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Glycopenia-induced sympathoadrenal activation in Diabetes mellitus and uncontrolled arterial Hypertension

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Dedication

To my wonderful boys
Alexei Ayomide and Michel Adeoluwa Adeagbo

Abstract

Glucose monitoring helps to individualize the management of insulin-treated patients and good glycemic control is strongly associated with better outcomes for patients with T1DM and T2DM. Despite advancements in diabetes technology, hypoglycemia still plays a limiting factor preventing the attainment of the desired glycemic control.
This present study investigated a possible association between hypoglycemia and arterial hypertension with a primary focus on the role of the sympathoadrenal system.
In a prospective, observational cohort study involving 65 insulin-treated diabetes patients (type 1, type 2, type 3c), we hypothesized that hospitalized insulin-treated diabetes patients with hypertensive crisis have more hypoglycemic episodes than counterparts without hypertensive crisis on admission.
The study patients were divided into three subgroups, Group 1 patients in the target group presented with hypertensive crisis as cause of admission, and the negative control patients in Group 2 had neither hypertensive crisis nor hypoglycemia on admission, Group 3 with hypoglycemia on admission, served as the positive controls. All patients were subjected to continuous flash glucose monitoring using FGM sensors (FreeStyle Libre, Abbott Diabetes Care, Abbott GmBH, Wiesbaden, Germany), to a 24-hour ambulatory blood pressure monitoring, 24-hour electrocardiogram recordings, and laboratory tests including plasma catecholamines.
53 patients, thereof 19 Group-1, 19 Group-2, 15 Group-3 patients, completed this study. Group-1 patients had the highest maximum systolic blood pressure, a higher daily cumulative insulin dose at admission, a higher body-mass index, and higher plasma norepinephrine than control patients of Group 2. Group-3 patients had more documented hypoglycemic episodes (0.8 ± 0.5 per 24 hours) than Group-2 patients (0.2 ± 0.3 per 24 hours), however, they were indifferent to the ones in Group-1 patients (0.4 ± 0.4 per 24 hours). Plasma norepinephrine and mean arterial blood pressure were not different between Group-1 and Group-3 patients, though higher than in Group-2 patients. By discharge, the daily cumulative insulin dose decreased in Group-1 (-18.4 ± 24.9 units) and Group-3 patients (-18.6 ± 22.7 units) but remained unchanged in Group-2 patients (-2.9 ± 15.6 units). In conclusion, for Group-1 patients, the results are consistent with an association between hypoglycemic events and uncontrolled hypertension.

Referat und bibliographische Angaben

Kontinuierliche Gewebe-Glukoseüberwachung trägt dazu bei, das Management von insulinbehandelten Patienten zu individualisieren. Eine damit erreichte, bessere glykämische Kontrolle ist mit besseren Ergebnissen für Patienten mit T1DM und T2DM in Bezug auf Folgeerkrankungen, Sterblichkeit, Krankenhausaufnahmen assoziiert. Trotz technologischer Fortschritte, spielt Hypoglykämie nach wie vor eine begrenzende Rolle, der das Erreichen der gewünschten glykämischen Kontrolle verhindert. Die vorliegende Studie untersuchte einen möglichen Zusammenhang zwischen Hypoglykämie und arterieller Hypertonie mit einem Schwerpunkt auf der Rolle des Sympatho-adrenalen Systems.

In einer prospektiven, Beobachtungs-und Kohortenstudie mit 65 insulinbehandelten Diabetes-Patienten (Typ 1, Typ 2, Typ 3c), stellten wir die Hypothese auf, dass hospitalisierte insulinbehandelte Diabetes-Patienten mit hypertensiver Krise bei Krankenhausaufnahme mehr hypoglykämische Episoden haben als diejenigen, die keine hypertensive Krise bei der Krankenhaus-Aufnahme hatten. Die Studienpatienten wurden in drei Untergruppen aufgeteilt, wobei Patienten der Gruppe 1 in der vorgestellten Zielgruppe mit hypertensiver Krise bei Aufnahme waren, Negativkontrollpatienten in Gruppe 2 hatten weder eine hypertensive Krise noch eine Hypoglykämie bei Aufnahme, und Patienten der Gruppe 3 hatten eine nachgewiesene, symptomatische Hypoglykämie bei Aufnahme. Gruppe 3, diente als die Positivkontrolle. Alle Patienten erhieilten eine kontinuierliche Gewebe-Glukoseüberwachung mittels flash glucosemonitoring (FGM) (intrakutane FreeStyle Libre-Sensoren, Abbott Diabetes Care, Abbott GmBH, Wiesbaden, Deutschland), eine 24-Stunden ambulante Blutdruckmessung, ein 24-Stunden Elektrokardiogramm und Laboruntersuchungen einschließlich Plasma-Katecholamine.

53 Patienten, davon 19 Patienten der Gruppe 1, 19 Patienten der Gruppe 2 und 15 Patienten der Gruppe 3, schlossen diese Studie ab. In Gruppe 1 hatten die Patienten den höchsten maximalen systolischen Blutdruck, eine höhere tägliche kumulative Insulindosis bei der Aufnahme, einen höheren Body-Mass-Index und einen höheren Plasma-Noradrenalin-Wert als die Kontrollpatienten von Gruppe 2. Patienten der Gruppe 3 hatten mehr dokumentierte hypoglykämische Episoden (0.8 ± 0.5 pro 24 Stunden) als die Patienten der Gruppe 2 (0.2 ± 0.3 pro 24-Stunden), jedoch waren sie den Patienten der Gruppe 1 (0.4 ± 0.4 pro 24-Stunden) gegenüber statistisch nicht unterschiedlich. Plasma-Noradrenalin und der mittlere arterielle Blutdruck waren bei Patienten der Gruppe 1 und Gruppe 3 nicht unterschiedlich, wenn gleich jeweils höher als bei Patienten der Gruppe 2. Bei Entlassung, sank die tägliche kumulative Insulindosis bei Gruppe-1 (-18.4 ± 24.9 units) und bei Gruppe–3 Patienten (-18.6 ± 22.7 Einheiten), blieb aber beiden Patienten der Gruppe 2 unverändert (-2.9 ± 15.6 Einheiten).

Als Schlussfolgerung, bei Patienten der Gruppe 1 sind die Ergebnisse mit einer Assoziation zwischen hypoglykämischen Ereignisse und unkontrolliertem Bluthochdruck als Folge einer hypoglykämie-vermittelten Sympatho-adrenalen Aktivierung vereinbar.

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Abbreviations

ABPM	Ambulatory blood-pressure monitoring
AKI	Acute kidney injury
BG:	Blood glucose
BP:	Blood pressure
CAN:	Cardiovascular autonomic neuropathy
CGM:	Continuous glucose monitoring
CI:	Confidence Interval
CKD:	Chronic kidney disease
CNS:	Central nervous system
DBP.	Diastolic blood pressure
DDD	Defined daily dose
DEGS:	Studie zur Gesundheit Erwachsener in Deutschland
ECG	Electrocardiogram
eGFR	estimated Glomerular filtration rate
FGM:	Flash Glucose Monitoring
HAAF:	Hypoglycemia assosciated autonomic failure
HbA1c	Glycated hemoglobin A1c
HRV:	Heart rate variability
HU:	Hypoglycemia unawareness
ISF.	Intersitital subcutaneous fluid
IU	Insulin units including units of insulin analogues

n	number
ns	not significant
Rt-CGM:	Real-time CGM
SBP:	Systolic blood pressure
SDNN:	Standard deviation of the NN-Interval
SMBG:	Self-monitoring of blood glucose
SNS :	Somatic nervous system
T1DM:	Type 1 Diabetes Mellitus
T2DM.	Type 2 Diabetes Mellitus
TIR:	Time in range

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1 Introduction

1.1 Epidemiology of diabetes mellitus

Diabetes is a complex heterogeneous group of metabolic diseases characterized by chronic hyperglycemia due to an impairment of insulin secretion, insulin action, or both [1]. The disease burden related to diabetes is high and rising in every country, fueled by the global rise in the prevalence of obesity and unhealthy lifestyles [2]. Globally, about 1 in 11 adults worldwide now have diabetes mellitus, 90% of whom have type 2 Diabetes Mellitus(T2DM) [3]. The nationwide, population-based German National Health Interview and Examination Survey for Adults (DEGS1, Studie zur Gesundheit Erwachsener in Deutschland) of the Robert Koch-Institute (RKI) estimated the overall prevalence of type 2 diabetes in the whole German population between 18 and 79 years of age at a total of 7.4 percent [4]. Multiple genes and environmental factors contribute to the development of this spectrum of diseases [5]. The onset of type 1 diabetes for example has been observed over the years to be predominant in young people and is due to the selective autoimmune destruction of the pancreatic beta-cell often mediated by immune mechanisms, leading to insulin deficiency [6]. Type 2 diabetes on the other hand is due to a progressive insulin secretory defect on the background of insulin resistance [7]. It is commonly associated with metabolic syndrome and obesity remains an important factor associated with the increased prevalence of type 2 diabetes. The age at onset of T2DM has been falling in recent years, and the disease is now common among teenagers and young adults [5]. The hallmark of diabetes is the uncontrolled hyperglycemia resulting in symptoms such as polyuria, polydipsia, weight loss, polyphagia, blurred vision, and susceptibility to certain infections. Acute life-threatening consequences of uncontrolled diabetes are hypoglycemia, hyperglycemic crisis with ketoacidosis, or the non-ketotic hyperosmolar syndrome [2]. Long-term complications of diabetes include retinopathy with a potential loss of vision; nephropathy leading to renal failure; peripheral polyneuropathy with a risk of foot ulcers, amputations, and Charcot's joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [1]. Despite multifactorial lifestyle interventions along with intensive therapies to control blood pressure, lipids, antiplatelet therapy, and glycemic therapy, diabetes still accounts for the most important cardiovascular risk factor and the leading cause of chronic kidney disease and end-stage renal disease worldwide [8].

1.2 The impact of hypoglycemia in diabetes

Hypoglycemia in diabetes is mostly the effect of the complex interplay of relative or absolute therapeutic insulin excess and compromised physiological and behavioral defenses against decreasing plasma glucose concentrations [9, 10]. When defining hypoglycemia, an individualized approach is usually recommended, a glucose level of below 3, 9 mmol/l is the lower limit of the physiologic fasting range in non-diabetics, referred to as the alert level when the counter-regulatory responses are thought to come into play [6]. In fact, counterregulatory physiological responses come into play even earlier, if the decrease of tissue glucose is too steep. This observation has been coined the pseudohypoglycemic event [11]. Hypoglycemic episodes can, therefore, be defined as instances of defective counterregulatory hormone regulation, elevated circulating insulin levels due to excessive secretion of insulin or therapeutic hyperinsulinemia; accompanied by the failure to employ gluconeogenic substrates adequately [12]. Overall, hypoglycemia is less frequent in type 2 diabetes than in type 1 diabetes. However, as the patients approach the insulin-deficient end of the spectrum due to progressive beta-cell failure in long-standing advanced type 2 diabetes, the frequency and burden of iatrogenic hypoglycemia progressively increase, sharing similar trends with T1DM [13]. Most hypoglycemic episodes tend to be silent and undocumented, mostly occurring outside the confines of the healthcare settings. The ability of the patients to recognize the symptoms, therefore, depends on the adequacy of the hypoglycemia awareness and the effectiveness of the counterregulatory systems [14]. Hypoglycemia causes recurrent morbidity in most people with T1DM and many with advanced T2DM and sometimes has fatal consequences. Randomized clinical trials have proven that the use of intensive insulin therapy to target normal glycated hemoglobin A1C (HbA1c) did not offer cardiovascular benefits and has been shown to even increase the cardiovascular mortality for T2DM patients. The reason for this lack of benefit or even excess mortality may relate to hypoglycemia and its sequelae [15, 16]. Based on these observations, the glycemic goal of patients tends to be individualized and is therefore defined as the lowest achievable A1C-level that preserves the symptoms of hypoglycemia awareness while minimizing the occurrence of hypoglycemic episodes [9, 17]. The risk-reduction of hypoglycemia while maintaining an upside of good glycemic control involves adequate patient education and empowerment, frequent glucose monitoring, and flexible individualized therapy regimes that improve patient motivation and compliance [18].

1.3 Clinical manifestations of Hypoglycemia

Hypoglycemia causes the activation of the autonomic nervous system involving the adrenomedullary, sympathetic neural, and parasympathetic system. Autonomic or neurogenic symptoms tend to occur at plasma glucose concentrations below 70 mg/dL (3.9 mmol/L). The symptoms observable are non-specific and are mainly caused by the sympathoadrenal activation and are mediated by norepinephrine, acetylcholine, and epinephrine. The autonomic symptoms that emerge can be divided into adrenergic symptoms that include palpitations, tachycardia, anxiety, tremors; and cholinergic symptoms that include sweating, warmth, nausea, and hunger. The neuroglycopenic symptoms tend to occur as a direct result of brain glucose deprivation, usually at

plasma glucose concentrations of approximately 50 mg/dL (2.8 mmol/L) or less. The symptoms manifested include weakness, behavioral changes, visual changes, confusion, dysarthria, dizziness, amnesia, lethargy, seizure, and even loss of consciousness [19, 20, 21].
The overall disease burden of recurrent hypoglycemia is dependent on the duration of diabetes, hypoglycemia unawareness, high glycemic variability, and previous history of frequent hypoglycemic events. The other risk factors of recurrent hypoglycemia include concurrent comorbidities like chronic kidney diseases, long-standing chronic heart failure, and critical illness that require intensive care treatment [22].

1.4 The role of the counterregulatory systems in hypoglycemia

The Somogyi effect, also known as the "chronic Somogyi rebound," or "posthypoglycemic hyperglycemia," was first discovered in the 1930s by Dr. Michael Somogyi [23]. He proposed that when blood glucose levels drop too low during the late evening, activation of counterregulatory hormones such as adrenal catecholamines, corticosteroids, growth hormone, and glucagon may be observed, leading to activation of gluconeogenesis and resultant hyperglycemia in the early morning [24]. The activation of the sympathoadrenal system during insulin-induced hypoglycemia has also been previously demonstrated to accelerate blood pressure readings [25]. Feldman-Billiard and his co-workers in 2010 examined a total of 22 patients, mostly on antihypertensive medications with type 1 (n = 4) or type 2 (n = 18) diabetes using a continuous glucose monitoring system (Medtronic) and simultaneous ambulatory BP measurements in a small cohort study. Hypoglycemic events were found to be associated with a significant median 23% rise in systolic BP (SBP) and a non-significant 8% rise in diastolic BP with no concurrent increase in heart rate [26]. A close temporal relation between hypoglycemia and BP increase was found in this study. It was further hypothesized that hypoglycemia-induced hypertension could be amplified in patients experiencing frequent and severe hypoglycemia, which may increase the risk of a broad spectrum of hypertension-related complications [26].

1.4.1 Physiologic defenses against hypoglycemia

Insulin is secreted from pancreatic islet β-cells into the hepatic portal vein and leads to inhibition of lipolysis, suppression of glucagon secretion, limitation of gluconeogenic precursor (e.g., lactate, amino acids, glycerol) flux from muscle and fat to the liver (and kidneys), and the CNS-mediated activation of the parasympathetic outflow [9, 17]. Also, in the CNS, insulin has been shown to exert an anorexigenic effect [27]. Insulin and glucagon secretion are normally regulated primarily by changes in glucose concentrations within the pancreatic islets and secondarily by autonomic mechanisms.When blood glucose levels fall, there is a decrease in pancreatic β-cell insulin secretion and a concomitant increase in pancreatic α-cell glucagon secretion [28]. Additionally, the sympathoadrenal system triggers an increase in adrenomedullary catecholamines

in response to falling blood glucose levels. Epinephrine and norepinephrine released can rapidly stimulate hepatic glycogenolysis, hepatic- and renal gluconeogenesis, limit glucose clearance by insulin-sensitive tissues such as muscle, mobilize gluconeogenic precursors from muscle (lactate, amino acids) and fat (glycerol) to the liver and kidneys, and also suppress insulin secretion. The adrenomedullary response is mostly critical in T1DM where a loss of glucagon response occurs within a few years of diabetes onset [19, 28, 29]. Cortisol and growth hormone in contrast to glucagon and epinephrine have a delayed form of response to hypoglycemia, usually occurring after several hours [30, 31].The mechanisms involved during the physiological response to hypoglycemia and the central role of the counterregulatory systems is illustrated below in Fig. 1.1

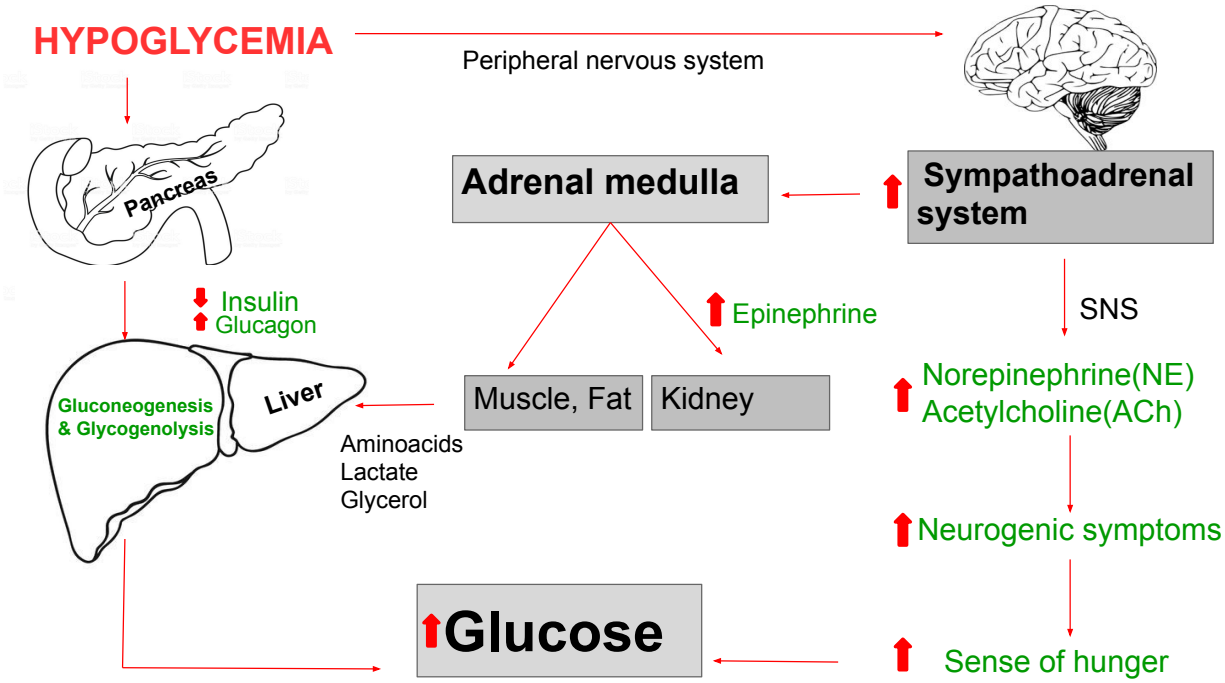

Figure 1.1: Diagrammatic representation of the central role of the counterregulatory system during the physiological response to hypoglycemia,SNS:sympathetic nervous system [32].

1.4.2 Compromised hypoglycemia-counterregulation in diabetics

Diabetic autonomic neuropathy as part of diabetic neuropathies and hypoglycemia unawareness represent serious complications of diabetes mellitus that may affect the hypoglycemia responsiveness of the autonomic nervous system. When the counterregulatory systems are defective and fail to abort an episode of hypoglycemia, there may be a failure to sense a significant drop in blood glucose levels, and/or a failure to exert autonomic warning symptoms known as the clinical syndrome of hypoglycemia unawareness (HU). This may cause dangerous levels of hypoglycemia resulting in neuroglycopenic symptoms and its sequelae. [17, 32, 33].

1.5 Continuous Glucose Monitoring Systems

Glucose monitoring is the hallmark of diabetes management, especially for insulin-treated patients. Continuous glucose monitoring may have a greater impact on diabetes management than optimized insulin delivery [34]. The Diabetes Control and Complications Trial (DCCT) Research Group and the UK Prospective Diabetes Study (UKPDS) Group demonstrated that a lowering of HbA1c mitigates the onset and progression of diabetic microvascular complications. Glycated hemoglobin (HbA1c), the gold standard for assessment of glycemic control, is however unable to reflect hypoglycemic risk or increased glucose variability [35, 36]. Self-monitoring of blood glucose (SMBG) through test strips using the finger-prick method multiple times a day is the traditional norm. It is estimated that a single patient carries out about 2000 SMBG per year on the average [37]. Apart from the psychological effects of frequent SMBG , induced pain, accumulated trauma to the fingers, and fear of hypoglycemia between measurements, the SMBG also provides limited information about the real-time blood glucose concentrations and fails to expose rapidly changing blood glucose levels. Episodes of hyperglycemia and hypoglycemia, might therefore be missed, and not factored into treatment decisions [38]. Continuous glucose monitoring (CGM) systems due to major advancements in diabetes technology are gradually becoming the new standard and represent a powerful tool for individualizing diabetes care. The evolution of the CGM systems since 1999 to facilitate self-management has shown to improve glucose control and reduced exposure to hypoglycemia despite insulin therapy intensification [39]. Currently, there are two types of CGM systems: real-time CGM (rt-CGM) and flash glucose monitoring (FGM).The flash glucose monitoring (FGM) system, was introduced in 2014 and it is a novel interstitial flash glucose monitor that uses a wired enzyme technology ((osmium mediator and glucose oxidase enzyme co-immobilized on an electrochemical sensor) to detect glucose levels in the interstitial subcutaneous fluid [40]. The use of subcutaneous interstitial fluid for glucose monitoring can provide closeness to the vasculature thereby providing relatively accurate information with small discrepancies compared to capillary blood glucose levels in real-time [41, 42]. Figure 1.2 shows the summary of the currently available rt-CGMS and FGM devices with special references to their properties and differences in modes of action.

1.5.1 Differences between the blood glucose- and the interstitial subcutaneous glucose monitoring

The CGM devices offer dynamic continuous measurements with a better overview of the glucose excursions of diabetic patients. Over the years, the physiologic differences between the blood glucose monitoring and the interstitial glucose monitoring resulting in discrepancies in both measurements (blood and subcutaneous) have been reported [44, 45, 46]. It is important to note that the measurements take place in different body compartments that follow different dynamics. The graphical illustration of the physiological delay between the capillary blood and the interstitial space is demonstrated in Fig. 1.3. G1 represents the glucose concentration in capillary blood and G2 represents the glucose concentration in intersitialfluid(G1). ΔG is the difference between

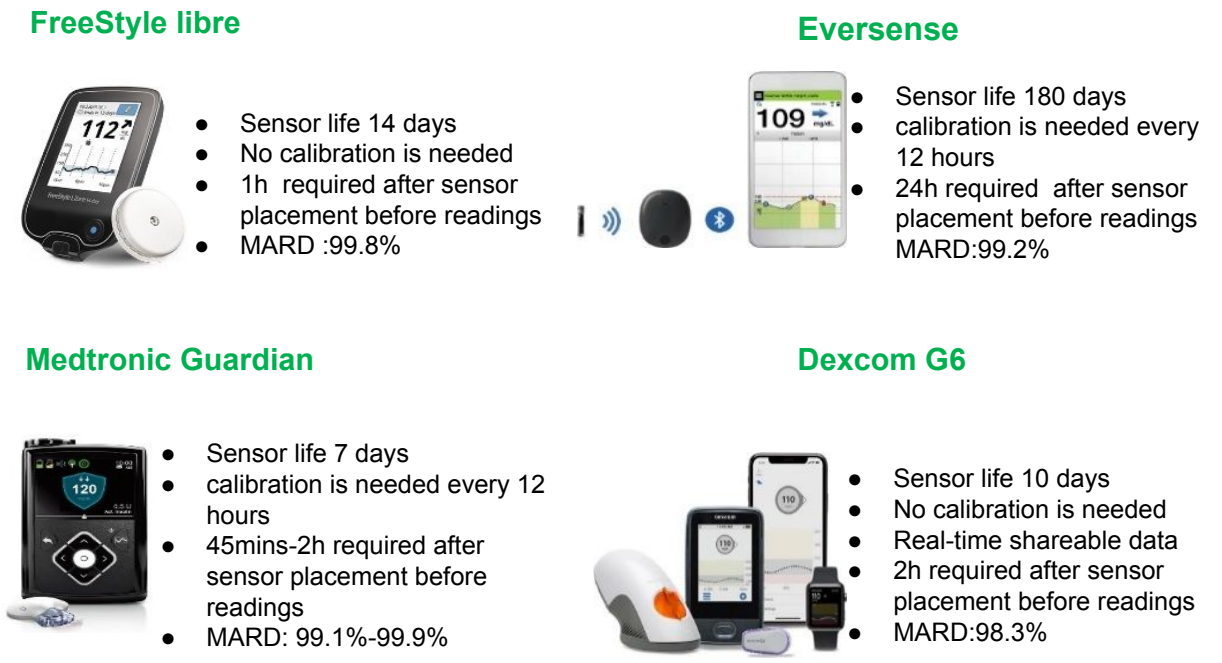

Figure 1.2: Overview of the currently available rt-CGMS and FGM devices with special references to their properties and differences in modes of action [43].

G1 and G2. During phases of stable glucose levels,the measurements are closely similar, drastic increments or decrements as seen in Fig.1.3 will cause more discrepancies in measurements as it takes time for glucose to equilibrate across both compartments. This time-lag, ΔT is defined as the physiologic interstitial fluid glucose delay, that exists between the blood measurement and the interstitial subcutaneous fluid measurement [44, 45]. During steady-state conditions, this time lag is relatively negligible, making the blood measurements and the subcutaneous measurements more comparable. The steady-state time lag has been proposed by several authors to be between 5 -10min [47, 38].

1.5.2 Description of flash continuous glucose monitoring

The FreeStyle Libre system uses two components: a disposable sensor that is inserted into the user's upper arm and a separate hand-held touchscreen reader device used to scan and retrieve glucose readings. The use of mobile phone devices serves as a practical alternative for the reader devices and with the aid of the FreeStyle Libre software, a detailed summary of glucose reports can be generated [40]. The sensor which is usually placed on the back of the upper arm can be worn for up to 14 days. Following insertion, the sensor requires a 1-h warm-up period before glucose data are available. The sensor continuously samples and measures interstitial glucose levels, generating a new glucose value each minute [48, 49]. The current glucose concentration, trend arrows, and the most recent 8 h of sensor glucose readings are usually displayed in 15-min intervals. Patients are advised to ensure at least an 8-hourly scan to ensure continuous measurements for 24 hrs. The reader can store historical data for up to 90 days. Retrospective

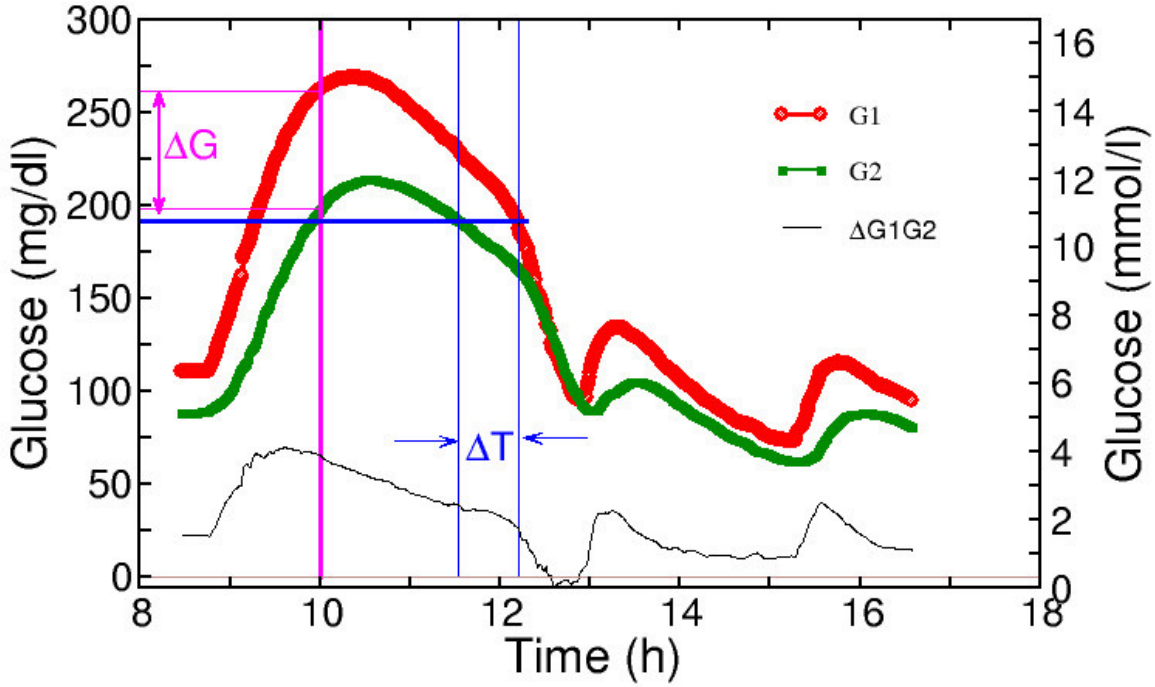

Figure 1.3: Graphical illustration of the differences between glucose concentration in blood and intersitialfluid [43].

analysis of CGM data include estimated glycosylated hemoglobin (HbA1c), monthly-pooled glucose profiles, glycemic variability, glucose average, number and duration of hypoglycemic events, daily profiles and information on the number of scans. This information enables patients and their clinicians to identify glycemic patterns that support and facilitate informed therapy decisions. Unlike rtCGM systems, the FreeStyle Libre requires users to manually scan their sensors. The Freestyle Libre 2 recently developed in October 2018 now offers a low-glucose real-time alarm as a variable option. This is particularly beneficial in patients who are unaware of their hypoglycemic episodes, especially during sleep [50].

1.5.3 Current evidence of the efficacy of the flash glucose monitoring device

There are 3 major randomized clinical trials for the FreeStyle Libre device. The first study is the IMPACT trial, a randomized controlled multicenter trial that enrolled a total of 328 adult participants across 23 European diabetes centers between 2014 and 2015. The patients had well-controlled [HbA 1c$\leq$59 mmol/mol (7.5%)], Type 1 diabetes, and an intact awareness of

hypoglycemia. After the screening and baseline phase, 120 participants were randomly assigned to the intervention group (Freestyle group) and 121 to the control group (self-monitoring of blood glucose SMBG). FreeStyle Libre use was associated with a 38% reduction in time spent in hypoglycemia (<3.9 mmol/l) without deterioration in the HbA1c levels [39].

The FreeStyle Libre has also been assessed in people with Type 2 diabetes on intensive insulin therapy in a large multicentre European study of 224 participants at 26 European diabetes centers in 2016. Despite less frequent sensor scans than were seen in the IMPACT study (8 vs 15 per day), the time spent in hypoglycemia (<3.9 mmol/l) was reduced by 43% with the HbA 1c remaining unchanged. Treatment satisfaction was higher in users and no device-related serious adverse events were reported [41].

1.5.4 Advantages of the flash glucose monitoring system

The FreeStyle Libre CGM is a user friendly device and its installation is quite easy. Compared to other CGMs, the Freestyle libre has a 14-day longevity making the cost relatively minimal [51]. There are no parts of the system that need to be sanitized and reused among patients thereby preventing the risks of patient-to-patient transmission of infections. FreeStyle Libre allows valuable glucose data to be collected even if patients are inconsistent with their blood glucose monitoring. The device is factory calibrated and does not need finger-stick glucose calibration during use. The proprietary manufacturing process minimizes sensor –to- sensor variation [52].

1.5.5 Limitations of the flash glucose monitoring system

Hypersensitivity reactions such as allergic contact dermatitis, attributed to isobronyl acrylate, a potential allergen in the protective plastic shell around the needle of the Freestyle sensor has been reported [53]. As a further limitation, sensors sometimes tend to fall off before the intended 14-day period of use. To avoid this, some patients use special adhesive wipes like skin-tac, armbands to secure the sensors in place properly [53]. There is usually an approximately 5-10 minute lag (physiological lag time) between capillary measurements and interstitial subcutaneous fluid measurements as previously explained, posing a limitation in the direct comparison of the CGM-generated values and the traditional SMBG [38]. The time lag is relatively dependent on the metabolic state of the individual with increased differences observable during exercise and post-prandial states. As a technical limitation, CGM devices detect severe hypoglycemia less accurately and tend to underestimate hyperglycemia. In cases of extreme glucose excursions, patients are therefore generally advised to confirm subcutaneous measurements with SMBG. [54]. Of Note,dehydrated patients may also not get accurate readings from this measuring system [40].

2 Aims of the Study

Based on previous work done showing a possible association between blood pressure elevation and hypoglycemia,this current study aimed to investigate a possible correlation between hypoglycemic episodes and arterial hypertension with a primary focus on the role of the sympathoadrenal system.
We hypothesized that insulin-treated patients hospitalized for hypertensive crisis have a propensity for more hypoglycemic episodes in prospective FGM Monitoring than counterparts hospitalized for other reasons. As a secondary hypothesis, plasma norepinephrine concentrations were expected to be elevated both in insulin-treated diabetes patients with hypertensive crisis on admission and in diabetes patients who presented on admission with hypoglycemia.

3 Materials and Methods

3.1 Subjects and Methods

The Glycopenia-Hypertension Study is a prospective observational and explorative cohort study that tried to determine a possible relationship between hypertensive crisis and the counter- regulatory responses during hypoglycemia in diabetics with the aim of generating a hypothesis. Insulin-treated diabetes patients (type 1, type 2, type 3c) , hospitalized in the University Hospital Halle (Saale) between 1.6.2017 and 31.12.2019 were screened and enrolled for participation based on the inclusion and exclusion criteria. The number of recruited study participants was limited by in- and exclusion criteria and by the scheduled time frame of study. Based on the nature of the clinical study, an observational and explorative study carried out for the purpose of hypothesis generation, therefore a power calculation for study size was not possible. The attending staff physicians directly involved in the clinical management of the patients did not participate in the study. Thus all decisions made in study patients reflected the staff physician‘s clinical judgement and were not influenced by the organizers or by persons conducting this study. All patients provided written informed consent. The independent ethics committee of the Medical Faculty of the Martin-Luther University Halle-Wittenberg approved this study protocol (Study number 2017-28). Data acquisition was performed according to the principles of the Declaration of Helsinki and Good Clinical Practice (E6, revision 2) from 2015.

3.2 Study design

Out of the 65 screened patients, 6 patients withdrew their consents, the remaining 59 recruited patients were divided into 3 groups, Groups 1, 2 and 3. After the procedure of screening and study enrolment, the patients received flash glucose monitoring devices, which were used to closely determine the occurrence of hypoglycemic events over a period of 5-10 days. The patients in Group 1 (target group) were insulin–treated diabetics (Type 1, 2, 3 c) who presented with hypertensive crisis without hypoglycemia at the time of admission and did not have any of the underlisted exclusion criteria. The patients in Group 2 (negative control group) were defined as insulin-treated diabetics who did not have any hypertensive crisis or hypoglycemia at the time of admission, or any of the underlisted exclusion criteria. These patients were admitted to the hospital due to other medical reasons. The patients in Group 3 (positive control group) were defined as insulin-treated diabetics who presented with hypoglycemia at the time of admission, and did not have any of the underlisted exclusion criteria.

3.2.1 Inclusion criteria

- Insulin-treated diabetes patients (type 1, type 2 or Type 3c Diabetes mellitus), diagnosed for at least one year before study enrollment,
- Age: 18 - 99 years
- Male or female

Cohort specific inclusion criteria:

- Group 1: hypertensive crisis at admission (systolic blood pressure > 180 mmHg)
- Group 2: absence of hypertensive crisis or symptomatic hypoglycemia at admission.
- Group 3: symptomatic hypoglycemia at admission

3.2.2 Exclusion criteria

- Age below 18 years or more than 99 years
- An active tumour disease or curative care within 5 years, Pregnancy or women with child-bearing potential with no safe forms of contraception,
- Severe pain (visual analogue scale from 1-10: >3),
- known secondary cause of arterial hypertension,
- Septicaemia,
- Allergies to adhesives, inability to use FGM
- Current use of glucocorticoids,
- Psychiatric disorders and all forms of dementia with lack of ability to provide an informed consent,
- Stage-5 chronic kidney disease (defined by estimated GFR < 15ml/min)
- Acute kidney injury (AKI) with need for renal-replacement therapy
- Acute or chronic heart failure (New York Heart Association class higher than 2).

3.3 Study visits

The standard study visits (V's) are scheduled during the period of hospital admission as illustrated in Fig. (3.1).

- **Visit 1(V1): Study visit within 24h after admission.**
  During the V1, an informed consent taking was performed. This was followed by study recruitment and clinical assessment of the study patients, involving history taking and clinical examination. After patients received instructional materials and behavioural counseling regarding diabetes care, the certified diabetes care specialists performed the flash glucose monitor sensor placement (FreeStyle Libre, Abbott Diabetes Care, Abbott GmbH, Wiesbaden, Germany). The patients also received the 24h -ABPM and the 24h Holter ECG monitoring devices.

- **Visit 2 (V2): Study Visit within 48h after admission.**
  The blood samples to determine the levels of catecholamines and cortisol were taken using standard defined methods further explained below (see section on laboratory measurements).All other required laboratory values were extracted from the routine laboratory work-up tests carried out by the attending physicians. The 24h ABPM and Holter- ECG were removed and analyzed. The patients were asked if there were any challenges with the monitoring devices. The sensor usage was assessed. The Amedtec ECG Pro V. 4.80.006 software program was used in the analysis of the obtained blood pressure and ECG measurements and the acquired data was stored anonymously in Excel format.

- **Visit 3 (V3 or Closing visit): 5d- 7d but can be up to 14d after V1 or prior to discharge:**
  The FGM sensor was removed, Data was retrieved and analyzed. Concomitant medications including daily cumulative insulin dose and laboratory parameters including serum creatinine, estimated glomerular filtration rate (eGFR), if applicable, were recorded. In case of an evolving AKI as shown by an increase (>0.3 mg/dL) of serum creatinine by discharge, eGFR was not calculated. In case of an AKI prior to hospitalization as shown by a decrease (>0.3 mg/dL) of serum creatinine by discharge or in case of no change (>0.3 mg/dL) of serum creatinine by discharge, eGFR at discharge was provided.

- **Unscheduled visits:**
  This was also carried out regularly to ensure appropriate usage of the devices and to inquire challenges or possible adverse effects. The unscheduled visits were done at the discretion of the investigator.

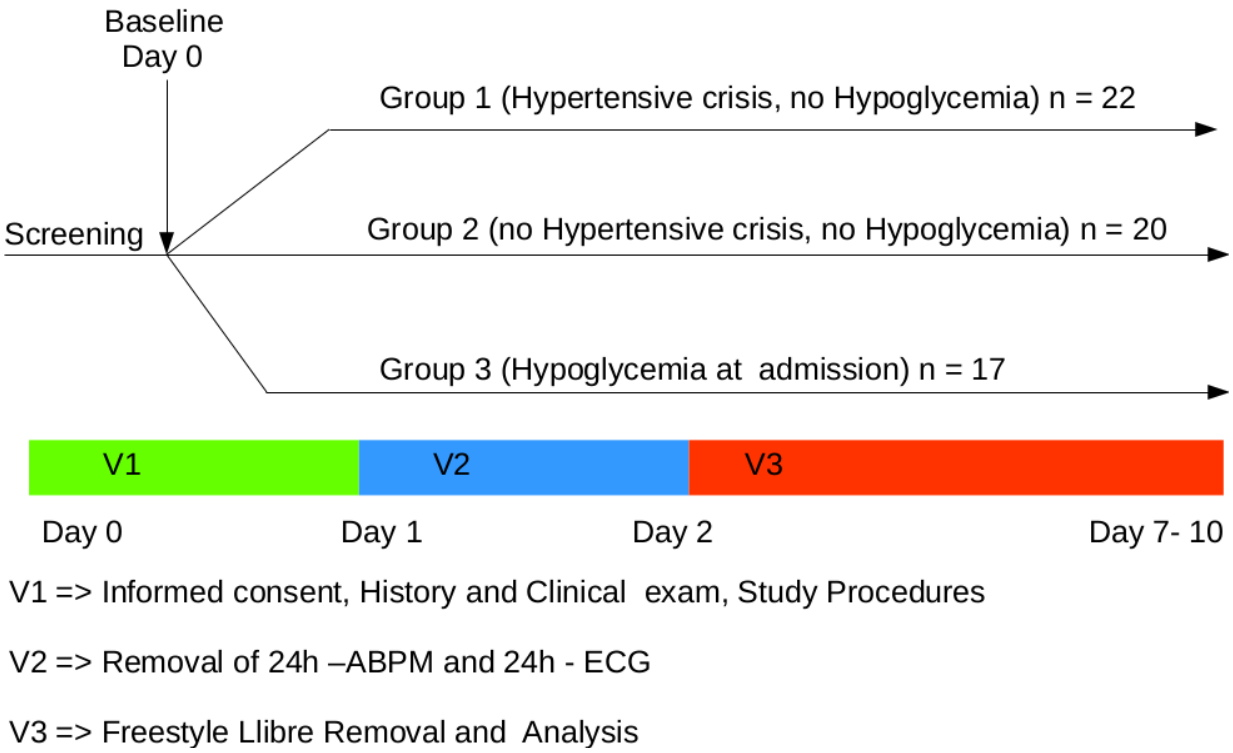

Figure 3.1: Flow chart illustrating the standard study visits (V1,V2 and V3) scheduled during the period of hospital admission

3.4 Study procedures

The laboratory investigation was carried out during the study visit 1. In order to avoid multiple blood sample takings, the laboratory workup was done simultaneously with routine laboratory tests carried out by the prescribing physicians. The blood samples for the catecholamines was taken from a freshly inserted intravenous cannula. In order to avoid false positive values of the stress hormones, the patients were required to lie down in a supine position, avoiding all forms of physical activity or agitation for a period of about 30 mins. Using specialized tubes anticoagulated with EGTA, the blood samples were immediately transferred to the central laboratory in chilled boxes for centrifugation. The EGTA-Plasma was frozen and transferred to Amedes Science Laboratories (external laboratory) for the final analysis. Samples for the determination of cortisol were taken first in the morning in sodium-lithium-heparin tubes and the analysis was determined using immunoassay systems. All others laboratory values such as ,creatinine, urea, plasma glucose and HbA1c was carried out at the clinical chemistry department of the Universitätsklinikum, Halle using standardized and validated procedures in accordance with the good laboratory practice

3.4.1 General description of the Freestyle Libre Sensor placement

A certified diabetes care specialist was at the bedside to train all the patients on the FreeStyle Libre technology. The patients were educated in detail on how to independently use the measur-

ing devices. The sensor placement was carried out by the specialized healthcare instructor. The method of placement was carried out as recommended by the manufacturing company, Abbott Diabetes Care Inc.

- The sensor pack and the sensor applicator is firstly confirmed to have the same code. The area of skin, usually behind the upper arm will be disinfected with an alcohol wipe.
- Areas with stretch marks, lumps and scars are usually avoided. Once the area of skin is dry, the sensor pack is opened. Using a hard surface the sensor applicator is pressed firmly onto the cap until an audible click is heard. The sensor applicator now contains a filament and this is pressed firmly on the disinfected skin. The sensor applicator is then removed leaving the sensor firmly attached to the skin
- If bleeding occurred, the sensor is immediately replaced and inserted at a different site.
- The sensor reader will then be allowed to calibrate for 60 mins before the measurements could commence. The patients will be implored to scan at least every 8 hours so the reports will include all the data. For the measurements, the reader is usually held about 4cm from the sensor and the reading usually takes about 5 seconds [55].

  Figure (3.2) shows a pictorial illustration of the aforementioned steps during the FGM placement.

3.5 Case Report Forms

The following data was acquired during the period of the clinical study after the process of screening and recruitment.

3.5.1 History taking and Clinical examination

The date of birth, sex, age at admission, date of admission and discharge Weight, height, body mass Index were assessed. History of co-morbidities such as cardiovascular co-morbidities, drug history which includes details of the concomitant medications, daily cumulative insulin doses at admission and by discharge, number of anthypertensives at admission and by discharge and the duration of diabetes were recorded.

3.5.2 Laboratory parameters investigated

- HbA1c (mmol/mol)
- Serum Cortisol (nmol/l)
- Serum Epinephrine(pmol/l)

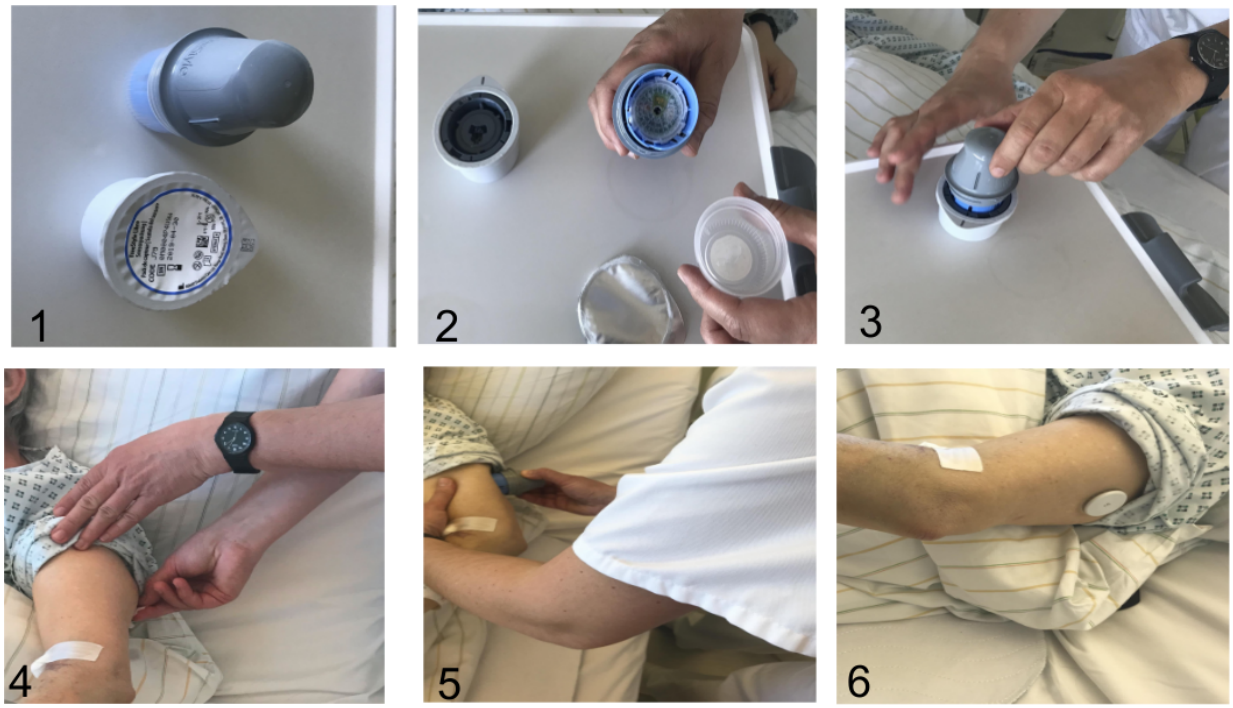

Figure 3.2: Steps of the FGM sensor placement (1) Confirmation of the code numbers (2) Removal of the protective coverages (3) Sensor applicator attached onto the cap (4) Skin selection and disinfection (5) Sensor applicator pressed firmly on the area of skin (6) Sensor firmly attached to the skin

- Serum Norepinephrine(pmol/l)
- Serum Creatinine (umol/l)
- Serum Urea (mmol/l)

3.5.3 Holter Monitoring

- 24-hour ambulatory blood pressure monitoring for the assessment of the following parameters:
- maximum systolic pressure (day-time)
- maximum diastolic pressure (day-time)
- maximum systolic pressure (night-time)
- maximum diastolic pressure (night-time)
- average systolic pressure (day-time)
- average diastolic pressure (day-time)
- average systolic pressure (night-time)

- average diastolic pressure (night-time)
- Blood pressure variability (Dipper/Non-Dipper/Reverse dipper)

3.5.4 24h –Holter -ECG for the assessment of the heart rate variability

Heart rate variability (HRV) is the fluctuation in the time intervals between adjacent heartbeats . HRV indexes neurocardiac function and is generated by heart-brain interactions and the autonomic nervous system (ANS) processes.
The SDNN score,the gold standard for medical stratification of cardiac risk,is the parameter of investigation to determine the heart rate variabilitiy.The normal range of values is defined as between 50-100ms. Based on the 24- h monitoring, patients with SDNN values below 50 ms are classified as having impaired heart rate variability [56].

3.6 Flash-Glucose-Monitoring: Analysis and interpretation

Hypoglycemia was defined as either confirmed hypoglycemia (blood sugar level of < 3.9 mmol/l) or an hypoglycemic episode requiring third party assistance. The followimg parameters were investigated during the analysis of the FreeStyle Libre device.

- duration of the continuous glucose monitoring in days
- total number of hypoglycemic events
- average number of hypoglycemic events in 24h
- average number of nocturnal hypoglycemia in 24h

Figure (3.3) shows the daily glucose profile over a 3-day period of a study patient with details of the interstitial glucose values. The timing of the hypoglycemic event (diurnal or nocturnal) and the number of episodes of hypoglycemia are easily identifiable.

3.7 Adverse events

Adverse events were sought by non-directive questioning at each visit and were also recorded when mentioned by the patient during or between visits or during physical Examination. A clinical inspection of the sensor placement area was carried out during the clinical visits.

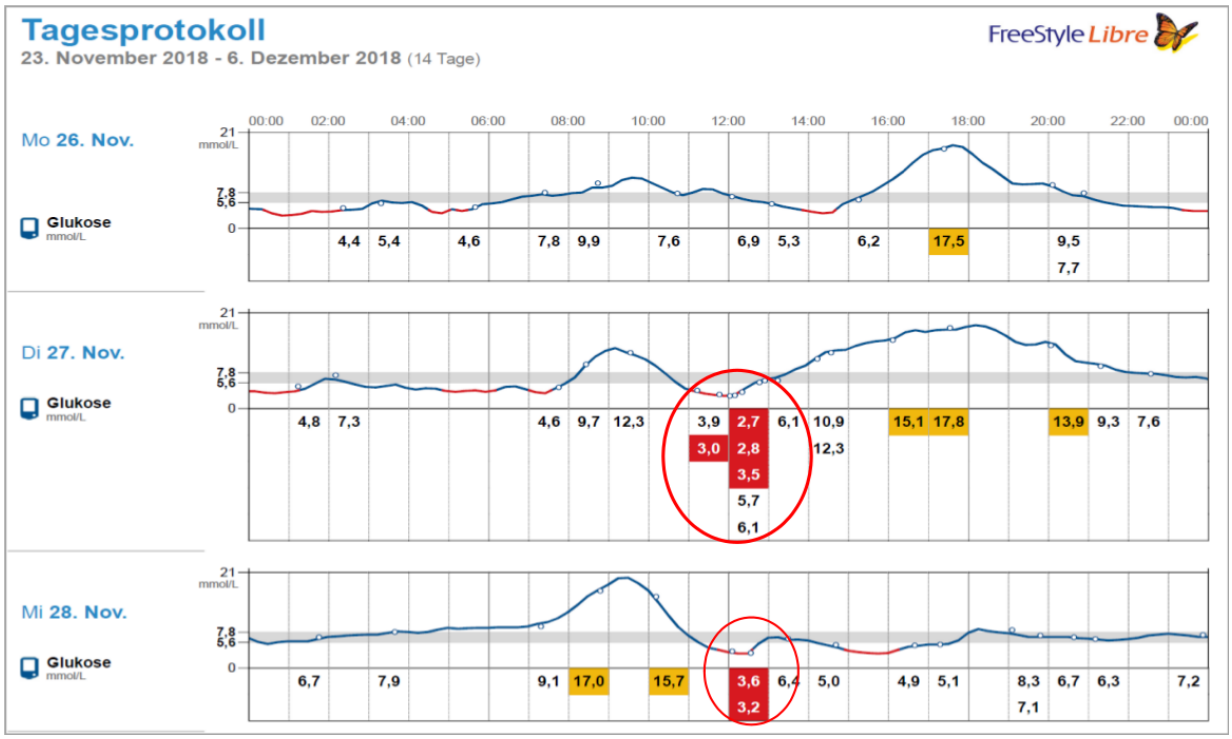

Figure 3.3: Daily glucose profile in the FGM, showing the IG values and the hypoglycemic episodes.

3.8 Data management and data protections rights

The data relevant to the clinical study are stored in a pseudonymized form and could only be saved by the investigators. By signing the patient information and declaration of consent, the investigators were given the legal permission to process and use the patient's personal data and the data collected during the study for analysis . The results of the study were allowed to published in a statistically prepared form (i.e. cohort-wise, not as a single result), presented to the public and used for educational purposes. Information that could be used to identify patients (such as name, date of birth) was not used in any publication or presentation.

3.9 Statistical analysis

If applicable, both the hypertensive crisis and symptomatic hypoglycemic events at admission were used for cohort allocation and were not considered as an event for analysis. In addition, information on missing data was provided (in Table 4.2). Continuous data were given as mean ±standard deviation. To test for normality, Kolmogorov-Smirnov test was used. For group-wise comparisons, an ordinary one-way Analysis of Variance test was used as a parametric test, Kruskal-Wallis test was used, if data showed no Gaussian distribution. As post-hoc tests, Tukey or Dunn's test were used, where appropriate. The following outcomes for the study were investigated:

- **Primary Outcome parameters**:
  Number of post-admission hypoglycemic episodes (tissue glucose level < 3.9 mmol/L) per 24h of FGM

- **Secondary Outcome parameters**:
  - Change in concomitant classes and defined daily dose (DDD) of antihypertensive medications by discharge.
  - Change in daily cumulative insulin dose by discharge.
  - Comparison of plasma catecholamines, heart rate variability (the standard deviation of RR Intervals derived from Holter electrocardiogram) and comparison of HbA1c among groups.

4 Results

4.1 Study population

The participating patients were recruited in line with the respective study protocol. The overview of the study population is represented in Fig.(4.1). In total, 65 patients were screened and enrolled in the study. Only 6 (9.2%) patients withdrew their consent. 59 (90.8%) patients were allocated to the three different groups based on the aforementioned criteria. In Group 1, a total of 22 (37.3%) patients were enrolled in the study, in total 3 patients were withdrawn from the study due to cancer diagnosis and death during the hospital stay and 2 cases of severe acute renal failure requiring hemodialysis were also excluded. A total of 19 patients completed the study and were analyzed accordingly. In Group 2, a total of 20 (33.9%) patients were enrolled and 1 patient who could not complete the study due to FGM data was excluded. The remaining 19 patients completed the study and were analyzed accordingly. However in Group 3, a lesser number of patients, 17(28.9%) in number were recruited, the patients presented with hypoglycemia out of which 2 patients had to be withdrawn from the study due to unavailable FGM data. A total of 15 patients completed the study and were analyzed accordingly.

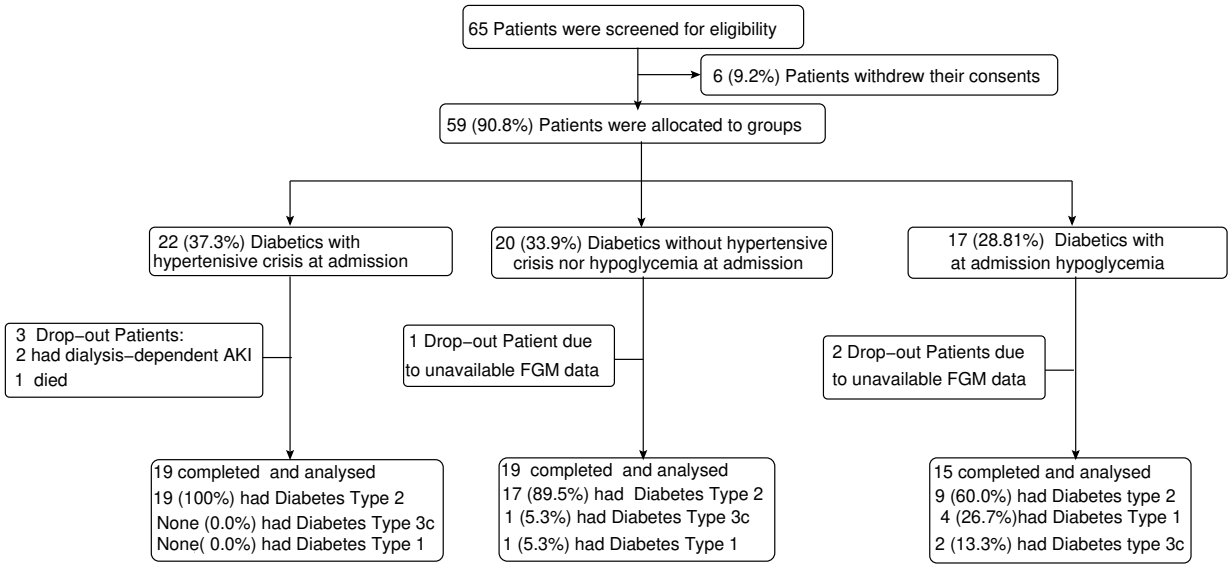

Figure 4.1: Flow chart which demonstrates screening and study recruitment of diabetes patients to this study.

4.2 Patient dispostion, demographic data and baseline characterisitics

A total of 41.5% of the study patients were males and the female patients accounted for 58.5% respectively. Patients with type- 1, type- 2, and type- 3 c Diabetes were recruited for the project with mostly type-2 diabetics accounting for the majority of the study population. 13.2% of the study population were type 1-diabetics and a minor percentage of patients (0.04%) accounted for the type 3 c diabetics. 86.8% representing the largest portion of the recruited patients were type- 2 diabetics. The age of the study population was well balanced in all the groups, with Group1 patients having a median age of 69.9 years, Group -2 patients having a median age of 64.1 years, and Group -3 patients with 62.2 years. Regarding the body mass indices of the study groups, the Group -1 patient was more obese in comparison to the Groups 2 and 3 patients with median values of –Group -1: 38.8 kg/m^2, Group -2: 29 kg/m^2 and Group- 3: 27.4 kg/m^2 respectively. The differences between the HbA1c-levels among the groups were alike indicating similar disease burden. Suffice to say that the median values of the HbAic in all groups 1,2 and 3 were 8.6%, 8.9%, and 7.7% respectively and this indicates the patients all had poorly-controlled diabetes. The details of the patient disposition and the baseline characteristics are summarized in Table 4.1.

4.3 Hypoglycemia-induced blood pressure elevation

The maximum systolic blood pressure was highest in Group-1 patients both at daytime and at night-time (Fig. (4.2), upper panel). The most relevant difference was seen in the maximum systolic pressure elevation at day time with median values of –Group -1: 191.6 mmHg, Group -2: 157.7 mmHg, and Group 3: 172.8 mmHg respectively as seen in as seen in Table (4.2) This conforms to the selection criteria for the Group- 1 Patients who presented with hypertensive urgency at admission. However, the average systolic arterial blood pressure in Group 1 and Group 3, both at daytime and nighttime were similar and this was not an expected finding (Fig. (4.2), lower panel). The negative control patients in Group 2 based on the selection criteria did not show any relevant blood pressure elevation, neither on admission nor by discharge. Similar patterns in the blood pressure variations in patients with hypertensive crisis compared to patients with hypoglycemia at admission was observed.

4.4 Comparison of antihypertensives classes and Effect on BP Control across groups

We compared the number of the classes of antihypertensives and the defined daily dosages(DDD) of the antihypertensives used for the treatment for all study populations. By discharge, the use of antihypertensives was observed to have been intensified in Group 1 (Figs. (4.3 and 4.4)). There

Table 4.1: Patient Characteristics (at admission and at discharge) of hospitalized diabetes patients with a hypertensive crisis at admission (Group- 1), without a hypertensive crisis or a symptomatic hypoglycemia at admission (Group 2), with symptomatic hypoglycemia at admission (Group 3).

	Group 1			**Group 2**			**Group 3**			
	n	Mean ±SD	n^a	n	Mean ±SD	n^a	*n*	Mean ±SD	n^a	*p*
Men/Women(*n*)	7/12	NA	NA	9/10	NA	NA	6/9	NA	NA	NA
Diabetes Type 1/2/3c	0/19/0	NA	19	1/17/1	NA	19	6/8/1	NA	15	NA
Age(years)	19	69.9 ± 9.8	19	19	64.1 ± 15.8	19	15	62.2 ± 21.9	15	0.3449
Body mass index (kg/m^2)	19	38.1 ± 14.0	19	19	28.5 ± 10.5	19	15	27.4 ± 6.0	15	0.0028
Antihypertensive classes per patient at admission (*n*)	19	3.9 ± 1.6	19	19	2.3 ± 1.6	19	15	2.5 ± 1.6	15	0.1435
Antihypertensive DDD at admission (n)	19	6.2 ± 5.4	19	19	3.0 ± 3.4	19	15	4.7 ± 6.0	15	0.0662
Daily cumulative insulin dose (units/d)	19	60.9 ± 41.5	19	19	30.5 ± 27.6	19	15	46.5 ± 23.3	15	0.0229
HbA1c (%)	19	8.6 ± 2.8	19	19	8.9 ± 2.8	19	15	7.7 ± 1.5	15	0.8592
Urea (plasma; mmol/L)	19	10.5 ± 5.1	19	19	6.6 ± 5.0	19	15	6.9 ± 4.2	15	0.0105
Creatinine (serum; μmol/L)	19	132.8 ± 55.3	19	19	89.5 ± 37.2	19	15	110.5 ± 55.2	15	0.0106
Cortisol (plasma; pg/mL)	19	395.4 ± 156.9	17	19	331.1 ± 126.7	18	15	387.6 ± 176.1	13	0.4079
Epinephrine (plasma; pg/mL)	19	34.9 ± 26.1	18	19	32.6 ± 20.4	18	15	32.6 ± 24.4	14	0.9326
Norepinephrine (plasma; pg/mL)	19	788.6 ± 411.9	17	19	437.5 ± 239.3	17	15	644.3 ± 378.7	14	0.0191

[a] *indicates final number of diabetes patients subjected to statistical analysis. This number can be lower than the maximum number due to the lack of data.*

p indicates analysis of blood pressure parameters, age, cortisol, norepinephrine using ANOVA test. All other parameters analyzed using Kruskal-Wallis testi.

were no relevant changes between the antihypertensive dosages (defined daily dose: DDD) in Group 2 and Group 3 when compared to admission in Fig. (4.4). Similar findings were also noted when the antihypertensive classes were compared together (Fig. (4.3)). Before discharge, the average mean arterial pressure was already well controlled in all study patients with Group 1 with 95.3 mmHg, Group 2 with 89.3 mmHg, and Group 3 with 97.1 mmHg (p=0.12) respectively as seen in Table (4.2). In our study, intensification of the antihypertensives was observed only in Group-1 patients with hypertensive crisis.

Table 4.2: Outcomes of hospitalized diabetes patients with a hypertensive crisis (Group -1), without a hypertensive crisis or a symptomatic hypoglycemia a (Group- 2), with symptomatic hypoglycemia a at admission (Group 3.) Hypoglycemia at a plasma glucose concentration of <3.9 mmol/L.

	Group 1			**Group 2**			**Group 3**			
	n	Mean ±SD	n^a	n	Mean ±SD	n^a	*n*	Mean ±SD	n^a	p^*
Renal function										
Creatinine at discharge (serum; μ mol/L)	19	114.5 ± 36.6	17	19	89.8 ± 32.6	19	15	100.2 ±46.5	13	0.0754
Change of serum creatinine (baseline versus discharge; μmol/l)	19	-17.9 ± 41.6	17	19	0.3 ± 44.1	19	15	-12.4 ±65.4	13	0.6705
Estimated glomerular filtration rate (ml/min/1.73m^2) at discharge	19	55.2 ± 18.6	16	19	76.7 ± 14.9	16	15	67.3 ±22.4	12	0.0068
Blood pressure during hospitalization										
Systolic blood pressure (maximum, day-time; mmHg)	19	191.6 ± 20.7	19	19	157.7± 20.2	18	15	172.8 ±22.2	15	<0.0001
Systolic blood pressure (mean, day-time; mmHg)	19	142.5 ± 13.8	19	19	124.6± 16.1	19	15	138.7 ±18.2	15	0.0030
Diastolic blood pressure (maximum, day-time; mmHg)	19	98.7 ± 13.4	19	19	88.6 ± 11.4	18	15	98.9 ±20.0	15	0.0749
Diastolic blood pressure (mean, day-time; mmHg)	19	75.3 ± 9.4	19	19	71.3 ± 8.3	19	15	79.1 ±15.4	15	0.1340
Mean arterial pressure (day-time; mmHg)	19	97.7 ± 8.9	19	19	85.9 ± 9.5	16	15	98.9 ±15.3	15	0.0035
Systolic blood pressure (maximum, night-time; mmHg)	19	167.7 ± 30.4	19	19	135.6± 15.1	15	15	151.5 ±23.2	15	0.0021
Systolic blood pressure (mean, night-time; mmHg)	19	141.7 ± 18.6	19	19	117.9± 12.1	16	15	125.1 ±17.8	15	0.0003
Diastolic blood pressure (maximum, night-time; mmHg)	19	90.9 ± 15.7	19	19	81.3 ± 8.2	16	15	90.8 ±15.7	15	0.1339
Diastolic blood pressure (mean, night-time; mmHg)	19	72.9 ± 11.3	19	19	68.7 ± 8.6	16	15	71.7 ±12.9	15	0.5279

[a] *indicates final number of diabetes patients subjected to statistical analysis. This number can be lower than the maximum number due to the lack of data.*

p^* *indicates analysis of blood pressure parameters, age, cortisol, norepinephrine using ANOVA test. All other parameters analyzed using Kruskal-Wallis test. (See supplementary Appendix for the details of the Post-hoc tests).*

Table 4.2 continued..

	Group 1			Group 2			Group 3			
	n	Mean ±SD	n^a	n	Mean ±SD	n^a	*n*	Mean ±SD	n^a	p^*
Mean arterial pressure (night-time; mmHg)	19	95.9 ± 11.9	19	19	85.9 ± 9.5	16	15	89.5 ± 13.8	15	0.0507
Antihypertensive classes per patient at discharge (*n*)	19	4.1 ± 1.6	19	19	2.2 ± 1.6	19	15	2.2 ± 1.2	15	0.0011
Change of antihypertensive classes (baseline versus discharge; *n*)	19	1.1 ± 1.1	19	19	0.2 ± 1.1	19	15	-0.3 ± 1.0	15	0.0017
Antihypertensive DDD at discharge (n)	19	11.0 ± 8.7	19	19	3.0 ± 3.3	19	15	4.9 ± 6.5	15	0.0012
Change of antihypertensive DDD (baseline versus discharge; n)	19	4.8 ± 6.1	19	19	0.1 ± 1.9	19	15	0.2 ± 3.8	15	0.0028
Holter-ECG parameters										
Minimal heart rate (bpm)	19	66.7 ± 13.2	13	19	58.8 ± 13.3	11	15	63.0 ± 14.8	9	0.3844
Maximum heart-rate (bpm)	19	107.6 ± 18.8	13	19	102.2 ± 23.3	11	15	119.1 ± 24.5	9	0.2373
Mean heart rate (bpm)	19	79.2 ± 13.4	13	19	72.8 ± 15.8	11	15	82.8 ± 19.6	9	0.3751
Heart rate variability, standard deviation of heart-beat intervals (ms)	19	65.1 ± 52.5	14	19	60.5 ± 40.4	15	15	56.4 ± 35.5	9	0.9584
Diabetes-related parameters										
Length of FGM (d)	19	5.1 ± 0.7	19	19	5.3 ± 1.5	19	15	6.2 ± 0.9	15	0.0004
Hypoglycemic episodesa (during FGM, *n*)	19	2.2 ± 1.9	19	19	0.7 ± 1.4	19	15	4.5 ± 2.3	15	<0.0001
Hypoglycemic episodes per nighta (during FGM, *n*)	19	0.8 ± 1.0	19	19	0.2 ± 0.5	19	15	1.5 ± 1.4	15	0.0051
Daily cumulative insulin dose at discharge (units/d)	19	42.5 ± 33.0	19	19	27.6 ± 24.3	19	15	27.9 ± 19.0	15	0.2960
Change of daily cumulative insulin dose (baseline versus discharge; units/d)	19	-18.4 ± 24.9	19	19	-2.9 ± 15.6	17	15	-18.6 ± 22.7	15	0.0479
Primary Outcome										
Hypoglycemic episodes (tissue glucose<3.9 mmol/l) per 24h (*n*)	19	0.4 ± 0.4	19	19	0.2 ± 0.3	17	15	0.8 ± 0.5	15	<0.0001

[a] *indicates final number of diabetes patients subjected to statistical analysis. This number can be lower than the maximum number due to the lack of data.*

p^* *indicates analysis of blood pressure parameters, age, cortisol, norepinephrine using ANOVA test. All other parameters analyzed using Kruskal-Wallis test. (See supplementary Appendix for the details of the Post-hoc tests).*

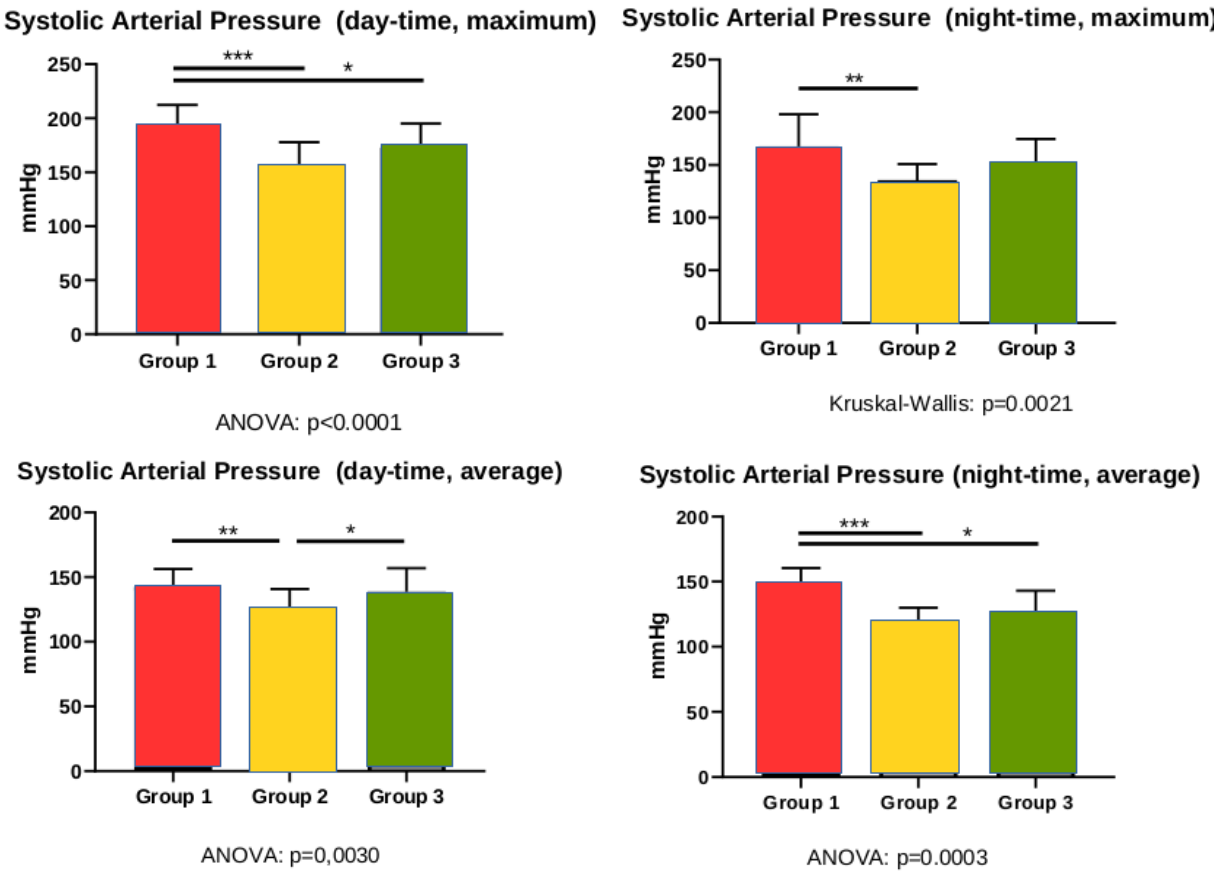

Figure 4.2: Maximum systolic blood pressure both at daytime and at nighttime (upper panel) and average systolic arterial blood pressure both at daytime and at nighttime (lower panel). Asterisks signify relevant differences according to post-hoc analysis. (* (p<0.05), ** (p<0.01), *** (p<0.001) and **** (p<0.0001))

4.5 Evidence of sympathoadrenal activation

Plasma Norepinephrine was higher in Group-1 patients when compared to control patients of Group 2, median values of –Group 1: 788 pmol/l, Group 2: 428 pmol/l, and Group 3: 644.3 pmol/l (Fig. 4.5)respectively. Of note, there was no relevant difference in plasma norepinephrine concentration between Group-1 and Group-3 patients. Norepinephrine elevation observed in the two groups poses evidence for sympathetic activation that may have occurred. Epinephrine levels however did not show any relevant changes among all groups (median values: Group 1: 33.9 pmol/l, Group 2: 36.1 pmol/l and Group 3: 32.6 pmol/l) as seen in Table (4.2).

4.6 Heart-rate variability across groups was not compromised

Heart rate variability (HRV) determined using the SDNN score is the gold standard for medical stratification of cardiac risk.The normal range of values is generally defined between 50-100ms.

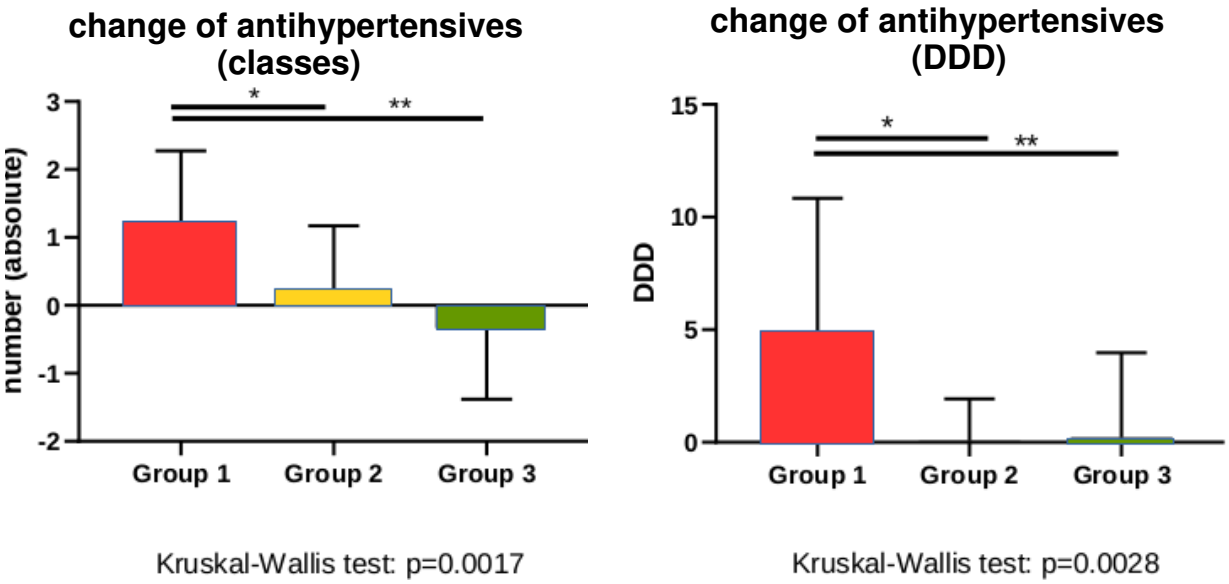

Figure 4.3: Comparison of the Antihypertensives classes among groups. Asterisks signify relevant differences according to post-hoc analysis. (* ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) and **** ($p<0.0001$))

Based on a 24 -Holter monitoring, patients with SDNN values below 50 ms are classified as having impaired heart rate variability. In our study, a compromised heart rate variability among the three groups was not observed(Fig. 4.6) Furthermore, relevant differences or correlation in the SDNN values of the respective groups was also not seen (median SDNN values of –Group 1: 65.1ms, Group 2: 60.5ms and Group 3: 56.4ms respectively). Additionally, there were no relevant changes between mean heart rate values across the respective groups. (mean heart rate values values of –Group 1: 79.2bpm, Group 2: 72.8bpm and Group 3: 82.8bpm respectively).Summarily, a relevant heart rate variability suggesting the presence of autonomic neuropathy and/or a compromised sympathodrenal response to hypoglycemia was not observed.

4.7 Impact of hypoglycemia across groups-Primary Outcome

The number of hypoglycemic episodes per 24 hours of FGM was highest in the positive-control Group 3 and Group-1 and Group-3 patients did not differ with regards to hypoglycemic episodes per 24 hours (Fig. 4.7left). When comparing the average number of nocturnal hypoglycemic episodes per 24 hours of FGM, the same proportion of hypoglycemic episodes was found (Fig. 4.7right). Again, patients of Group 1 and Group 3 did not differ.The absolute values of our primary outcome are demonstrated in Table (4.2). In other words, diabetes patients both with a hypertensive crisis (Group 1) and with symptomatic hypoglycemia at admission (Group 3) had a high number of hypoglycemic episodes during hospitalization. By discharge, the daily cumulative insulin dose decreased to the same extent in Group-1 and Group-3 patients

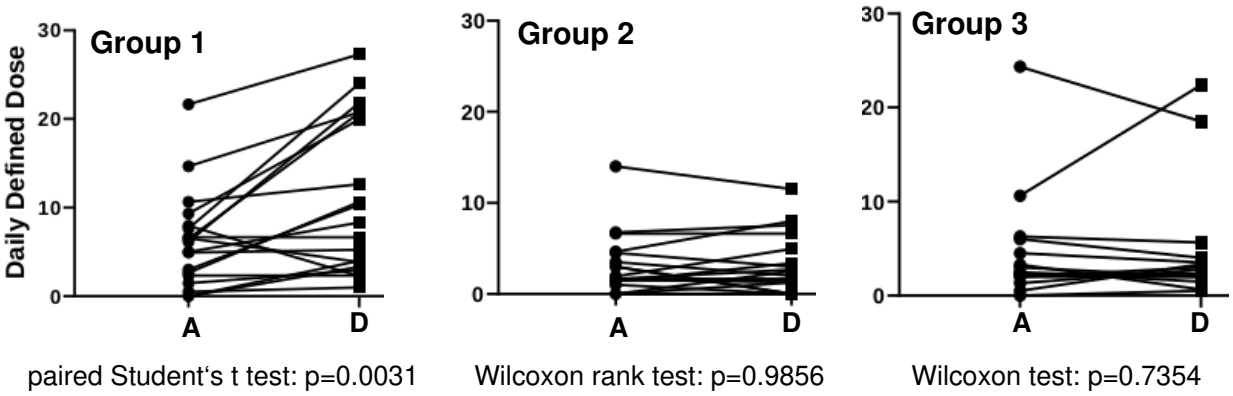

Figure 4.4: Comparison of the Antihypertensives in DDD among Groups (1,2 and 3) at admission (A) and Discharge(D).

(Fig. 4.8).Table (4.2) shows the details of the absolute values obtained. By discharge, insulin therapy was abandoned completely in 15.8% of Group-1 patients, in 10.5% of Group-2 patients, and 13.3% of Group-3 patients.

4.8 Reduced renal function as a risk factor for hypoglycemia

At admission, the Group 1 patients recruited had impaired renal function with a median GFR of 50.6 ml/min. The median values of creatinine were as follows: –Group 1:114.5 μmol/l, Group 2: 89.8 μmol/l, and Group 3: 100.2 μmol/l as seen in Table (4.2) with relevant differences across groups as demonstrated in (Fig. 4.9)with Group 1-Patients having the highest creatinine and urea values . Based on the KDIGO Classification, the Group -1 patients had a chronic kidney disease Stage 3a. Group- 2 patients had a median value of 76.8 ml/min and the Group -3 patients a median value of 66.6 ml/min respectively. During hospitalization, 1 out of 19 Group-1 patients, 3 out of 19 Group-2 patients, and 1 out of 15 Group-3 patients had an evolving acute kidney injury. After the exclusion of serum creatinine of patients with an evolving AKI, eGFR at discharge was still less in Group-1 patients than in Group-2 patients. However, Group-1 and Group-3 patients were not different in terms of eGFR at discharge (see Table 4.2). In summary, Group 1- patients had an impaired renal function when compared to Groups 2 and 3. By discharge, group-wise changes of serum creatinine and urea were however not different among groups (Table 4.2). Chronic renal disease being a risk factor for hypoglycemia may have contributed to the hypoglycemia burden observed in the hypertensive diabetics (Group -1 Study patients). Renal function was impaired in study patients with hypertensive crisis at admission.

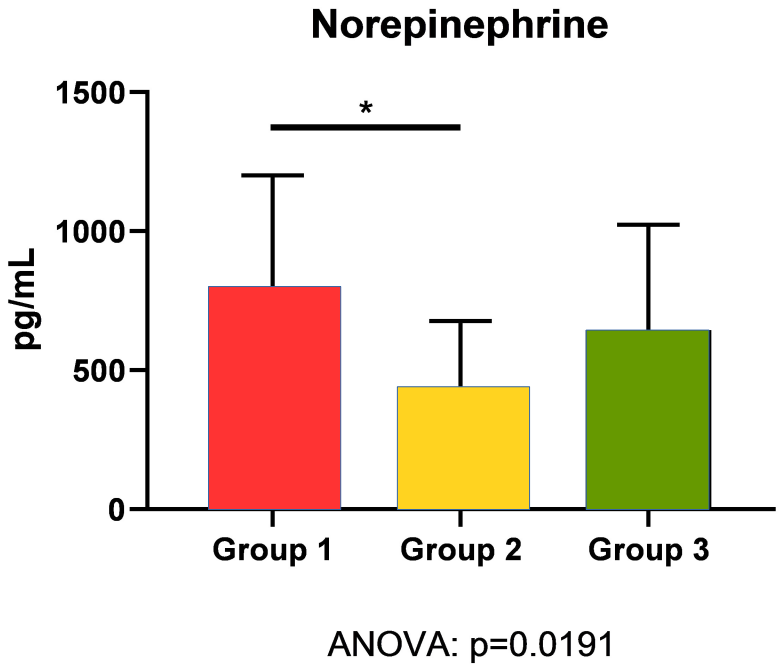

Figure 4.5: Plasma Norepinephrine levels at admission among groups. Asterisks signify relevant differences according to post-hoc analysis. (* (p<0.05), ** (p<0.01), *** (p<0.001) and **** (p<0.0001))

4.9 Effect of hypoglycemia on Cortisol levels

Plasma Cortisol elevation is a supposed correlate of the sympathetic activation [57]. Its important role in hypoglycemia is probably to increase glucose production by decreasing its utilization and accelerating lipolysis [58]. On the other hand, co-morbidities such as Addison's disease also increase the risk of hypoglycemia [12]. In our study, relevant elevations and differences among the groups with median values of –Group 1: 382 nmol/l, Group 2: 337.2 nmol/l and Group 3: 387.6 nmol/l respectively, were not observed (see (Fig. 4.10.) Cortisol did not show any significant correlation among groups. The reason for this observation remains unclear.

4.10 Adverse events

During the period of the whole study, only 2 cases (0.04%)of adverse events due to bleeding complications from the insertion site were seen. The patients were both observed to be on oral anticoagulants and both had impaired bleeding time at the time of sensor placement. There were no cases of allergic reactions to the sensor but its important to note that a post-admission follow up was not done and events after discharge were not monitored. 3 other cases of patients (0.06%) lost their sensors within 48 hours due to loosening of the adhesives and had to have new placements done. This was commonly observed during the summer months most likely due to

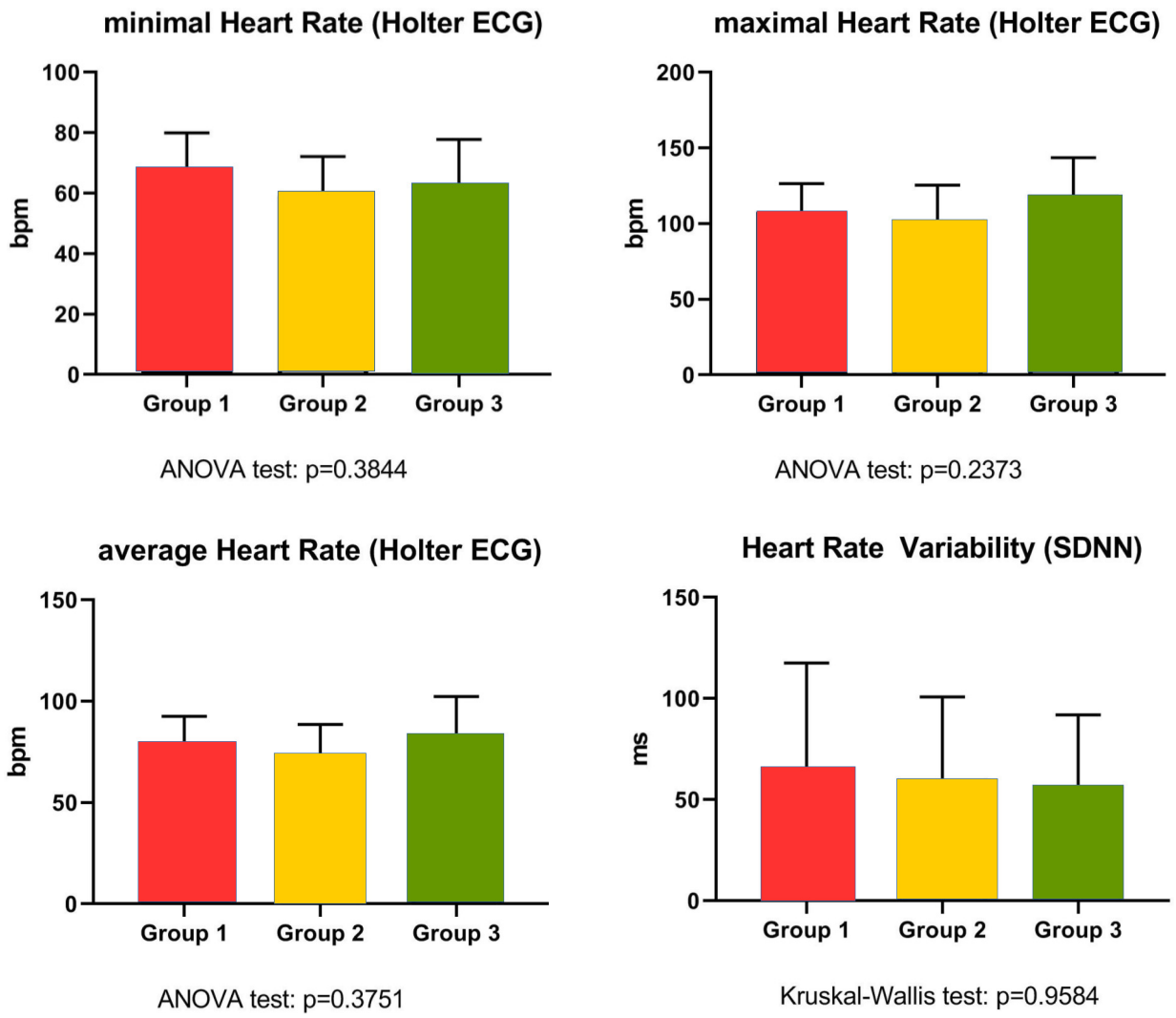

Figure 4.6: Comparison of minimum heart-rate values, maximum heart-rate values,average heart-rate values and heart-rate variability(SDNN) across groups, derived from the Holter-ECG Monitoring.

heavy sweating. This was however not classified as an adverse event.

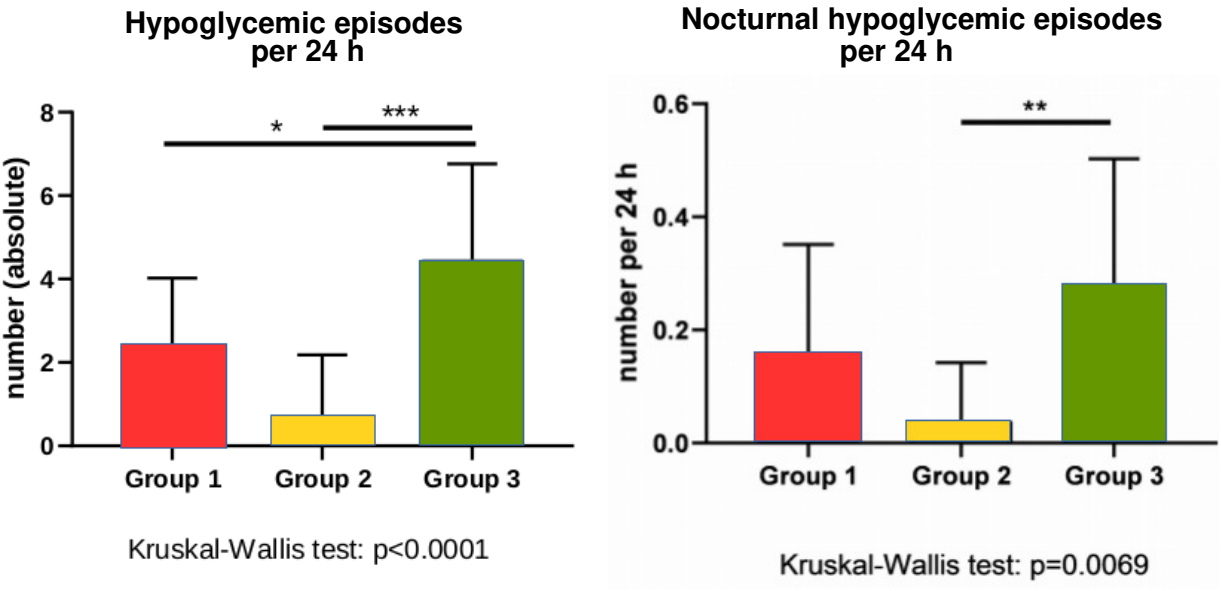

Figure 4.7: Hypoglycemic episodes per 24h flash glucose monitoring (FGM) among groups. Asterisks signify relevant differences according to post-hoc analysis. (* (p<0.05), ** (p<0.01), *** (p<0.001) and **** (p<0.0001))

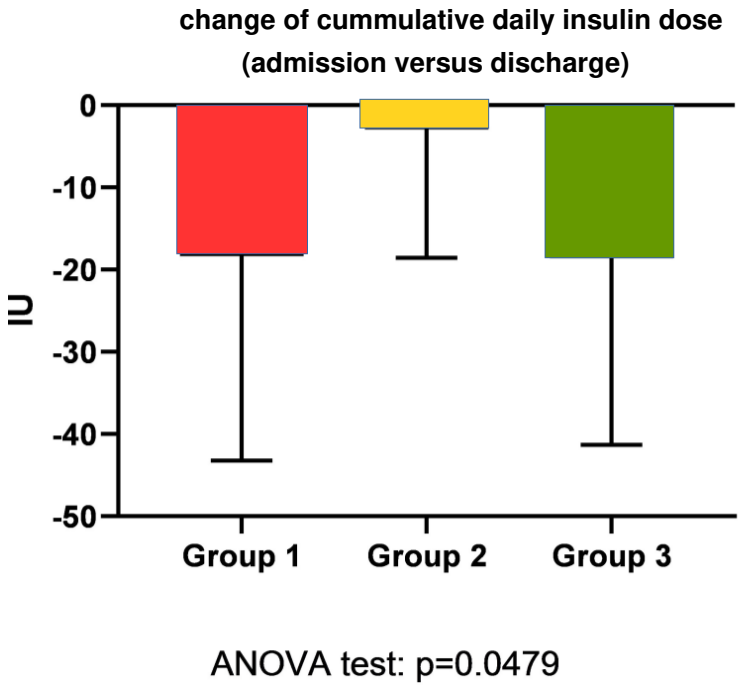

Figure 4.8: Change of cumulative daily insulin units (IU) from the time of admission versus discharge. Asterisks signify relevant differences according to post-hoc analysis. (* (p<0.05), ** (p<0.01), *** (p<0.001) and **** (p<0.0001))

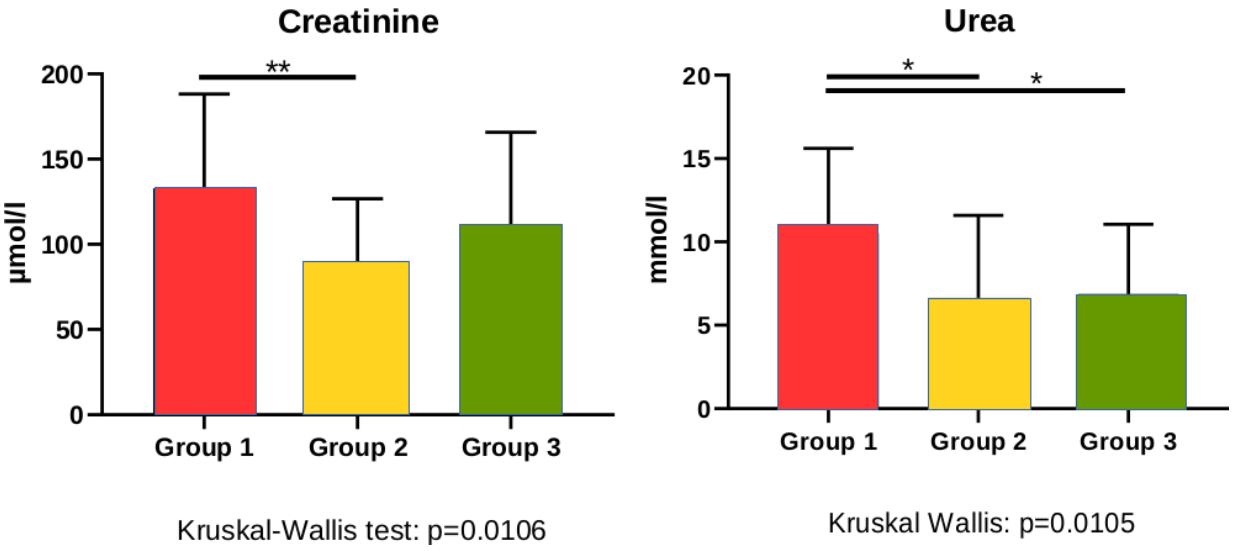

Figure 4.9: Serum Creatinine and Urea levels at admission among groups. Asterisks signify relevant differences according to post-hoc analysis. (* ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) and **** ($p<0.0001$))

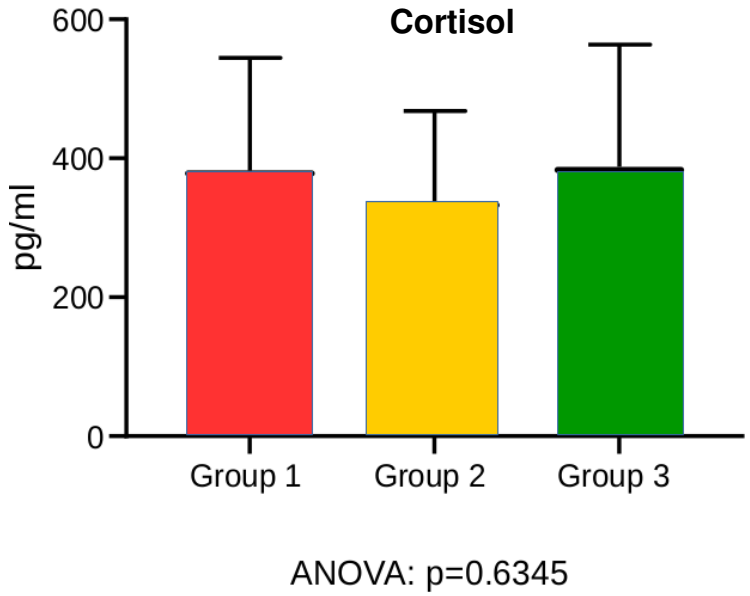

Figure 4.10: Plasma Cortisol levels at admission among groups showing no significant statistical differences.

5 Discussion

Despite advancements in diabetes technology, hypoglycemia still plays a limiting factor preventing the attainment of the desired glycemic control required in preventing the micro- and macrovascular complications in patients with diabetes. This study aimed to shed light on the blood-pressure regulation in insulin-treated patients with diabetes mellitus of any type. We investigated 65 insulin-treated diabetes patients (type 1, type 2, and type 3c) and hypothesized that hospitalized insulin-treated diabetes patients with hypertensive crisis have more hypoglycemic episodes than counterparts without a hypertensive crisis on admission. A continuous flash glucose monitoring using a FGM sensor, a 24-hour ambulatory blood pressure monitoring, 24-hour electrocardiogram recordings, and laboratory tests including plasma catecholamines were carried out. The results derived showed that blood pressure variations were not different in diabetes patients with hypertensive crises compared to diabetes patients with hypoglycemia on admission, who served as the control group. Besides, Insulin-treated diabetes patients with hypertensive crisis (Group 1) and with symptomatic hypoglycemia (Group 3) were not different in terms of the hypoglycemic burden during hospitalization. Furthermore, plasma norepinephrine, a correlate of sympathetic system activation was observably elevated in the study group with hypertensive diabetics (Group1). The intensification of the antihypertensives was observed only in diabetes patients with hypertensive crisis. The impaired renal function in diabetics with a hypertensive crisis at admission (Group1) may have acted as one of the contributing factors to the increased hypoglycemic episodes. A detailed discussion of the results obtained is elucidated below.

5.1 Sympathoadrenal activation during hypoglycemia

Neuroglycopenia is known to trigger neurohumoral activation involving both a hypothalamic-pituitary-axis- and sympathoadrenal activation which in turn induces the release of adrenal catecholamines [59, 10]. Evidences supporting the close temporal relationship between hypoglycemia and arterial hypertension has also been demonstrated in a small cohort study of type-1- and type-2-diabetes patients [26]. In this present study, similar trends were observed, supporting the role of sympathoadrenal activation during hypoglycemia. Confirming the prespecified inclusion criteria,the maximum systolic arterial pressure was highest in Group-1 patients. Likewise, hypoglycemic episodes per 24 hours FGM were more often detected in the overtly hypoglycemic Group-3 patients when compared to control patients of Group 2. Of note, the first symptomatic hypoglycemia occurring at admission was the inclusion criterion for Group-3 patients and did not count as a result. As a novel finding, Group-1 and Group-3 patients had comparable plasma

norepinephrine levels and supposedly, a comparable state of activity of the SNS. Also, Group-1 and Group-3 patients had a similar phenotype concerning the mean arterial blood pressure. These results support the hypothesis that Group-1 patients may have had an activated SNS with higher plasma norepinephrine levels in comparison to Group-2 patients. For Group-1 patients, it is tempting to speculate that hypoglycemia triggered the norepinephrine release within the framework of the Somogyi effect. If this hypothesis holds in Group-1 patients, the systolic blood-pressure excursions and the hypertensive crisis at admission may be, in part, due to a pronounced Somogyi effect in terms of catecholamine release and SNS activation following hypoglycemia.

5.2 Heart rate variability as a correlate of the sympathoadrenal activation

Cardiovascular autonomic neuropathy (CAN) is an underdiagnosed complication of diabetes that can cause the loss of HRV. During hypoglycemia, a measurable change in the HRV has been observed both in patients with or without cardiovascular autonomic neuropathy [56, 60]. Overall in our study, we did not find a compromised HRV across the groups. Although cardiovascular autonomic neuropathy was not an exclusion criteria in this study, a prevalent cardiac autonomic neuropathy among study participants is unlikely because the 24-hour HRV was not reduced, and the resting heart rate was also not increased. Nevertheless, future studies on hypoglycemia counterregulations need to comprehensively assess the existence and, if applicable, the manifestations of both diabetic autonomic neuropathy and hypoglycemia unawareness.

5.3 Role of Cortisol as a counterregulatory hormone

The counterregulatory cortisol or growth hormone response is mainly regulated through the central nervous system (CNS). Elevated levels of cortisol is a known critical counterregulatory response to severe hypoglycemia. Rhyu et al investigated the prevalence of impaired cortisol counterregulatory response in T2DM during severe hypoglycemia. Out of the 112 T2DM patients investigated in this retrospective study, 20.5% of the study population showed an impaired cortisol response during hypoglycemia [57]. The exact mechanism of impaired cortisol or growth hormone response to hypoglycemia is however unclear [61]. Further work is needed in order to clarify the role of cortisol in hypoglycemia associated sympathoadrenal activation. Regarding our study, Groups-1 and- 3 patients had a higher hypoglycemia burden. The expected cortisol elevation due to hypoglycemia was however not observed [62].

5.4 Effect of a higher-than-optimal cumulative insulin dose

A retrospective study carried out in Germany between 2013 and 2015 involving of 284,878 T2DM patients showed an unexpectedly high number of patients without previous antidiabetic drugs therapy receiving insulin monotherapy as against the recommended guidelines [63]. Infact, a study carried out between 2010 and 2011 further reiterated this fact. Germany was found to rank as the second country after Finland with the highest per-capita use of insulin in Europe [64]. Our study focused on the hypoglycemic burden in insulin-treated diabetes patients of any causality with the primary aim of determining the number of hypoglycemic episodes per 24 hours of flash glucose monitoring. In Group-3 patients, the hypoglycemic burden is evident by the proven hypoglycemic episodes during FGM, and by the reduced daily cumulative insulin dose by discharge. Therefore, we conclude that Group-3 patients had a higher-than-optimal daily cumulative insulin dose at admission. Surprisingly, hypertensive Group-1 patients and initially hypoglycemic Group-3 patients showed no difference concerning hypoglycemic burden in the FGM results. Likewise, as evidence for a higher-than-optimal insulin dosage, the daily cumulative insulin dose was decreased in Group-1 patients by discharge as well. Regarding the daily cumulative insulin dose, as a limitation, the within-day dosing issues were not considered in this study. Nevertheless, once hypoglycemic episodes became evident, therapeutic decisions likely led to an insulin reduction. Therefore, the yielded hypoglycemia rate during FGM was unlikely to be increased by the open-label FGM used in this study In the case of a higher-than-optimal daily cumulative insulin dose, the reduction of insulin may reduce both hypoglycemic events and an ensuing hypertensive crisis as a consequence of neurohormonal activation occurring in the framework of the Somogyi effect. If not, patients with an individually higher than optimal daily cumulative insulin dose are more likely to suffer from repetitive hypoglycemic events, or, if hypoglycemia still is compensated for by neurohormonal activation, from hypertensive events [65].

5.5 Renal impairment could be a risk factor for the hypoglycemic events

The presence of chronic renal insufficiency, commonly diabetic nepropathy is an additional risk factor for hypoglycemia in people with diabetes. Renal glucose release accounts for 20% of overall endogenous glucose release, which is responsible for about 40% of all gluconeogenesis [66]. The increased risk of hypoglycemia is mainly due to the impaired renal glucose release during the process of counterregulation. Additionally, the reduced clearance of insulin evident when the GFR falls below 15-20 ml/min/1.73m2 plays a crucial role [19]. In a 10-yr prospective, longitudinal cohort study carried out by Yun et al, the presence of baseline macroalbuminuria (defined as urinary albumin excretion $\geq$ 300 mg/day) in form of a pre-existing di-

abetic nephropathy was shown to be an independent risk factor for the future development of severe hypoglycemia in T2DM patients with apparently normal or only minimally decreased renal function (e.g., GFR>60 mL/min/1.73 m2) irrespective of whether or not they were receiving insulin [67]. Suffice to say, CKD independently increases the propensity for hypoglycemia even without diabetes. The excessive mortality associated with hypoglycemia makes this complication a significant threat to patient safety in CKD [68]. In our study, increased renal impairment was found in our Group-1 patients indicating a pre-existing diabetic nephropathy. This could have contributed to the increased hypertensive crisis seen and possibly the similar hypoglycemia burden observable in the hypertensive diabetics (Group 1) in comparison with Group-3 patients with only hypoglycemia at admission. More importantly, an elevation in sympathetic nerve activity (SNA) has been reported by several authors as a contributing factor to hypertension and plays a detrimental role in the progression of CKD independent of any increase in blood pressure [67]. Additionally, the elevated body-mass index seen in Group-1 patients could have posed factor in the context of metabolic syndrome, as a contributing factor to our findings.

5.6 Strengths and Limitations

The major strength of this study was the use of the flash glucose monitoring system that made it possible to observe asymptomatic- and nocturnal hypoglycemic episodes more closely. The use of this system made it possible for us to detect more hypoglycemic episodes that could have been missed. Our study groups were also well defined and focused more on T2DM accounting for 87% of the study population making comparisons between the groups easier and reproducible. As a major limitation, the sample size investigated was relatively small. Besides, FGM and ABPM results were not blinded and could have influenced therapy decisions. Furthermore, the Holter-BP Monitoring was carried out for only a period of 24 hours. All other blood pressure excursions that occurred during the remaining period of the continuous interstitial glucose monitoring were missed. The use of a Holter-BP Monitoring for longer periods of time, with regards to our study patients, was less feasible. In addition, a follow-up of the patients on an outpatient basis to monitor the efficacy of insulin reduction on the hypoglycemia burden and blood pressure readings was not carried out. In this study, association between hypoglycemic events, evidence of SNS activation, and uncontrolled arterial hypertension in diabetes patients on insulin therapy were found. These findings align with our initial hypothesis but more randomized clinical trials with double-blinded investigations and much larger study populations still need to be carried out to confirm the hypothesis.

6 Summary and Conclusion

In this present investigation, a prospective observational and explorative cohort study was carried out to determine a possible correlation between hypoglycemic episodes and hypertensive crisis with a primary focus on the role of the sympathoadrenal system. Insulin-treated T2DM diabetics with arterial hypertension were mainly included. We hypothesized that hospitalized insulin-treated diabetes patients with hypertensive crisis have a propensity for more hypoglycemic episodes than counterparts without hypertensive crisis on admission in post-admission continuous glucose monitoring using the FGM system. Counterregulatory hormones investigated were expected to be elevated if the hypothesis holds true both in insulin-treated diabetes patients with the hypertensive crisis on admission and in diabetes patients who presented on admission with hypoglycaemia. Our results showed similar trends with respect to blood pressure excursions in both hypertensive diabetics and diabetics who presented with hypoglycemia at admission. A similar hypoglycemia burden was also observable in both groups in comparison with negative controls. To further support this hypothesis of sympathoadrenal activation, elevated plasma epinephrine levels in hypertensive diabetics in comparison with other groups was demonstrable. In summary, a link between sympathoadrenal activation during hypoglycemia and uncontrolled arterial hypertension was found. The role of cortisol despite its known counterregulatory effect during hypoglycemia remains unclear. The intensification of insulin therapy in order to achieve a tight glycemic control may lead to a higher-than-optimal cumulative insulin dose usage. Consequently, an increased hypoglycemic burden especially in patients with pre-existing renal insufficiency, most importantly diabetic nephropathy.

6.1 Future outlooks

Numerous research studies have begun to uncover the mechanisms by which the central nervous system responds and adapts to hypoglycemia. Randomized clinical trials assessing surrogates of SNS tone in diabetes patients are still needed to gain a better understanding of both the normal and possibly attenuated responsiveness to hypoglycemia in diabetes patients. It is not uncommon to see hypoglycemia associated autonomic failure in patients with type -1 diabetes and advanced type -2 diabetes who, as a result of this failed counterregulatory response, are prompted into a vicious cycle of recurrent hypoglycemia and its fatal sequelae [32]. In future studies, the consideration of more surrogate parameters of SNS activity including the low-frequency band intensity in power spectral analysis of heart rate and, if feasible, direct measurements of sympathetic nerve activity may further resolve this puzzle. Given the fact that Germany is a country with one of the

highest per-capita use of insulin in Europe [64], the results of these future studies may contribute to developing strategies to avoid or further minimize the incidence of hypoglycemic episodes. To gain a comprehensive picture of the hypoglycemia – hypertension relationship, better coverage of blood-pressure and tissue glucose monitoring should be achieved. As the use of long-term FGM becomes more feasible, future studies may even provide insights on the initial hypoglycemic episode leading to hospitalization. The prioritization of hypoglycemia prevention strategies will go a long way into achieving control goals while avoiding the morbidity and mortality associated with hypoglycemia. In conclusion, the results of this pilot study may help in the early detection of patients at high risk for hypoglycemia and increase awareness among health-care professionals when treating hypertensive diabetics on insulin therapy.

Supplementary Appendix

Table 6.1

Post-hoc Test for hypoglycemia per 24h. Kruskal-Wallis-Test used for analysis of variance

Dunn's multiple comparisons test	Mean rank Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	11.4		No	ns	0.0566
Group 1 vs. Group 3	-11.1		No	ns	0.0961
Group 2 vs. Group 3	-22.4		Yes	****	<0.0001

Post-hoc test for change of cumulative insulin dose. ANOVA test used for analysis of variance (admission versus discharge)

Tukey's. multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-15.4	-32.1 to 1.29	No	ns	0.0761
Group 1 vs. Group 3	0.232	-17.6 to 18.0	No	ns	0.9995
Group 2 vs. Group 3	15.7	-2.14 to 33.4	No	ns	0.0950

Post-hoc test test for nocturnal hypoglycemia per total FGM Duration
Kruskal-Wallis-Test used for analysis of variance

Dunn's multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	8.500		No	ns	0.1691
Group 1 vs. Group 3	-6.768		No	ns	0.4604
Group 2 vs. Group 3	-15.27		No	**	0.0038

Table 6.2

Post-hoc Test for hypoglycemic episodes during FGM
Kruskal-Wallis-Test used for analysis of variance

Dunn's multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	10.7	No	No	ns	0.0796
Group 1 vs. Group 3	-13.6	Yes	Yes	*	0.0249
Group 2 vs. Group 3	-24.3	Yes	Yes	****	<0.0001

Post-hoc Test for length of FGM duration
Kruskal-Wallis-Test used for analysis of variance

Dunn's multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-2.08		No	ns	>0.9999
Group 1 vs. Group 3	-16.6		Yes	***	0.0006
Group 2 vs. Group 3	-14.5		Yes	*	0.0035

Post-hoc Test for change of antihypertensives in DDD
Kruskal-Wallis-test used for analysis of variance

Dunn's multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	14.5		Yes	*	0.0113
Group 1 vs. Group 3	15.9		Yes	**	0.0085
Group 2 vs. Group 3	1.41		No	ns	>0.9999

Post-hoc Test for the change in antihypertensive classes in DDD at discharge
Kruskal-Wallis-Test used for the analysis of variance

Dunn's multiple comparisons test	Mean rank difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	17.8		Yes	**	0.0011
Group 1 vs. Group 3	13.0		Yes	*	0.0453
Group 2 vs. Group 3	-4.86		No	ns	>0.9999

Post-hoc-Test for the change of antihypertensive classes
Kruskal-Wallis test used for analysis of variance

Dunn's multiple comparisons test	Mean rank difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	12.0		Yes	*	0.0358
Group 1 vs. Group 3	17.4		Yes	**	0.0018
Group 2 vs. Group 3	5.40		No	ns	0.8596

Table 6.3

Post-Hoc Test for antihypertensive classes at discharge

Kruskal-Wallis test used for analysis of variance

Dunn's multiple comparisons test	Mean rank difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	15.9		Yes	**	0.0037
Group 1 vs. Group 3	16.2		Yes	**	0.0060
Group 2 vs. Group 3	0.302		No	ns	>0.9999

Post-hoc Test for mean arterial pressure at day-time

ANOVA test used for analysis of variance

Tukey's.multiple comparisons test	Mean rank difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	11.7	2.38 to 21.1	Yes	*	0.0106
Group 1 vs. Group 3	-1.29	-10.8 to 8.21	No	ns	0.9426
Group 2 vs. Group 3	-13.0	-22.9 to -3.12	Yes	**	0.0072

Post-hoc Test for average systolic blood pressure at night-time

ANOVA-test used for the analysis of variance

Tukey's. multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	23.8	10.2 to 37.4	Yes	***	0.0003
Group 1 vs. Group 3	16.6	2.77 to 30.4	Yes	*	0.0152
Group 2 vs. Group 3	-7.20	-21.6 to 7.20	No	ns	0.4534

Post-hoc Test for maximum systolic blood pressure at night-time

ANOVA-test used for the analysis of variance

Dunn's multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	17.4		Yes	**	0.0013
Group 1 vs. Group 3	7.61		No	ns	0.3914
Group 2 vs. Group 3	-9.76		No	ns	0.1865

Post-Hoc Test for mean systolic blood pressure at day-time

ANOVA-test used for the analysis of variance

Tukey's.multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	17.9	5.39 to 30.4	Yes	**	0.0032
Group 1 vs. Group 3	3.81	-9.51 to 17.1	No	ns	0.7700
Group 2 vs. Group 3	-14.1	-27.4 to -0.774	Yes	*	0.0359

Table 6.4

Post-Hoc Test for the maximum systolic blood pressure at day-time
ANOVA-test used for the analysis of variance

Tukey's.multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	33.9	17.2 to 50.5	Yes	****	<0.0001
Group 1 vs. Group 3	18.8	1.27 to 36.3	Yes	*	0.0330
Group 2 vs. Group 3	-15.1	-32.8 to 2.65	No	ns	0.1098

Post-hoc Test for the eGFR on discharge
Kruskal-Wallis-Test for the analysis of variance

Dunn's multiple comparisons test	Mean Difference	95.00% CI of difference	Significant?	Summary	Adjusted P Value
Group 1 vs. Group 2	-14.3		Yes	**	0.0048
Group 1 vs. Group 3	-7.70		No	ns	0.3434
Group 2 vs. Group 3	6.55		No	ns	0.5373

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Theses

1. Sympathoadrenal activation plays an important role in blood pressure regulation and in response to hypoglycemia. The activation of the sympathoadrenal system during insulin-induced hypoglycemia may lead to hypertensive blood-pressure episodes

2. Cardiovascular autonomic neuropathy (CAN) is an underdiagnosed complication of diabetes that can cause the loss of heart-rate variability. A relevant heart rate variability suggesting the presence of autonomic neuropathy or compromised sympathoadrenal response to hypoglycemia was however not observed.

3. Elevated levels of cortisol is a known critical counterregulatory response to severe hypoglycemia. From the data in the current study, its exact role remains unclear.

4. In insulin-treated diabetes patients, a higher than optimal insulin dose can increase the hypoglycemic burden. Here, a continuous glucose monitoring may help to tailor insulin therapy and improve outcomes (less hypoglycemic events).

5. Renal Impairment could be a risk factor for hypoglycemic events.

6. Insulin-treated patients hospitalized for hypertensive crisis have a propensity for more hypoglycemic episodes in prospective FGM Monitoring than counterparts hospitalized for other reasons.

7. Comparable plasma norepinephrine concentrations, due to sympathoadrenal activation, were seen in insulin- treated diabetes patients with hypertensive crisis and in diabetes patients who presented with hypoglycemia.

8. Similar patterns in the blood pressure variations in patients with hypertensive crisis compared to patients with hypoglycemia were observed.

9. Randomized clinical trials assessing surrogates of sympathetic-nervous-system tone in diabetes patients are still needed to gain a better understanding of responsiveness to hypoglycemia in diabetes patients.

10. Hypothetically, repetitive hypoglycemia may associate with autonomic failure in patients with type-1 diabetes and advanced type-2 diabetes with possibly fatal sequelae. Long-term studies are needed to study the responsiveness of the autonomic nervous system to repetitive hypoglycemia.

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Statutory declaration

I declare that I have written this thesis independently, that I have not used any other than the declared sources, and that I have explicitly marked all materials which have been quoted literally or by content from the used sources.

Abimbola Bukola Adeagbo

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Place, date Signature