

According to all the graphs of Figure 4.1.1, QTc prolongation was observed proportionally with BG decrease. For each patient, different situations have occurred during IHT test. Therefore, regularly increase or decrease was not observed for BG and QTc. These situations will be discussed in the next section. For IHT test, the minimum BG target value should be 40 mg / dL, but this value could not be obtained in all patients. Because the patients metabolism represent different response to decreasing of BG. Therefore, different symptoms and severity of Hypoglycemia was occurred in all patients.

Table 4.1.1 The table shows the changes in QTc from baseline, BG maximum and nadir value, and the maximum value of the QTc during IHT test.

Patient	**BG [max-min] (mg/dl)**	**QTc [max-min] (ms)**	**Difference (ms)**	**Max Value (ms)**
1	86-40	563-438	125	727
2	96-55	582-495	87	727
3	82- 41	428-391	37	805
4	81-51	499-447	52	710
5	111-51	534-453	81	736
Group mean ± SD	91±13 48 ±7	521 ±60 445± 37	76 ±34	741 ±37

As seen from the Table 4.1.1 obtained BG and QTc values are suitable with literature. The amount of increase in QTc interval and the maximum value of QTc was demonstrated in Table 4.1.1. The average maximum value of QTc was 725 ms during entire test and this value was very high.

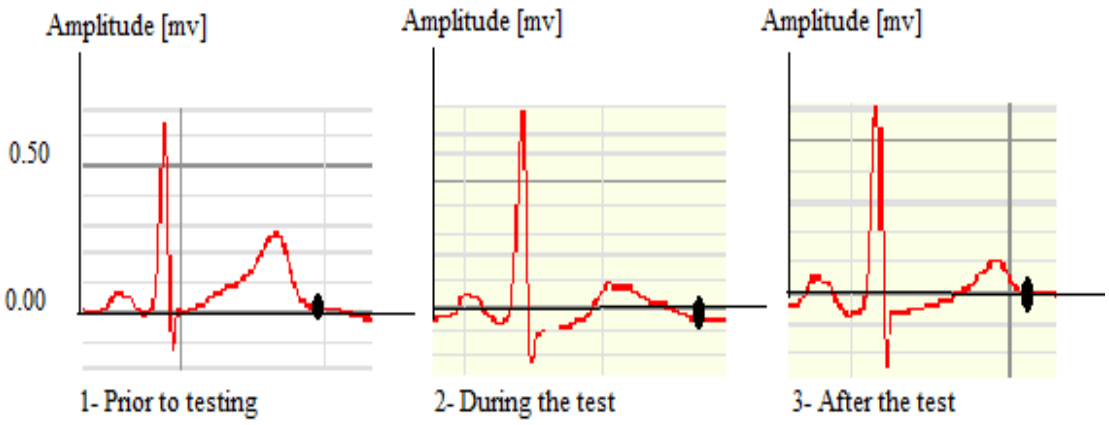


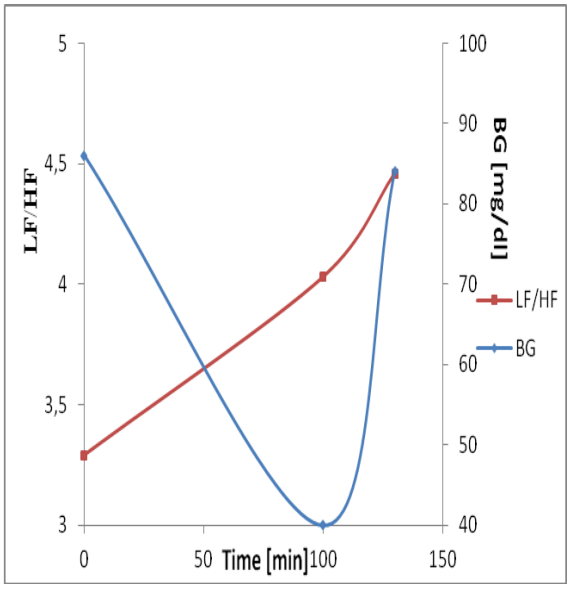
Figure 4.1.2 Three median beats from a patient at baseline (left), during (middle) and after (right). The '*' indicates the end of the T-wave and flattening and lengthening of the Twave.

In Figure 4.1.2, Three median beats from a patient at baseline is shown at different phase, prior, during and after. In addition, lengthening and flattening of the T wave is shown in this Figure. At " during " phase, QT lengthening and T wave flattening are seen in the time domain. As seen, QT lengthening and T wave flattening is detectable with visual analysis. We conclude that QTc lengthening is potentially good indicators to evaluate the electrophysiological responses during Hypoglycemia, but it should be tested for more patients. Therefore, QTc can be used for early detection of Hypoglycemia.

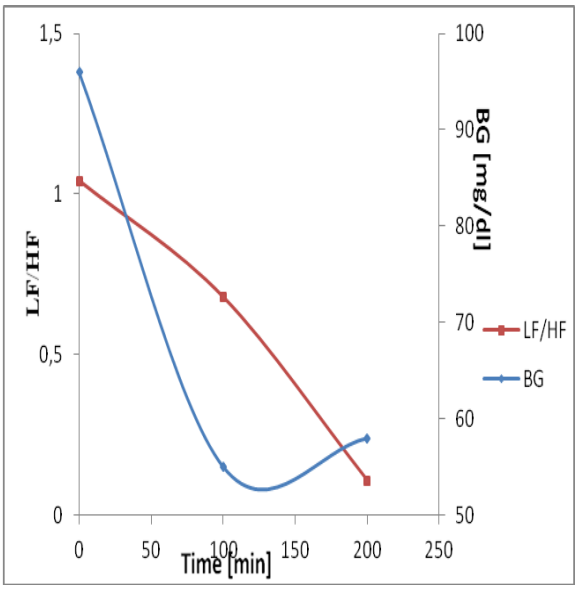
4.2 Results for HRV and PRV Signal Analysis

In literature analysis of HRV signal and the LF/HF ratio is used to evaluate the Hypoglycemia. In the thesis first of all HRV analysis was done to prove the if the results concistent with the literature or not. An increase in this ratio corresponds to an increase in the SNS.

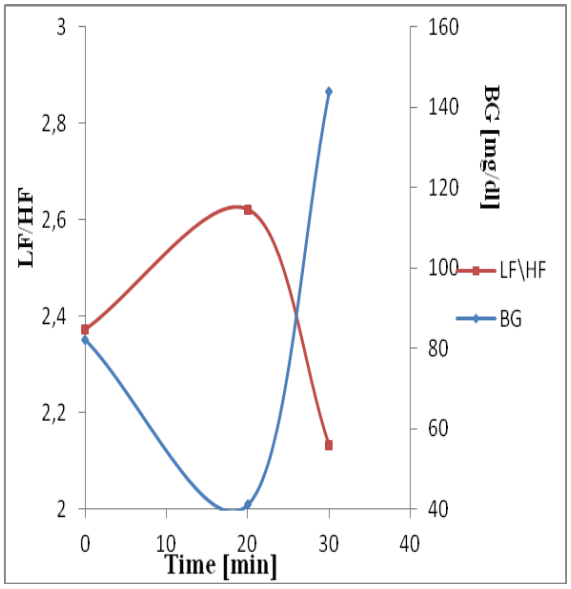
In contrast to, a decrease in this ratio corresponds to an incrase in PNS. The LF / HF ratio that computed from HRV and PRV signal is shown in Table 4.2.1 and Figure 4.2.1.



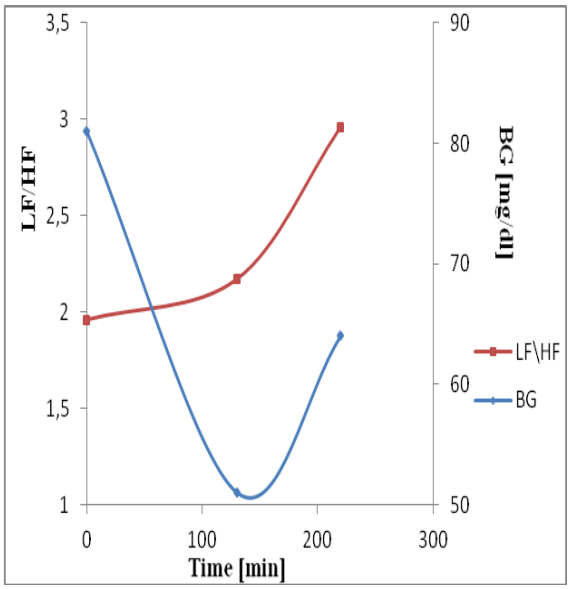
a.The first patient



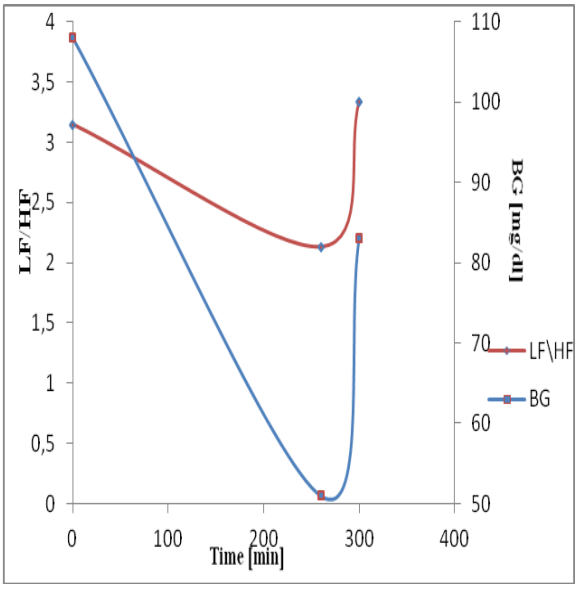
b. The second patient



c.The third patient



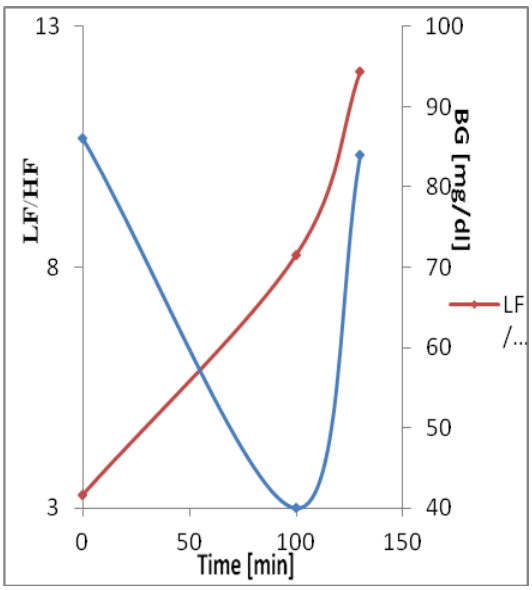
d. The fourth patient



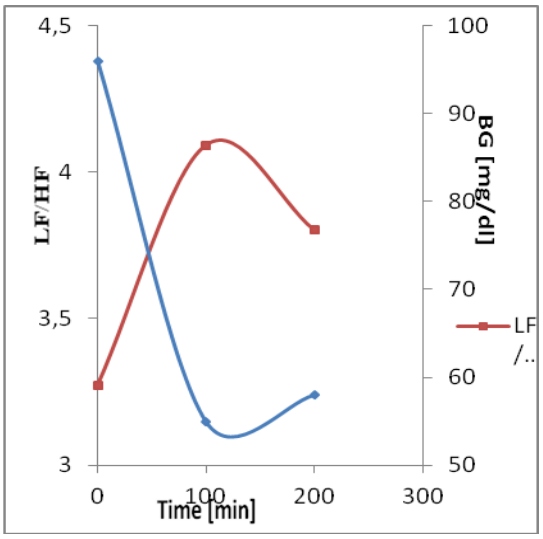
e. The fifth patient

Figure 4.2.1 Graphics (a,b,c,d,e), for the LF/HF ratio obtained from HRV

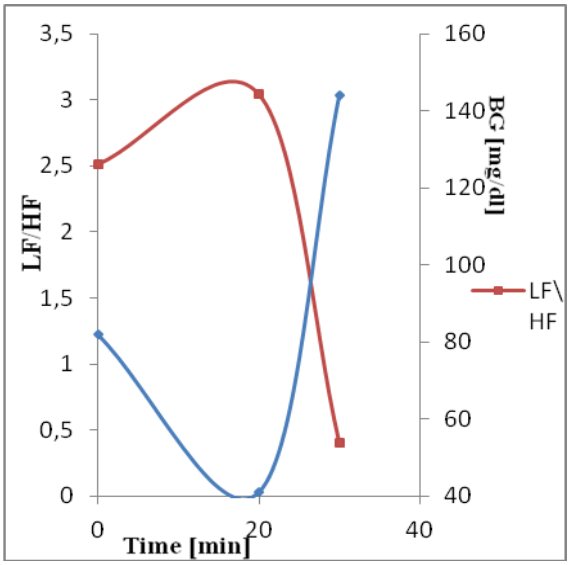
As seen in Figure 4.2.1, LF/HF ratio was invreased when of the BG was decreased. To demonstrated the hypotesis is that “PRV can use instead of HRV” LF/HF ratio was computed from PRV signals.



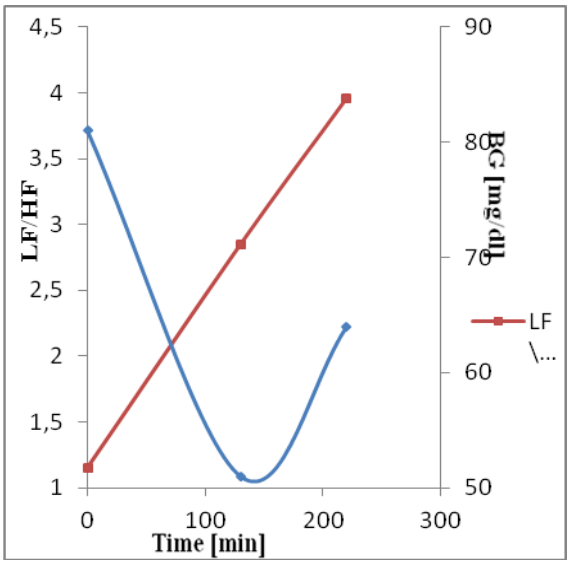
a.The first patient



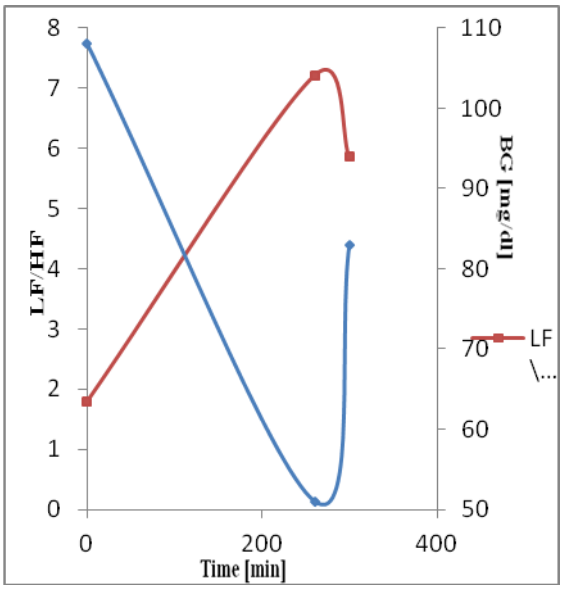
b.The second patient



c.The third patient



d.The fourth patient



e.The fifth patient

Figure 4.2.2 Graphics (a,b,c,d,e), for the LF/HF ratio obtained from PRV.

As seen in Figure 4.2.2, LF/HF ratio was increased when of the BG was decreased.

The obtained results for PRV is shown in Figure 4.2.2. When the Figure 4.2.1 and Figure 4.2.2 is computed , results for LF/HF ratio are seen to be compatible in point of HRV and PRV signals.

Table 4.2.1 Descriptive Results, Analysis for the Parameters obtained from HRV for all patients.

	HRV				
	Phase	**LF/HF**	**Mean RR**	**Std RR**	**Rms RR**
1	**Prior**	3,29	0,418	0,030	0,419
	During	4,03	0,849	0,115	0,856
	After	4,46	0,792	0,087	0,797
2	**Prior**	1,041	0,657	0,028	0,658
	During	0,679	0,621	0,043	0,623
	After	0,108	0,640	0,040	0,641
3	**Prior**	2,373	0,878	0,067	0,880
	During	2,621	0,767	0,076	0,771

	After	2,132	0,768	0,099	0,774
4	**Prior**	1,96	0,895	0,030	0,896
	During	2,170	0,891	0,045	0,892
	After	2,96	0,873	0,048	0,874
5	**Prior**	3,148	0,986	0,072	0,989
	During	2,13	0,934	0,084	0,937
	After	3,34	0,891	0,071	0,894
Group mean ± SD	**Prior**	2.36	0.77	0.05	0.77
	During	2.32	0.81	0.07	0.82
	After	2.6	0.79	0.06	0.8

Table 4.2.2 Descriptive Results, Analysis for the Parameters obtained from PRV for all patients.

	PRV				
	Phase	**LF/HF**	**Mean RR**	**Std RR**	**Rms RR**
1	**Prior**	3,279	0,421	0,041	0,423
	During	8,26	2,222	10,851	11,074
	After	12,07	9,564	27,119	28,616
2	**Prior**	3,274	0,654	0,035	0,655
	During	4,092	0,666	0,698	0,965
	After	3,803	0,745	0,669	1,000
3	**Prior**	2,510	0,878	0,067	0,880
	During	3,043	0,914	0,745	1,179
	After	0,407	0,775	0,146	0,788
4	**Prior**	1,152	0,892	0,037	0,893
	During	2,85	0,899	0,217	0,925
	After	3,96	0,884	0,220	0,911
5	**Prior**	1,801	0,436	0,175	0,470
	During	7,21	1,158	1,036	1,554

	After	5,86	1,104	1,331	1,729
Group mean ± SD	**Prior**	2.40	0.66	0.07	0.66
	During	5.09	1.17	2.70	3.14
	After	5.22	2.61	5.9	6.6

In the Table 4.2.1 and 4.2.2, LF/HF ratio are demonstrated for three phase, "prior, during and after". LF / HF ratio is given as an average value for all phases because ECG and PPG signal contains more noise. Noise ratio is eliminated in this way. At the same time, Mean RR, Std RR and Rms RR value are calculated for each phase.

According to all the graphs, we observed changes in LF\HF ratio obtained from HRVand PRV proportionally with BG decrease. The LF/HF obtained from PRV and HRV is higher at the "during the test" phase for all patient. These resluts confirms that there is an increased SNS activity during IHT test. Similarity between the LF/HF ratio obtained from HRV and PRV were not observed, but there was an increase in both. We notice that the mean LF\HF ratio increases during the IHT test or hypoglycemia for all patients.

Results of the across phase and all patients comparison demonstrate that there is a difference among different phases and patients. But, results of the LF/HF ratio across HRV and PRV comparison demonstrate that there is not a difference among different HRV and PRV. We conclude that LF/HF ratio may a good indicator to evaluate hypoglycemia, but it should be tested for more patients.

4.3 Results for GSR Signal Analysis

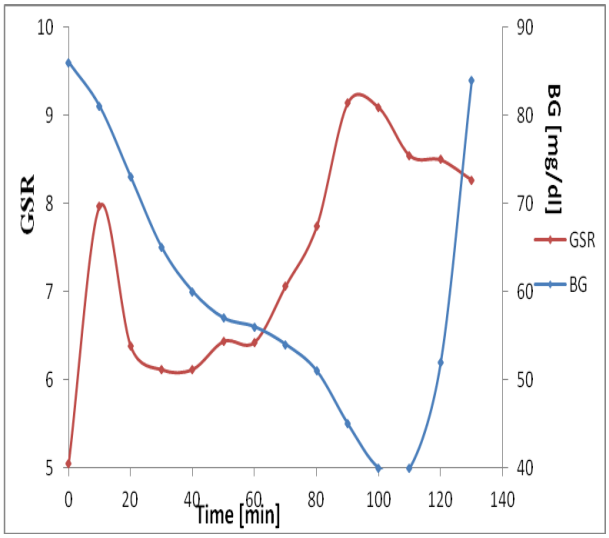
In the thesis, mean and standard deviation values of the GSR signals recorded for each patient, are calculated. Results for GSR signals with phase comparison is shown in Table 4.3.1 ("prior, during and after").

Table 4.3.1 Results for GSR signals with phase comparison

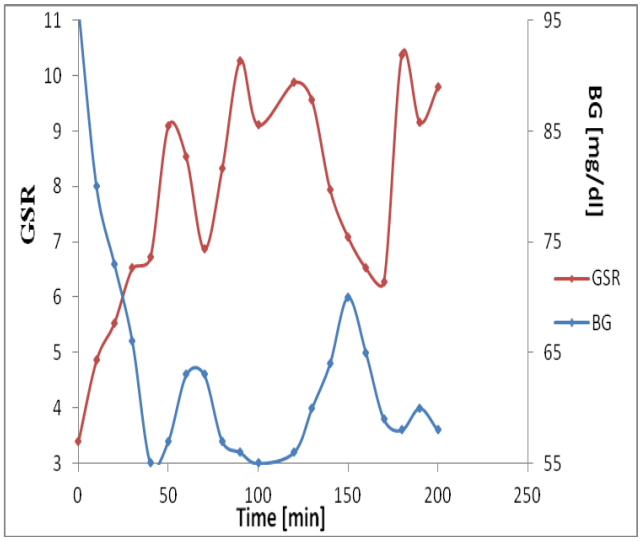
Patient	**Phase**	**Mean**	**Std**
1	**Prior**	5.055	0.246
	During	7.469	1.215
	After	8.273	0.500
2	**Prior**	3.412	0.132

	During	8.209	1.948
	After	9.628	0.860
3	**Prior**	10.109	0.955
	During	11.709	2.243
	After	10.192	1.829
4	**Prior**	4.911	0.434
	During	4.188	0.564
	After	4.450	0.451
5	**Prior**	5.145	0.138
	During	7.00	1.571
	After	10.252	0.69
Group mean ± SD	**Prior**	5.7264	2.5
	During	7.715	2.7
	After	8.559	2.43

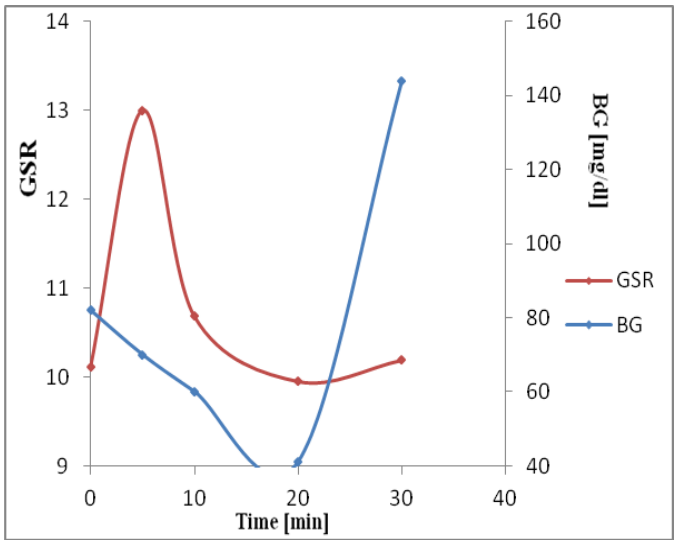
As seen in Table 4.3.1, mean value of GSR signals are higher at the "during and after" phase for all patients. These resluts confirms that there is an increased SNS activity during IHT test. We find out that the mean value of GSR signals get its maximum value during the IHT test and after the IHT test for all patients. This result is compatible with literature. Because the increased SNS activity leads to increased GSR amplitude. The mean skin conductance for "during the test or hypoglycemia" phase is higher than the mean skin conductance of other phase.



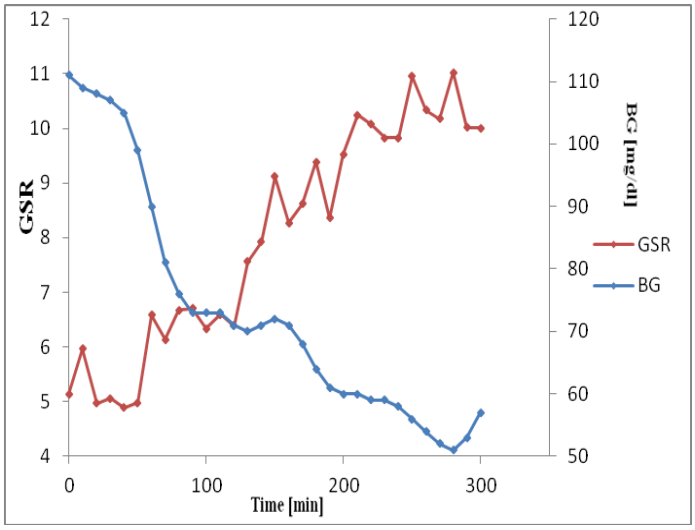
a.The first patient



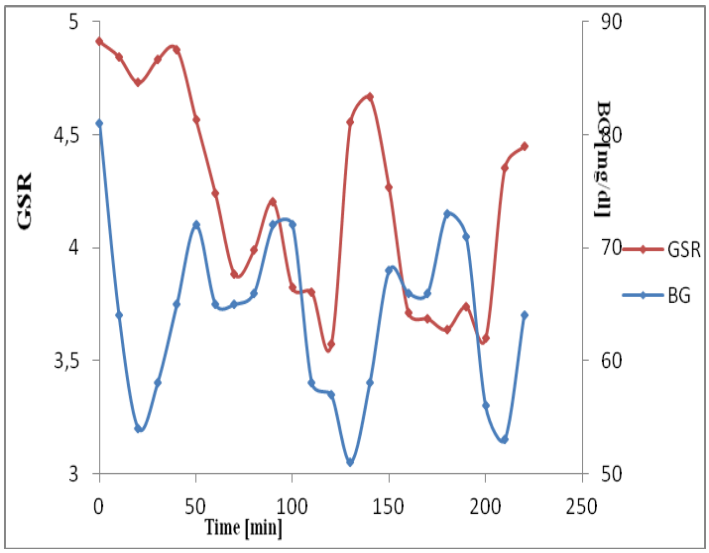
b.The second patient



c.The third patient



d.The fouth patient



e.The fifth patient

Figure 4.3.1 Graphics (a,b,c,d,e), for the seg_mean value obtained from GSR

As seen in Figure 4.3.1, the GSR signal was divided into 10-second long segments, and the mean values for each of these segments that it is called seg_mean were calculated. As seen in Figure 4.3.1, mean value of seg_mean were increased proportionally with BG decrease. We conclude that skin conductance (GSR amplitude) and seg_mean are good indicators to assess hypoglycemia, but it should be tested for more patients. Therefore, GSR signal can be used for early detection of Hypoglycemia.

4.4 Reaction Time Analysis Results

In the thesis, the Reaction Time signals recorded for each patient, mean and standard deviation values are calculated. Results of the analysis of the RT signals for phase comparison is shown in Table 4.4.1 and 4.2.1(“prior, during and after”).

Table 4.4.1 Comparison of Reaction Times. A shows " prior" phase and B shows "after" phase.

Subject's	**Random Data (Non- Dominant Hand)**			
Number	**1th Stimulus (s)**	**5th Stimulus (s)**	**10th Stimulus (s)**	**Mean (s)**
1. A	0.422	0.252	0.216	0.323
1. B	0.474	0.702	0.446	0.446
2.A	0.438	0.446	0.308	0.382
2.B	0.634	0.470	0.488	0.488
3.A	0.624	0.462	0.354	0.485
3.B	0.632	0.554	0.546	0.571
4. A	0.702	0.448	0.294	0.471
4. B	1 not used	5 not used	0.506	0.617
5.A	0.662	5 not used	0.518	0.602
5.B	0.812	0.646	0.716	0.745
Calculate the Means A:	0,569	0,402	0,338	0,452
Calculate the Means B:	0,638	0,593	0,540	0,573

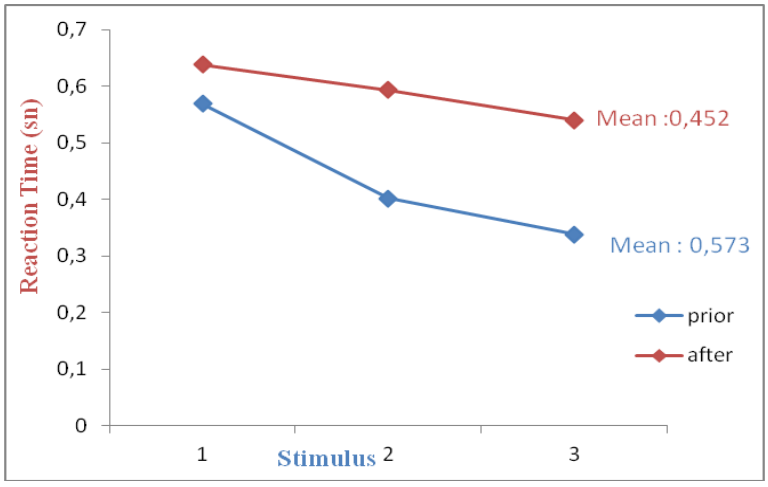


Figure 4.4.1 Graphic, for Reaction Time value obtained from first, fifth and tenth stimulus.

As seen in Figure 4.4.1, Reaction Time values were increased after the Hypoglycemia. The results were represented that hypoglycemia increases stimulus- response time and it makes difficult to learning.

Table 4.4.2 Calculate the variance and standard deviation for 5 subjects with data from Random Data

Subjects	**Mean Reaction time for Subjects** (X_j)	**Group Mean A,B** $(\bar{x})$	**Deviation** $(x_j - \bar{x})$	**Deviation2** $(x_j - \bar{x})^2$
Subject 1A	0.323	0,452	0,129	0,016
Subject 1B	0.446	0,573	0,127	0,016
Subject 2A	0.382	0,452	0,070	0,0049
Subject 2B	0.488	0,573	0,085	0,007225
Subject 3A	0.485	0,452	0,033	0,001089
Subject 3B	0.571	0,573	0,002	0,000004
Subject 4A	0.471	0,452	0,019	0,000361
Subject 4B	0.617	0,573	0,044	0,001936
Subject 5A	0.602	0,452	0,15	0,0225
Subject 5B	0.745	0,573	0,172	0,029584
	Sum the data for all students A = **Sum the data for all students B =**		$\sum_{j=1}^{n}(x_j - \bar{x})^2$	=0,04485 =0,054749
Variance (σ^2) =	**Multiply by 0.25 =**		$\frac{1}{n-1}$	=0,011 =0,0137
Standard Deviation =	**Take the square root of the variance A=** **Take the square root of the variance B=**			=0,0001 =0,0001

As seen in Table 4.4.2, the mean of the Reaction Time is relatively higher at the " after IHT test" phase for all patients, which confirms that there is imbalance of activity in ANS. As seen in Table 4.4.2, mean value of RT signals are higher at the “after the test” phase for all patients.

As seen in end of the Table 4.4.1, RT values decreased towards from Stimulus 1 to Stimulus 10 with learning process. These values increased in "after test" phase. As seen in end of the Table 4.4.1, RT values decreased towards from Stimulus 1 to Stimulus 10 with learning process. We were observed with this results that hypoglycemia has a negative impact on the learning. Upon reviewing Table 4.4.2, we notice that the mean of "prior to testing" phase minus the mean of the" after the test" phase estimated to be 0,121 second. All patients have delayed reaction times at " after the test" phase. When the results were analyzed statistically, we were concluded that hypoglycemia increases stimulus- response time and it makes difficult to learning.

DISCUSSION AND CONCLUSIONS

Hypoglycemia is medical emergency that necessitates quick diagnosis and treatment to preclude organ and brain injury. Especially nocturnal hypoglycemia may leads to sudden death, therefore early detection for Hypoglycemia has vital significance. In the studies conducted for early detection of hypoglycemia, researchers studied with the ECG signal.

In the literature, to enable detection of hypoglycaemia, the QT prolongation in the ECG signal is investigated. To this end, researchers have been developed decision algorithms for detect QT prolongation.

At the same time, in some studies, LF component and HF component of HRV signal obtained from ECG is investigated. Continuous non-invasive hypoglycemia monitor systems have been developed for early detection of hypoglycemia. This systems uses some physiological parameters; heart rate, QTc interval and skin impedance, breathing and heart pulse, sweating, skin temperature, motion & tremor.

In this study, Electrophysiological signals associated with Hypoglycemia was evaluated, in order to develop systems for early detection of Hypoglycemia. To this end, ECG, PPG and GSR signals were analyzed.

Firstly, in this study the changes in the hypoglycaemia- induced QT interval were shown (during IHT test).

It is evident from the results of this study that hypoglycaemia causes an increase of QTc. The computed hypoglycaemia- induced QTc is significantly different from the prior to testing.

During experimental hypoglycaemia QTc interval can raise by a mean 60–80 ms from baseline in healthy people and diabetic individuals outside autonomic neuropathy [73]. Nevertheless, hypoglycaemia in individuals with autonomic neuropathy results more less increase in QTc interval of nearly 25 ms, probably related to attenuated sympatho-adrenal responses in these patients [73].

In this study, during hypoglycaemia QTc interval can raise by a mean 76,4 ms from baseline in healthy people. Therefore, results supports data in the literature. In IHT test, the minimum BG target value is 40 mg / dL, but this value could not be obtained in all patients. Therefore, different symptoms and severity of Hypoglycemia was occurred in all patients.

Consequently, the rate of increase in QTc has been different each patient. The average maximum value of QTc was 725 ms during entire test and this value was very high. Thus the results from our study indicate that QT interval prolongation in patient might not be limited to low BG level, but is experienced also when the BG is declining after an insulin injection. We conclude that QTc can be used for early detection of Hypoglycemia.

It is known that the sympathetic activation increases during hypoglycaemia, as second feature, LF / HF ratio obtained from HRV nad PRV signal were investigated. PPG signal can be used as an alternative signal for estimating respiratory rate, heart rate and heart rate variability [74, 75].

In this study, we compared LF/HF ratio obtained from PRV and HRV during hypoglycemia. Sympathovagal balance is typically reflected in the ratio of LF/HF components.

In this study, for patient showed a divergence between LF/HF ratio obtained from HRV and PRV, but similarity between the LF/HF ratio obtained from HRV and PRV were not observed. All features obtained for all patients show that SNS was more active during hypoglycemia. Making this result more comprehensible is only possible by increasing the number of subjects participating in the study.

For all patient, the LF/HF ratio from PRV increased in comparison with the LF/HF ratio estimated from HRV. This increase could be related to noise of PPG signal, the tremor during hypoglycemia is affected PPG signal. During hypoglycemia, the mean NN interval decreased when compared to euglycaemia, which reflects an increased heart

rate during hypoglycemia. SDNN which is mathematically similar to the total power of the spectrum was found to be an order of magnitude higher during hypoglycemia in comparison with the euglycaemia.

As a measure derived from interval differences, we calculated RMSSD, the square root of the mean squared differences of successive NN intervals. The RMSSD which reflects high frequency variations in heart rate, decreased during hypoglycemia, indicating less parasympathetic activity during hypoglycemia. In the patient during hypoglycemia, the LF/HF ratio, regardless of the source, increased in magnitude. This increase reflects more sympathetic activity during hypoglycemia. But, results of the LF/HF ratio across HRV and PRV comparison indicate that there is not significant difference among different HRV and PRV. We conclude that LF/HF ratio obtained from HRV may a good indicator to evaluate hypoglycemia, but it should be tested for more patients for PRV.

In this study, was used Welc metod in analyzing the frequency axis. This method are used analysis in especially non stationary signals such as electrophysiological signals. More efficient methods such as wavelet transform is recommended in analysis of non-stationary signals for different studies. Investigate the effect of signal analysis methods in PRV analysis will be the subject of new studies.

Simplicity, mobility and comfort of PPG make it ideally suited non invasive monitoring of patients, especially at daily life, therefore in different studies, accuracy of PRV will be assessed as an estimate of HRV for early detection of hypoglycemia.

The most common feature used in GSR analysis is the mean amplitude value of the signal, namely "skin conductance level"**.** As seen in result, mean value of GSR signals are higher at the hypoglycemia for all patients. Glycemia was measured with glucometer every 10 minutes following the injection during this test. Therefore, GSR signal was first divided into 10-second long segments, and the mean values for each of these segments (seg_means) were calculated.

Mean value of seg_mean were increased proportionally with BG decrease. Skin conductance (GSR amplitude) and seg_mean are potentially good indicators to evaluate hypoglycemia. Therefore, GSR signal can be used for early detection of Hypoglycemia.

The mean of the Reaction Time is relatively higher at the after the hypoglycemia for all patients, which confirms that there is imbalance of activity in ANS, imbalance of hormonal system. RT values decreased with learning process. These values increased in after the hypoglycemia.

We notice that the mean of prior to hypoglycemia minus the mean of after the hypoglycemia estimated to be 0,121 second, all patients have delayed reaction times at after the hypoglycemia. At phase "prior to hypoglycemia" RT values were decreased from 0,569 to 0,452 seconds with learning process. The difference was 0.117 seconds. But, at the after hypoglycemia, this values were decreased from 0,638 to 0,573 seconds. The difference was 0.065 seconds. Learning time is reduced because all patients is now learned this test. This findings supports hypoglycemia increases stimulus- response time. and it makes difficult to learning.

We would like to underline following biomedical engineering contributions in our study:

1- We have recorded and analyzed several biomedical signals (ECG, PPG, GSR) at three phases during IHT test (before, during, and after). This comprehensive multiparameter signal analysis approach is "original" in terms of evaluation of electrophysiological counter-regulatory responses to hypoglycemia.

2- It is seen that increased sympathetic activation during hypoglycemia when analyzing the average of all features values .

3-QTc prolongation was demonstrated during hypoglycaemia. This findings supports to literature, therefore QTc can be used for early detection of Hypoglycemia.

4- LF/HF ratio across HRV and PRV comparison indicate that there is not significant difference among different HRV and PRV. LF/HF ratio obtained from HRV may a good indicator to evaluate hypoglycemia, but it should be tested for more patients for PRV.

5- The most obvious effect of hypoglycemia are observed on the GSR signals. in new studies, in order to develop systems for early detection of hypoglycemia should be used GSR signals.

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I worked in one of their research project entitled "Production of enzyme based compounds for pharmaceutical industry" which helped me learn and practice some basic molecular biology such as PCR, electrophoresis, digestion and ligation of DNA, transformation into *E.coli.,* and SDS Polyacrylamid gel electrophoresis. I also worked in other research project named "Investigation of biogeochemical and microbiological characteristics of acid mine drainage in Balikesir-Balya region by molecular biology techniques". 16S rRNA species analysis of collected sediment samples and oxidation analysis with *Acidithiobacillus thiooxidans* pure culture on sulfur metals such as sphalerite, pyrite, and galena were performed.

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I worked on scientific research project "Protein Engineering of Toluene Monooxygenases for bioremediation and biotechnology applications". In this project, some mutations were created on enzyme active and substrate canal region by saturation mutagenesis technique and mutant library were screened on selected substrates such as

toluene, nitrobenzene, naphthalene for increased activity. I also worked on other project named “A novel beta-lactamase from subsurface sediments”. Characterization of novel β-lactamase gene of *Bacillus licheniformis* by MIC test was performed.

Technical Skills:

Microbiological technology, isolation of genomic/chromosomal DNA, extraction and manipulation of plasmid DNA, isolation of RNA, cDNA synthesis, molecular cloning, gel electrophoresis of DNA, SDS-PAGE, western blot, directed evolution techniques such as saturation mutagenesis.

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