Diplomarbeit

Evaluation of Subcutaneous Glucose Monitoring Systems under Routine Environmental Conditions

eingereicht von

Hesham Elsayed

zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt an

Klinische Abteilung für Endokrinologie und Diabetologie

unter der Anleitung von

Ass.-Prof. Priv.-Doz. Dr. med. univ. Julia Mader,

Ass. Dr. Verena Schwetz

Graz, am 19.03.2017

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 19.03.2017 *Hesham Elsayed eh.*

Danksagungen

Mein besonderer Dank gilt meinen beiden Betreuerinnen, Ass. Prof. Dr. med. univ. Julia Mader und Dr. med. univ. Verena Schwetz für die exzellente Betreuung, umfangreiche Unterstützung und Motivation für das Fach für Endokrinologie und Diabetologie.
Außerdem möchte ich dem gesamten Team des Clinical Research Center für die Integration ins Team und für die regelmäßigen Schulungen bedanken, insbesondere bei Dr. med. univ. Gurban Jamala, Adelheid Puffing, BSc. Sarah Bischof, BSc. und Melanie Motschnig, BSc.
Ganz besonders gebührt meinen Eltern der Dank: Danke, dass Ihr immer für da wart, egal wie schwierig es für mich wurde. Meiner Schwester, Dr. Hend Elsayed möchte ich insbesondere für ihre nervliche Unterstützung, Rat und ihre Geduld bedanken. Meinen restlichen Geschwistern Heba, Hagar und Hamza gebührt ebenfalls ein besonderer Dank für eure unermüdliche Unterstützung und Motivation.

Zusammenfassung

Hintergrund

Die Inzidenz von Diabetes mellitus Typ 1 und Typ 2 steigt jährlich rapide an. WHO und ADA schätzen jährlich bis zu 1,5 Millionen Todesfolgen. Um das Risiko für Folgeerscheinungen und metabolische Entgleisungen zu reduzieren bzw. die Lebensqualität zu erhöhen, muss für jeden Patienten/jeder Patientin mit insulinabhängigem Diabetes eine adäquate Insulintherapie zur Normalisierung des Blutzuckerspiegels zu erreichen. Da im modernen Diabetesmanagement kontinuierliche Glukosemesssysteme etabliert sind, beschäftigt sich die Diabetesforschung mit der Verbesserung von bereits vorhandenen bzw. Entwicklung neuer, weniger invasiver Messmethoden, welche Therapien weitgehend positiv beeinflussen und so einen hohen Lebensstandard sichern sollen.

In einer monozentrischen Studie, wurde die Performance von drei auf dem Markt erhältlichen Systemen, Abbott FreeStyle libre, Dexcom G4 Platinum und Medtronic MiniMed 640G verglichen.

Material und Methoden

Die monozentrische Studie wurde bei zwölf Probandinnen und Probanden mit Diabetes mellitus Typ 1 durchgeführt. Relevante Einschlusskriterien umfassten Diabetes mellitus Typ 1 seit mindestens 6 Monaten, Therapie mit Insulinpen oder Insulinpumpe, Volljährigkeit, BMI < 35kg/m² und ein maximales HbA1c von 8.4%. Die Hauptausschlusskriterien waren Schwangerschaft, Erkrankungen, welche den Glukosehaushalt beeinflussen und die Einnahme von Medikamenten welche den Blutzuckerhaushalt beeinflussen könnten, exklusive Insulintherapie.

Die Studie wurde am Zentrum für medizinische Grundlagenforschung der Medizinischen Universität Graz durchgeführt und wurde von der Ethikkommission der Medizinischen Universität Graz genehmigt. Die getesteten Glukosesysteme wurden anhand der ISO-Kriterien 15197:2013, der mittleren absoluten relativen Differenz (MARD), und anhand Parkes Error Grid (PEG) bewertet.

Primäres Ziel dieser Studie war es, unter realitätsnahen Bedingungen die Genauigkeit und Zuverlässigkeit von Abbott, Dexcom und Medtronic zu beurteilen.

Primäres Ergebnis

Abbott, Dexcom und Medtronic erfüllten ISO Kriterien mit 73,2%, 56,1% und 52,0%. Die MARD lag für Abbott bei 13,2 ± 10,9%, für Dexcom bei 16,8% ± 12,3% und für

Medtronic bei 17,6%. Im PEG lagen alle Datenpaare von Abbott in den Zonen A + B, bei Dexcom waren es 99,3 und bei Medtronic 98,6%.

Diskussion

Abbott FreeStyle libre zeigt in allen glykämischen Bereichen zwar die beste Sensorperformance, jedoch zeigt sich Verbesserungsbedarf bei zukünftigen Generationen aller Geräte, vor allem im hypoglykämischen Bereich.

Abstract

Background

Since the incidence of both diabetes mellitus type 1 and type 2 are increasing rapidly, the WHO and ADA are estimating up to 1.5 million deaths annually, which are directly linked to the disease.

In order to prevent side effects and metabolic deficiencies, and in order to increase quality of life, it is necessary to establish an adequate insulin therapy to achieve normoglycemia in insulin-dependent diabetes.

Since continuous glucose monitoring systems (CGM) are established in modern diabetes management, research focuses on the improvement of already existing sensor technologies or the development of new, less invasive measuring methods, which should largely influence therapies positively and thus ensure a high standard living.

In the following open-label, single-center study, the performance of Abbott FreeStyle libre, Dexcom G4 Platinum and Medtronic MiniMed 640G, three CGM systems that are already available on the market, were compared in a head-to-head comparison.

Material und Methods

Twelve subjects with diabetes mellitus type 1 (T1D) participated in this monocentric, open-label trial. Main inclusion criteria were: diagnosis of T1D at least 6 months prior to the study, insulin treatment with insulin pen or pump for at least 3 months, body mass index < 35kg/m² und glycated hemoglobin level <8.4%. Main exclusion criteria were: pregnancy, medical conditions influencing glucose metabolism other than T1D and use of medication that impacts glucose metabolism other than insulin therapy.
The study was performed at the clinical research center at the Medical University of Graz and was approved by the ethical committee of Medical University of Graz. Sensor performance was determined by fulfillment of ISO 15197:2013 criteria, calculating mean absolute relative difference (MARD) for Abbott, Dexcom and Medtronic and illustrated by Parkes Error Grid (PEG).

Primary aim of the study was to evaluate the accuracy and reliability of three CGM systems.

Primary Outcome

Abbott, Dexcom and Medtronic fulfilled ISO criteria by 73.2%, 56.1% and 52.0%. The MARD for Abbott was 13.2 ± 10.9%, for Dexcom 16.8% ± 12.3% and for Medtronic 17.6%. Illustrated by PEG, 99.3% of reference pairs of Abbott, 99.3% of Dexcom and 98.6% of Medtronic fell within zones A and B.

Discussion

FreeStyle libre by Abbott has shown the best sensor performance in all glycemic ranges, however there is a need for improvement in future generations of all tested devices, especially in the hypoglycemic range.

Inhaltsverzeichnis

Table of Contents

Glossary and abbreviations

ADA	American Diabetes Associations
AGES	Austrian Agency for Health and Food Safety
AP	Artificial Pancreas
ARD	Absolute Relative Difference
ATP	Adenosine tri phosphate
BMI	Body Mass Index
CGM	Continuous Glucose Monitoring
DFS	Diabetic Foot Syndrome
DG4P	Dexcom G4 Platinum
DM	Diabetes Mellitus
EGA	Clarke Error Grid Analysis
FGM	Flash Glucose Monitoring
GCP	Good Clinical Practice
GLP-1	Glucagon-like peptide
GLUT	Glucose transporter
HbA1c	Glycated hemoglobin
ICU	Intensive Care Unit
ISF	Interstitial Fluid
JDRF	Juvenile Diabetes Research Foundation
MARD	Mean Absolute Relative Difference
medARD	Median Absolute Relative Difference
NI-CGM	Noninvasive Glucose Monitoring
OGTT	Oral Glucose Tolerance Test
rt-CGM	Realtime Continous Glucose Monitoring
SMBG	Self-Monitoring of Blood Glucose
T1D	Diabetes Mellitus type – 1
T2D	Diabetes Mellitus type – 2
WHO	World's Health Organization

List of Figures

List of tables

1 Diabetes Mellitus

Diabetes mellitus (DM) is defined as a group of common metabolic disorders, characterized by chronic hyperglycemia. Various types of diabetes mellitus are caused by a complex interaction of genetic predisposition and/or environmental factors. The most common types are diabetes mellitus type 1 caused by markedly reduced insulin secretion or even absolute insulin deficiency and diabetes mellitus type 2 caused by a decrease in glucose utilization, due to insulin resistance and/or impaired insulin secretion and increased glucose production. Worldwide the prevalence of DM has risen dramatically over the past decades. In 2000, the American Diabetes Association (ADA) predicted that until 2030, a steady increase in prevalence is to be expected with a number of 366 million people affected.(1,10,11)

2 History of Diabetes

The term diabetes is derived from the ancient Greek denoting “flow through”, a description of the cardinal symptom of polyuria. Literature/ manuscripts of ancient Chinese, Egyptians, Persians and Indians mention first a honey-sweet liquid precipitation in either more often older, overweight and indolent or lean people, which translates into the two most common types of diabetes, diabetes mellitus type 2 and type 1 respectively. (1,12)

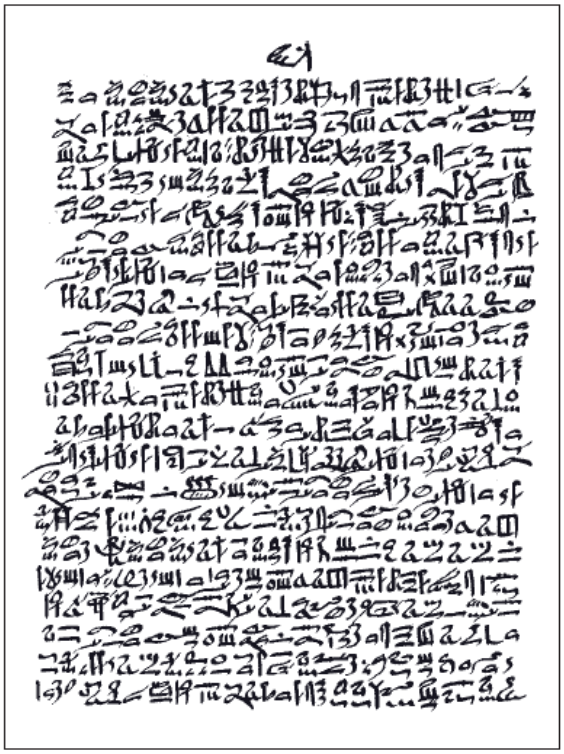

Fig. 1 The Ebers papyrus (1550 BC), The Wellcome Institute Library, London, UK (1)

Aretaeus of Cappadocia was the first to describe diabetes mellitus in its full clinical presentation, noting that an excessive flow of urine through the kidneys, thirst and weight loss exist. (1,13)

Until the 17th century, diabetes mellitus was largely considered as conspiracy theory and neglected, until an English physician, Thomas Willis, noticed the presence of sweet-tasting urine. In the 18th century, Matthew Dobson, also an English physician, proved that the honey-sweetness in urine is proportional to blood glucose values.(1,13,14)

In the 19th century, Claude Bernard, a French physician, discovered a link between elevated serum glucose levels and liver glycogen storage.(1,13,15)

In 1889, for the first time, the pancreas was described as central organ in the development of diabetes mellitus. In an experiment, Oskar Minkowski and Joseph von Mering removed the pancreas of a dog that consecutively developed all signs of diabetes mellitus type 1 and died shortly thereafter. (1,13)

Paul Langerhans was the first person to describe the histology of the pancreatic islets as a small cluster of cells. Édouard Laguesse took advantage of this discovery and further investigated its function, suggesting that they may produce a glucose-lowering substance. In 1921, insulin was discovered by Frederick G Banting and his student Charles H Best, who educed it from Langerhans islets of the pancreas of a healthy dog. Consecutively they demonstrated the reversal of induced diabetes by the application of the extract. The biochemists James B Collip improved the method to purify insulin and successfully treated the first patient, a 14 years old boy with symptoms of ketonuria and glycosuria, both of which almost disappeared after initialization of treatment. It was the first reported case of a patient who survived by chronic application of insulin the so far deadly disease. (1,13–15)

In 1936, Harold Himsworth first published a clear differentiation between the most common types of diabetes, namely diabetes mellitus type 1 (T1D) and type 2 (T2D). (13)

3 Regulation of Glucose Homeostasis

Euglycemia reflects the balance between hepatic glucose production and peripheral glucose utilization (uptake). The central regulator of the metabolic loop is insulin. It is produced in the beta-cells of the endocrine pancreas. The regulatory axis of glucose production, utilization and storage is formed by brain, liver, muscle, adipose tissue and beta cells of the pancreas that inter-communicate via neuronal and humeral pathways.
In case of low blood glucose levels and low insulin levels, glucagon secretion from pancreatic alpha cells triggers gluconeogenesis and glycogenosis and reduces glucose uptake in muscle and adipose tissue to normalize glycemia. Thereby glucagon promotes lipolysis of fatty acids and mobilization of amino-acids. (16)
Postprandial hyperglycemia is a trigger for beta cells to release insulin and to initiate a reversal process, promoting lipogenesis and protein synthesis by the storage of carbohydrates.(16,17)

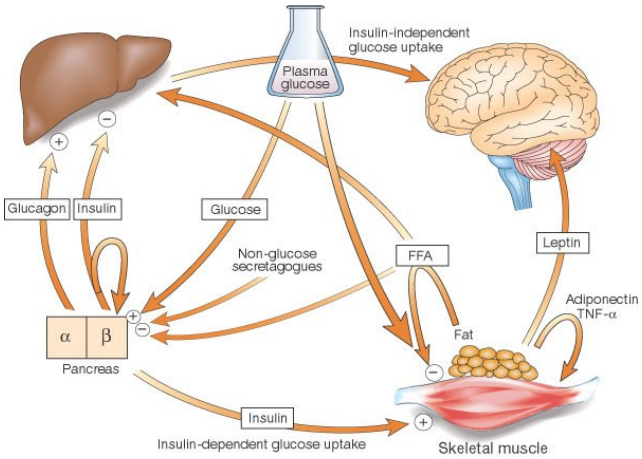

Fig. 2 Schematic overview of glucose homeostasis(2)

3.1 Insulin Biosynthesis

The beta cells of the pancreas produce preproinsulin, a precursor-polypeptide of insulin. Preproinsulin is single-chained and contains 86 amino acids. Subsequent proteolysis removes the signal-peptide “pre” in the endoplasmic reticulum. The resulting proinsulin contains three chains, A, B and C, which are connected by disulfide bonds. The Golgi apparatus forms vesicles, in which proinsulin is stored as insulin – zinc –complex. Proportional to rising glucose values, peptidases remove the C-peptide. Active insulin and C chain are consecutively stored together and if needed, co-secreted from beta cells. C-

peptide can be detected in blood serum and is - due to its longer half-life and no influence by exogenous administration in contrast to insulin itself - a useful marker of insulin secretion. (1,16,17)

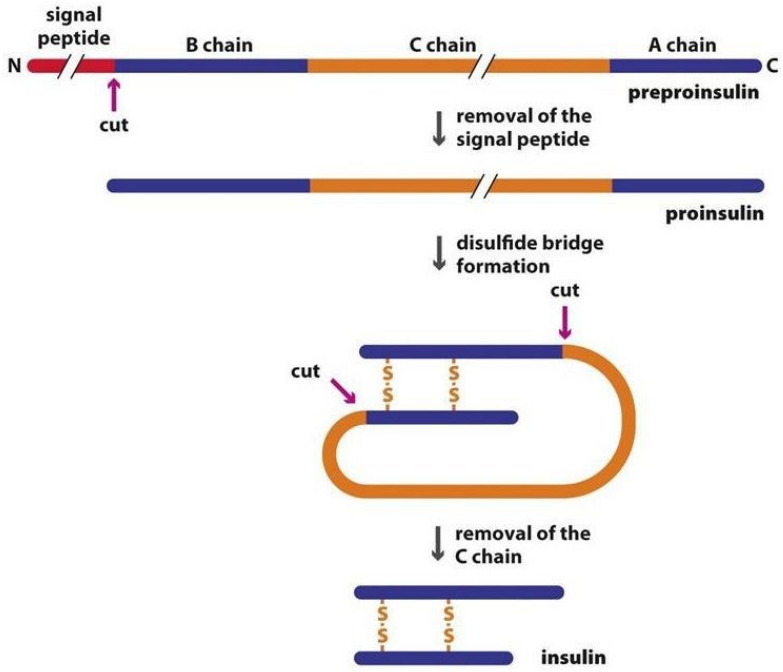

Fig. 3 Preproinuslin → Proinsulin → Insulin (3)

The total amount of stored insulin in the pancreas is 10mg, which can cover the requirements for five days. (17)

3.2 Insulin Secretion

Glucose levels above 70 mg/dL (key – regulator) and other nutrients (amino acids, ketones, neurotransmitters) regulate insulin secretion by enhancing protein translation and transcription. Glucose molecules are transported by glucose transporter (GLUT2) into beta cells and are phosphorylated. Further reactions to pyruvate generates adenosine tri phosphate (ATP) inhibiting the potassium ATP sensitive channel. This leads to a depolarization of the beta cell membrane, which causes to opening of calcium channels. The inflowing calcium stimulates insulin secretion from the vesicles of the beta cells. (1,16,17)

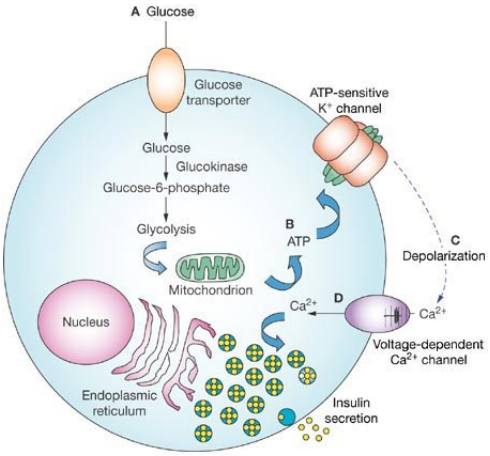

Fig. 4 Scheme of mechanism of glucose-induced insulin secretion (4)

Gastrointestinal peptides can also cause stimulation of the beta cells. The most potent protein, glucagon-like peptide (GLP-1), is released from the small intestine during meal intake. Its main effect is insulin synthesis and suppression of glucagon.(1)

3.3 Insulin Action

Once insulin is released into the blood stream, it binds to receptors on cell surfaces, promoting the uptake of glucose mainly in adipose tissues (lipogenesis), but also in liver and muscle tissue, where glycogen is synthesized. (1,16)
Furthermore, insulin receptor signaling induces inhibition of lipolysis and regulation, growth and proliferation of various insulin responsive cells. (16)

4 Diabetes Mellitus

4.1 Diabetes mellitus Type 1

Diabetes mellitus type 1 (T1D) is caused by the destruction of the pancreatic beta cells, which may be triggered by a combination of genetic, immunological and environmental factors.
The process of destruction leads to an absolute insulin deficiency that results in hyperglycemia and ketoacidosis, until insulin is sufficiently replaced. The onset of clinically overt T1D mostly occurs after a prodromal period, but the duration of this period may vary. (1,11,16)

4.1.1 Pathogenesis of Diabetes mellitus Type 1

The etiology of T1D is heterogeneous. It is a result of a combination of genetic, environmental and immunologic factors that leads to a chronic inflammatory infiltration of T cell lymphocytes and macrophages in the Langerhans islets ("insulitis") and cause beta cell destruction. Individuals with genetic predisposition who are exposed to a trigger that initiates the autoimmune process can develop the disease. In some cases patients may have islet – directed autoimmune disorders such as Addison's or Grave's disease, which are associated with T1D.(1,16)
Although all other cell types (α, δ and PP cells) are embryologically and functionally similar to beta cells they are not affected by the pathophysiologic process. The continuous reduction in beta cell mass may not affect the physiological insulin loop until approximately 80% of the beta cells are destroyed and the islets become atrophic. (1)
In patients with new onset of disease, circulating islet-related autoantibodies such as ICAs (autoantibodies against islet cells), IAAs (autoantibodies against insulin molecule), IA-2 (autoantibodies against tyrosine phosphatase) and GAD (autoantibodies against glutamic acid decarboxylase) are important immunologic markers.(1,16)
To date, no preventive or restorative intervention could be found to avoid or restore the onset of the disease. (16)

4.1.2 Development of Diabetes mellitus Type 1

Overt diabetes mellitus type 1 clinically presents after a prodromal period lasting months to years (i.e. the period until the beta cell destruction reaches 80%). Due to the progressive insulin deficiency, glucose utilization is impaired: Glucose transporters (GLUT2) that are needed to transport glucose molecules into liver, muscle and adipose tissue remain activated, resulting in hyperglycemia. In state of hyperglycemia (> 180mg/dL), glucose is excessively excreted into urine, because the renal threshold is exceeded. Symptoms of T1D often suddenly develop. Early symptoms due to the exceeded renal threshold are polyuria, polydipsia and insatiable thirst. Other important sudden onset symptoms are fatigue and weight-loss caused by the impaired insulin-dependent glucose homeostasis. The lack of insulin production triggers lipolysis and reduces glucose uptake into liver, muscles and adipose tissue.

Decreasing insulin levels consecutively lead to ketoacidosis, which in some cases may be the initial presentation. (1,16,18,19)

4.2 Diabetes mellitus Type 2

Diabetes mellitus type 2 (T2D) is characterized by insulin resistance, abnormal insulin secretion and increased gluconeogenesis. Abnormal fat metabolism is present in the majority of patients. The risk to develop T2D increases with age: starting from the age of 30, the risk is markedly increased at the age of 60. Unfavorable distribution of adipose tissue and environmental factors are associated with an increased risk also in childhood and adolescence.

In early stages, the glucose levels remain in the normal range, although insulin resistance is already present. This balance can be kept due to increased insulin secretion. Over time the excessive hepatic glucose release leads to beta cell failure by relative insulin deficiency resulting in hyperglycemia. Typical signs are polydipsia, polyuria and fatigue. Only in case of absolute insulin deficiency ketogenesis and weight loss might be present.(1,16,19)

4.3 Gestational Diabetes mellitus

During pregnancy, up to 14 % of all women develop glucose intolerance. One potential cause might be increased insulin requirements related to metabolic changes during pregnancy. Consecutive hyperglycemia might require dietary modifications and/or insulin treatment. Hyperglycemia during pregnancy is associated with an increased risk of fetal

complications such as preeclampsia or macrosomia or post-partum hypoglycemia (which may damage the central nervous system). (1,20)

4.4 Other Types of Diabetes mellitus

Other causes of diabetes mellitus can be caused by genetic mutations (e.g. cystic fibrosis, wolfram syndrome, maturity-onset diabetes of the young), pancreatic diseases (e.g. acute pancreatitis, chronic pancreatitis, chronic alcohol abuse, pancreatic cancer), corticosteroid therapy or endocrine conditions (e.g. Cushing syndrome). (1,16)

4.5 Acute Complications of Diabetes Mellitus

Depending on type of diabetes and degree of hyperglycemia and insulin deficiency, patients with diabetes mellitus require glucose-lowering therapy to avoid the occurrence of acute disorders such as diabetic ketoacidosis or hyperglycemic hyperosmolar state. (16)

4.5.1 Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) may be the presenting symptom of T1D. Absolute insulin deficiency causes hyperglycemia and hyperketonemia that consecutively lead to metabolic acidosis. Typical symptoms are nausea, vomiting, thirst, polydipsia, abdominal pain and shortness of breath with an odor of acetone. In 10% of cases DKA may result in coma. DKA most often occurs in patients with known diabetes, but it might also be the first presentation in a person who had not yet been known to suffer from diabetes. Usually an underlying condition such as intercurrent illness (pneumonia, diabetic foot syndrome, influenza, gastroenteritis and urinary tract infection), pregnancy, inadequate insulin administration (e.g. defective pump or pen) or myocardial infarction had led to DKA.
The lack of insulin and consecutive elevation of glucagon promotes the gluconeogenesis, glycogenosis and the release of free fatty acids from adipose tissue. Free fatty acids are converted via beta oxygenation into ketone bodies. The formation of ketone bodies leads to a metabolic acidosis, dehydration and a loss of electrolytes.

Ketoacidosis is a dangerous medical condition and that requires immediate treatment. The primary treatment of DKA is fluid- and electrolyte replacement as well as intravenous insulin therapy. (1,16)

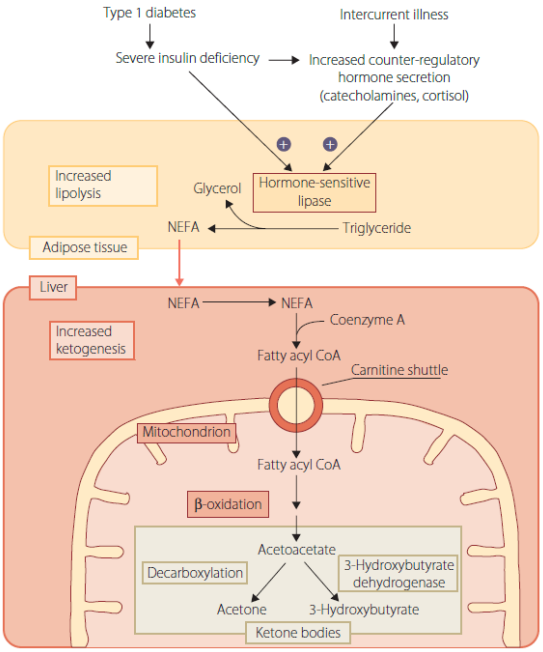

Fig. 5 Biochemical mechanism of ketoacidosis(1)

Severe complications of DKA are cerebral edema that even might cause cardiorespiratory arrest and thromboembolism.(1,16)

4.5.2 Hyperglycemic hyperosmolar state

The hyperglycemic hyperosmolar state (HSS) is an acute complication occurring mainly diabetes mellitus type 2. In HHS notable hyperglycemia (> 600 mg/dL) is the predominant clinical sign. Due to severe hyperglycemia massive dehydration occurs by osmotic diuresis (> 320mOsm/kg) and intravascular osmotic shift which subsequently leads to volume deficiency and electrolyte loss. In laboratory analysis besides hyperglycemia high bicarbonate levels are common but pH is usually not reduced. Patients often present with clinical signs of hypovolemia, hypotension and impaired tissue perfusion. HSS is similar to DKA a dangerous medical condition that requires immediate treatment. Main treatment modalities focus on fluid-, electrolyte- and insulin replacement. (1,16,21)

4.5.3 Hypoglycemia

Hypoglycemia is defined by abnormally low blood glucose levels (<70 mg/dL). Hypoglycemia most often occur in patients on insulin treatment but may also develop in patients treated with sulfonylureas. In most cases hypoglycemia is caused by accidental insulin overdosing in relation to actual insulin requirements: overestimation of carbohydrate content, exercise, vomiting, and stop of corticosteroid treatment or healing of an acute infection. There is no typical warning symptom prior to the occurrence of hypoglycemia. Symptoms of hypoglycemia can be divided into those caused by counter-regulatory hormones (epinephrine, adrenaline, glucagon) triggered by the decreasing glucose levels, and neuroglycopenic symptoms caused by insufficient glucose supply of the central nervous system. Typical symptoms caused by counter-regulatory hormones are e.g. shakiness, nervousness, sweating and rapid heartbeat. Neuroglycopenic signs comprise e.g. speak difficulties, atypical behavior, weakness, loss of coordination. Not all the above described signs are present in every hypoglycemic event. Also, there is no typical order of the appearance of the symptoms. In some cases, hypoglycemia might even be asymptomatic. Symptoms of hypoglycemia may vary by age, severity and the speed of the decline in glycemia. As worst case scenario hypoglycemia may result in seizures and coma, and in worst case even in death. Significant and repetitive hypoglycemia increases the risk of cardiovascular disease and damage to the central nervous system.

Hypoglycemia can be classified according severity (symptoms might be individual):

- mild hypoglycemia: 55 – 70mg/dL (frequent symptoms: hunger, trembling, sweating)
- moderate hypoglycemia: 40 – 55 mg/dL (frequent symptoms: mood changes, weakness, blurred vision)
- severe hypoglycemia: < 40 mg/dL (frequent symptom: coma

Treatment of hypoglycemia as recommended by the American Diabetes Association is the oral intake of 15-20 grams of glucose (e.g. glucose tablets, sugar-containing drinks) and regular blood glucose tests until glucose levels have normalized. In case of severe hypoglycemia subcutaneous glucagon administration or intravenous glucose application might be required (third-party help).

Especially in long-standing diabetes (both T1D and insulin-treated T2D) and patients with repetitive hypoglycemia there is a risk to develop hypoglycemia unawareness. Patients suffering from hypoglycemia unawareness do not feel symptoms of hypoglycemia despite low glucose values are present. Hypoglycemia unawareness is a considerable medical problem since patients might not adequately react by carbohydrate intake if they are experiencing hypoglycemia (1,11,16,22).

Hypoglycemia unawareness might be prevented by stable glucose control without relevant hypoglycemia and good patient education. (1,16,23)

4.6 Chronic Complications of Diabetes mellitus

Chronic hyperglycemia, as a result of poorly controlled diabetes mellitus can cause micro- and to some extent macrovascular complications.(16) Chronic hyperglycemia is predominantly a consequence of low patient compliance resulting in insufficient diabetes management. In T2D uncontrolled nutritional intake (high carbs, high fat) and lack of exercise promote hyperglycemia (1,17) Microvascular complications include the following: diabetic retinopathy – the main cause for blindness in industrialized countries, diabetic nephropathy – often requiring hemodialysis and diabetic neuropathies (e.g. peripheral and autonomous neuropathies). Diabetic foot syndrome (DFS) may develop as a consequence of peripheral polyneuropathy. DFS is the leading cause for atraumatic amputations. Macrovascular complications such as coronary heart disease, stroke and peripheral artery disease are mainly caused by arterial hypertension, hypercoagulability and hyperlipidemia and are only to a minor extent related to glycemic control.(1,16)

4.7 Complications of insulin therapy

Improper insulin application may trigger complications, such as hypoglycemia (most frequent complication) and hyperglycemia. Insulin therapy is often accompanied by weight gain due to anabolic effect of insulin or lipohypertrophy in case that insulin injection sites are not changed frequently.(1,24)

5 Diagnosis of Diabetes mellitus

The gold standard to establish the diagnosis of diabetes mellitus is the performance of an oral glucose tolerance test. The determination of fasting plasma glucose values or the assessment of glycated hemoglobin (HbA1c) might be alternative parameters to establish diagnosis.

Two random plasma glucose values above 200 mg/dL accompanied by symptoms such as polyuria, polydipsia, weight loss, are also appropriate for diagnosis. (1,16,25)

5.1 Assessment of fasting Plasma Glucose

Determination of fasting plasma must be performed in morning after an adequate period of fasting (eight to ten hours). The test should be performed from venous plasma and measured using a laboratory analyzer. According to WHO, plasma glucose values <110mg/dL (<6.1 mmol/L) are defined as normal glucose tolerance, values between 111mg/dL (6.2 mmol/L) and 125mg/dL (6.9 mmol/L) are suggestive of pre-diabetes and two plasma glucose values above 126 mg/dL (>7.0 mmol/L) are characteristic for diabetes mellitus.(1,16,26)

5.2 Oral Glucose Tolerance Test

The oral glucose tolerance test (OGTT) is the gold standard to establish the diagnosis of diabetes mellitus because of its accuracy. However, there is a broad spectrum (e.g. hunger, malnutrition, drugs and steroids) that can influence results by affecting glycemia. To avoid influence of meal intake, a fasting period of eight hours is recommended prior to the test. An oral glucose tolerance test is performed in three steps: first fasting plasma glucose is determined, followed by an oral intake of 75g glucose solution and then the determination of plasma glucose two hours after the consumption of the glucose solution. Two-hour post-challenge plasma glucose values <140mg/dL (<7.8 mmol/L) are suggestive of normal glucose tolerance, plasma glucose values >200 mg/dL (> 11.1 mmol/L) suggest establish the diagnosis of diabetes mellitus. (1,16,27)

In case of pregnancy, plasma glucose values have to be determined additionally at 60 minutes post-challenge. In that case, plasma glucose values at 1 hour post-challenge >180

mg/dL (>10 mmol/L) and after 2 hours post-challenge >153 mg/dL (< 8.5 mmol/L) establish the diagnosis of gestational diabetes(1,20)

5.3 HbA1c

Glycosylated hemoglobin (HbA1c) is a product of a biochemical, irreversible process, during which glucose molecules are attached to peptide side chains of erythrocytes, in relation to the blood glucose level.(1,17)Determination of HbA1c helps to identify average blood glucose concentration over a three-month period. The three-month period is to account for the lifespan of an erythrocyte (120 days).An elevated HbA1c value (> 6.5% or 46mmol/mol) is indicative for the presence of diabetes. Diagnosis should not solely be based on the determination of the HbA1c due to factors such as anemia, hemolysis, etc. that could influence results. HbA1c is not only used in the establishment of diagnosis but is also a marker of glycemic control after the diagnosis of diabetes has been established. HbA1c should be measured every three months in patients with diabetes and therapy should be adjusted accordingly. HbA1c treatment goals should be established according to patient age, diabetes duration, presence of late complications, comorbidities, and risk of hypoglycemia.(1,11,28)

6 Diabetes Therapy

The management of diabetes mellitus aims to normalize glycemia to avoid both acute and chronic complications and to allow a normal life style. To achieve these goals, not only medication but also education about lifestyle (weight loss, exercise and nutrition), self-glucose monitoring and insulin-administration is required. Treatment goals defined by the American Diabetes Association (ADA) is to safely establish glycemic control without increase in hypoglycemia. HbA1c targets depend on age, diabetes duration, comorbidities, cardiovascular risk and risk of hypoglycemia. Other comorbidities such as arterial hypertension, hyperlipidemia, obesity and smoking also need to be addressed to prevent cardiovascular events.(1,16,28)

6.1 Management of Diabetes mellitus Type 1

The most common treatment to date for patients with diabetes mellitus type 1 is basal-bolus insulin therapy. Basal insulin aims to supplement basal insulin requirements under fasting conditions to maintain euglycemia. For meals and in case of acute blood glucose excursions bolus insulin (rapid acting insulin) is administered depending on current blood glucose value, planned meal intake (carbohydrate content), insulin on board, activity level, and health status (e.g. infection). To be able to perform diabetes self-management, patients need to be well educated. Additionally, accurate blood glucose meters are required so that insulin dose adjustment can be performed safely. (1,11,16)

Insulin is injected subcutaneously by an insulin pen, insulin syringe or an insulin pump. Recommended suitable sites for insulin administration are the subcutaneous adipose tissue of the lower abdomen, upper outer thighs and arms. Injection sites have to be changed frequently to avoid the occurrence of lipohypertrophy (accumulation of adipose tissue), which causes slow and unreliable insulin absorption at the affected site.(1,11)

Insulin needs to be stored under controlled conditions: the insulin cartridge in use can be stored at room temperature for 21 days; insulin in stock needs to be stored between 2-8° Celsius.(1,11,29,30)

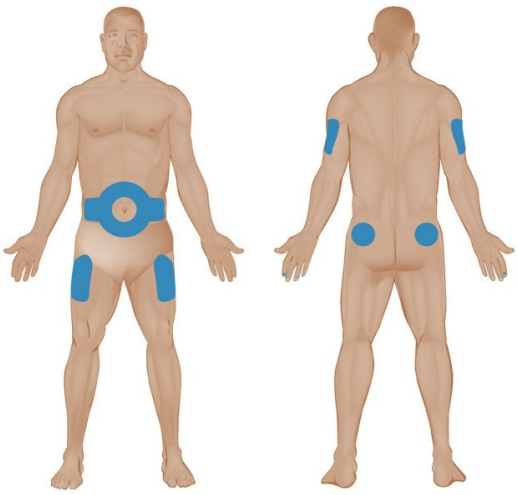

Fig. 6 Insulin injection sites(5)

- Regular insulin

Regular insulin is a form of short acting with no modification to effect prolongation. The absorption of regular insulin is slower than rapid acting. It takes about 30-60min until the insulin reaches the blood stream and begins to lower the glucose level. The maximum glucose lowering effect is reached after 2 – 5 hours.
Regular insulin is used to treat type – 1 diabetes, type – 2 diabetes, gestational diabetes or acute complications of diabetes such as hyperosmolar hyperglycemic state, hyperglycemia and diabetic ketoacidosis, as a postprandial insulin therapy or in combination as basal-bolus insulin(1,29–31)

Type of insulin/ brand names	**Onset**	**Peak**	**Duration**
Regular/ Novolin	30-60min	2-5 hours	5-8 hours

Tab.1a Regular insulin

- Rapid acting insulin

Short-acting or rapid-acting insulin is used to cover meals and to correct postprandial hyperglycemia. Rapid acting insulin is absorbed more quickly and can be directly before meal intake. Duration of rapid acting insulin is up to 5 hours. Rapid acting insulin can be used alone with meals (prandial insulin therapy) or in combination with basal insulin to establish basal-bolus insulin therapy. Because of its more stable and faster action profile mainly rapid acting insulin is used in the treatment of T1D.(1,11,29–31)

Type of insulin/ brand names	Onset	Peak	Duration
Lispro (humanolog)	15-30min	30-90min	3-5 hours
Aspart (novolog)	10-20min	40-50min	3-5 hours

Tab.1b Rapid-acting insulin

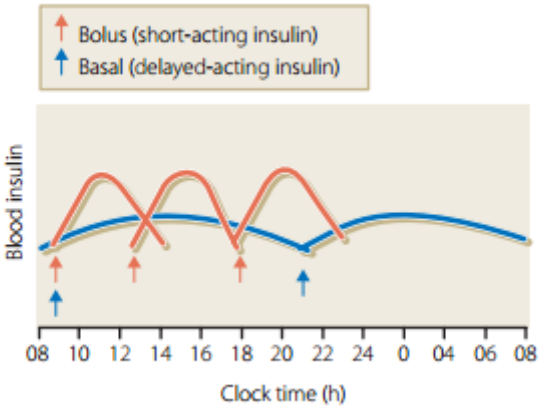

Fig.7 Basal-bolus insulin(1)

- Intermediate-acting insulin

Intermediate- acting insulin covers the need of about 12 hours. It is mainly used as basal insulin but due to is rather short duration of action needs to be injected twice daily in most cases. Onset of action is usually 90min after injection. One unfavorable property is that time to reach maximum effect may vary between 1.5-4 hours from day to day and patient to patient. Additionally the insulin is not flat but has a pronounced peak approximately 4 hours after injection what might cause hypoglycemia. Intermediate-acting insulin can be used as basal insulin therapy only in patients with T2D or can be combined with regular or rapid-acting insulin in basal-bolus insulin therapy. (1,29–31)

Type of insulin/ brand names	Onset	Peak	Duration
NPH	1-2 hours	4-12 hours	18-24 hours

Tab.1c Intermediate-acting insulin

- Long-acting insulin (basal insulin)

Long- acting insulin covers also cover basal insulin requirements as described in "intermediate-acting insulins". In contrast to intermediate acting insulins they have a flatter pharmacodynamics profile, longer duration of action of up to 24 hours and absorption properties are more stable. Time until onset can be up to 4 hours.(1,29–31)

Type of insulin/brand names	Onset	Peak	Duration
Insulin glargine	1-1.5 hours	No peak time	20-24 hours
Insulin detemir	1-2 hours	6-8 hours	Up to 24 hours
Insulin degludec	30-90min	No peak time	Up to 42 hours

Tab.1d Long-acting insulin

- Premixed insulin

Premixed insulin is a mixture of intermediate- and short-acting insulin in various ratios (e.g. 25:75, 30:70 or 50:50; short to intermediate-acting ratio). This type of therapy is most often used in patients who are not able to handle more complex insulin regimens. Mainly older patients with T2D or severely impaired patients with T1D use such insulin therapy. Because of the fixed, combination insulin therapy is less flexible as compared to multiple daily injections. Premixed insulin injections are usually injected one to three times a day with meals. Due to the peak approximately 2-3 hours after injection depending on the premixed ratio, snacks may be necessary to avoid hypoglycemia.(1,29,30,32,33)

Type of insulin/brand names	Onset	Peak	Duration
Humulin 70/30	30min	2-4 hours	14-24 hours
Novolin 70/30	30min	2-12 hours	Up to 24 hours
Novolog 70/30	10-20min.	1-4 hours	Up to 24 hours
Humulin 50/50	30min.	2-5 hours	18-24 hours
Humalog mix 75/25	15min.	30min – 2.5 hours	16-20 hours

Tab.1e Premixed insulin

6.2 Insulin Injection Techniques

Patients need to be well trained in the handling of their insulin injection device, insulin storage and in the injection technique to allow safe insulin administration. Insulin is dosed in forms of units. The correct insulin dose needs to be selected by the patient according to insulin sensitivity, carbohydrate factor, current blood glucose and planned meal intake. In some patients fixed insulin schemes might be appropriate.

The most commonly used injection devices are insulin pens. In some areas of the world patients still use insulin needle and syringe for insulin therapy due to reduced cost. The most expensive form of insulin delivery is the insulin pump. Two types of insulin pens exist: durable insulin pens where insulin cartridges (usually holding 3 mL of insulin at concentrations of 100-500IU/ml) need to be changed or disposable, prefilled insulin pens that can be discarded once the insulin reservoir is empty. The correct insulin dose is set by an adjustment wheel, which in some pens even allows the administration of half units. The length of the needle is selected based on body composition of the patient. In lean patients, short needles (4mm) should be used in lean, longer needles (up to 8 or even 12mm) in obese patients. Intramuscular injections should be strictly avoided due to the much faster insulin action in muscle tissue as compared to adipose tissue. Pens are due to the easy use and cost-effectiveness, the most commonly used insulin administration device in Europe. (1,6,11,31)

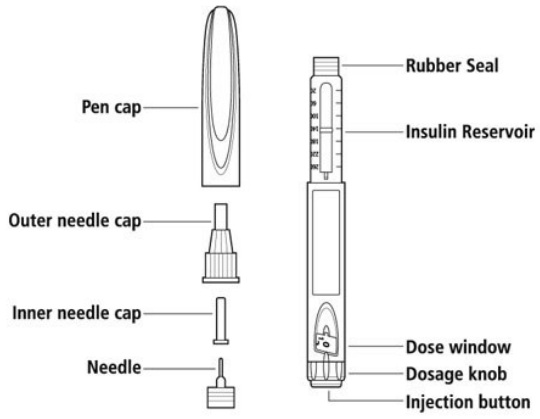

Fig. 8 Parts of an Insulin Pen(6)

Insulin pump therapy also known as continuous subcutaneous insulin infusion (CSII) is performed via an infusion pump worn externally. Design and configuration of insulin pumps may vary depending on manufacturer and model. An insulin pump includes:

- the pump (controls, processing module, and batteries)
- a disposable insulin reservoir (inside the pump)
- a disposable infusion set, including an infusion cannula which is inserted into the subcutaneous adipose tissue and a tubing system to connect the reservoir with the cannula.(1,11,34)

Recently, tubeless patch pumps have been developed including disposable or semi-disposable systems.(35)

Usually the insulin reservoir is filled with rapid acting insulin. It is then continuously infused according to the programmed basal rate. Bolus insulin can be administered on demand by the activating the bolus button. In this kind of pump therapy the patient has to program the pump himself and adjust basal- and bolus insulin according to blood glucose values. Early studies showed that the combined use of insulin pumps with continuous glucose monitoring (CGM) systems improves glycemic control. First systems integrating the CGM in the insulin pump (low glucose suspend, consecutively predictive low glucose suspend) automatically shut of basal insulin delivery in case of (impeding) hypoglycemia. Fully integrated systems (insulin pumps, CGM and control algorithm(10)) are called artificial pancreas systems (also closed-loop systems) and also address hyperglycemia.(1,11,36)

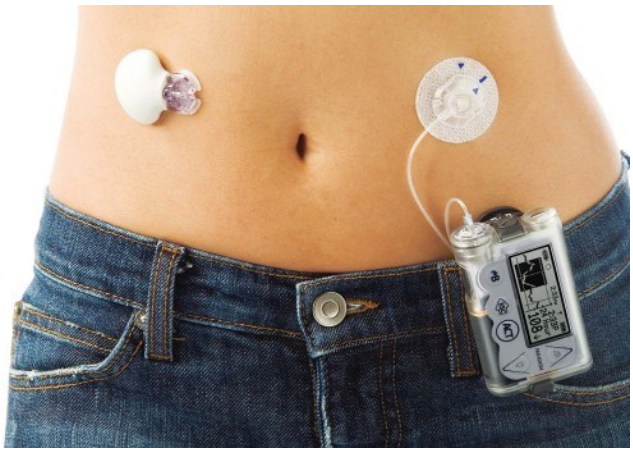

Fig. 9 CSII(7)

In several clinical studies, CSII improved glycemic control due to lowering HbA1c and decreasing hypoglycemic episodes. CSII is recommended for patients with a hypoglycemia unawareness, large variations in insulin requirements, small children and during pregnancy(1,11,34,37)

6.3 Closed-loop artificial pancreas

Even when using modern technology such as insulin pumps and CGM systems diabetes management is still burdensome. Success of insulin therapy is dependent from several factors such as correct insulin dosage, insulin absorption, timing of insulin administration, food intake, hormones other than insulin, physical activity and illness. (17,18,43,44). Adjunctive use of CGM/FGM in MDI as well as in insulin pump therapy (sensor augmented pump therapy, SAP) has been able to show improved glycemic control and reduced hypoglycemia rates.(38–41) As first steps towards closed-loop insulin delivery low-glucose suspend (LGS) and predictive low glucose suspend (PLGS) functions were introduced both of which improved glycemic control further without increased rates of DKA.(42,43)

As a next step, the first commercially available hybrid closed-loop insulin delivery is about to be introduced into the market in the USA (Medtronic 670G system). This system automatically adapts basal rates to avoid the occurrence of hypo- and hyperglycemia which shall help to further improve glucose control and facilitate diabetes management. The 670G system was evaluated in a clinical trial showing in a 3-month application during day and night no episode of severe hypoglycemia or ketoacidosis in 124 patients with T1D.(44) Still researchers focus on further improvement of closed-loop control because not all needs are yet addressed.

What is an artificial pancreas system?

An artificial pancreas system consists of externally worn medical devices consisting of a CGM, an insulin pump and a control algorithm that steers insulin delivery. (44,45)

The Juvenile Diabetes Research Foundation (JDRF) has defined the development of the closed-loop artificial pancreas in three generations, classified after degree of regulation.

The clinical evidence behind the AP, is the improvement showing of HbA1c reduction or reduction of hypoglycemic risks in children, adolescence and diabetic pregnancy. (44,46)

The first generation, non-closed loop systems, used to regulate insulin adjustment at low and high glucose values. In development stage one, the insulin pump automatically shuts down basal insulin delivery at very low glucose values for safety purposes if a patient is not responding to the warning signals (LGS). The second development step aimed to reduce occurrence of hypoglycemia by downregulation insulin delivery prior to hypoglycemia (PLGS). In the third step an algorithm to minimize both hypo- and hyperglycemia by up- or down-regulating insulin delivery accordingly was introduced. The second-generation systems, comprising development steps 4 and 5, comprise automated basal insulin delivery and adjustment. While in stage 4 mealtime insulin bolus still has to be applied manually, in stage 5 the necessity of manual mealtime insulin bolus shall be eliminated thus becoming a fully automated closed-loop system.
The third generation shall not only include insulin delivery but incorporate multiple hormones (e.g. also glucagon) which are fully automated delivered without necessity of intervention by the user. (37,44,45,47)

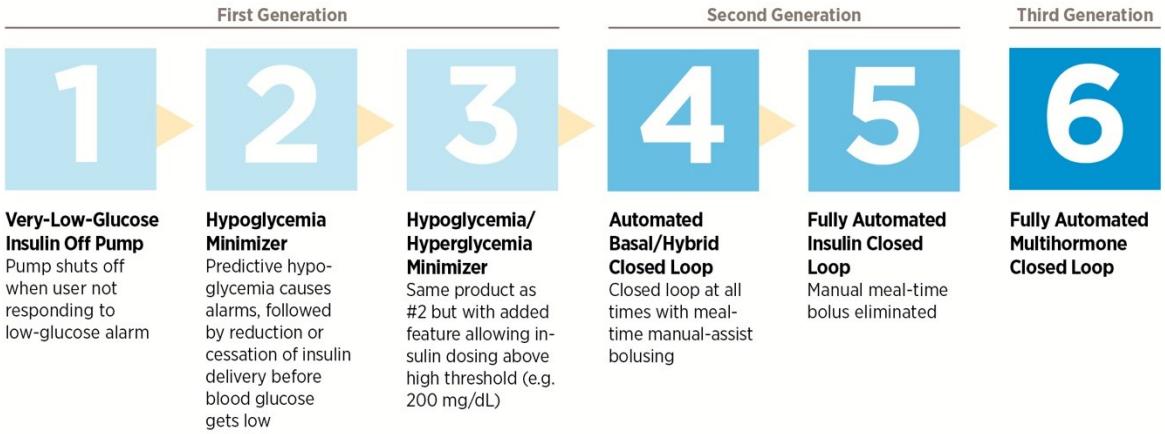

Fig.10 Schematic overview of the evolution of artificial pancreas systems (46)

For patients' comfort, future development focuses on the reduction of the size of the systems and to improve the hierarchic algorithm for insulin and/or glucagon titration to increase patients' safety.(37) Other approaches aim to develop single-port systems where only use one insertion site for both the CGM sensor and insulin catheter are required. This

single-port approach shall significantly improve patient comfort by reducing the numbers of insertions and insertion sites to be used. A recent study at the Medical University of Graz showed that insulin delivery at the site of glucose monitoring has no effect on sensor performance.(10,48)

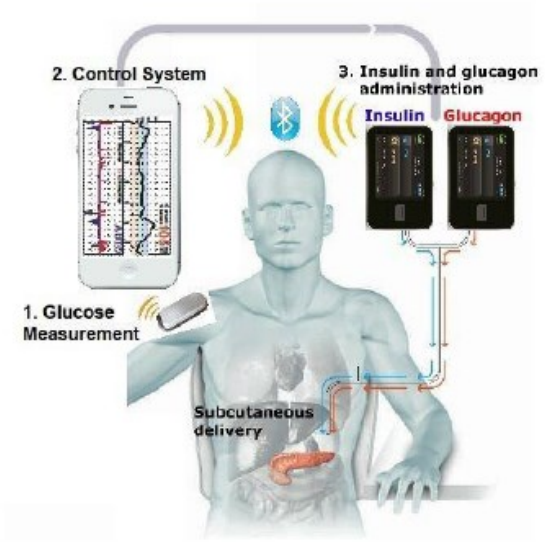

Fig. 11 Schematic presentation of closed – loop artificial pancreas device:
1. Glucose monitoring function carried out by CMG, 2. Digital Controller, analyzing and deciding adjustment of needed hormone therapy, 3. Dual Pump, which carries out Insulin or Glucagon administration (49)

7 Glucose Monitoring Systems

To allow safe establishment of glycemic control - especially in insulin treated diabetes mellitus – determination of current glycemia is important. Different methods of blood glucose measurement for home use (self-monitoring of blood glucose (SMBG), continuous glucose monitoring (CGM)) have been developed and introduced to the market in the last decades. More experimentally, researchers also aim to develop non-invasive glucose monitoring systems to reduce the burden of needle sticks or sensor insertion which is still present in current best practice. (50–53)

In contrast to the spot-measurements by SMBG CGM provides a continuous glucose signal and over time accuracy of SMBG vs. CGM based values has become comparable. SMBG and CGM differ in invasiveness: SMBG requires fingerstick measurements whenever a glucose value is needed whereas the minimally invasive CGM systems require sensor insertion every 5-14 days plus – depending on the system – fingerstick measurements for calibration.

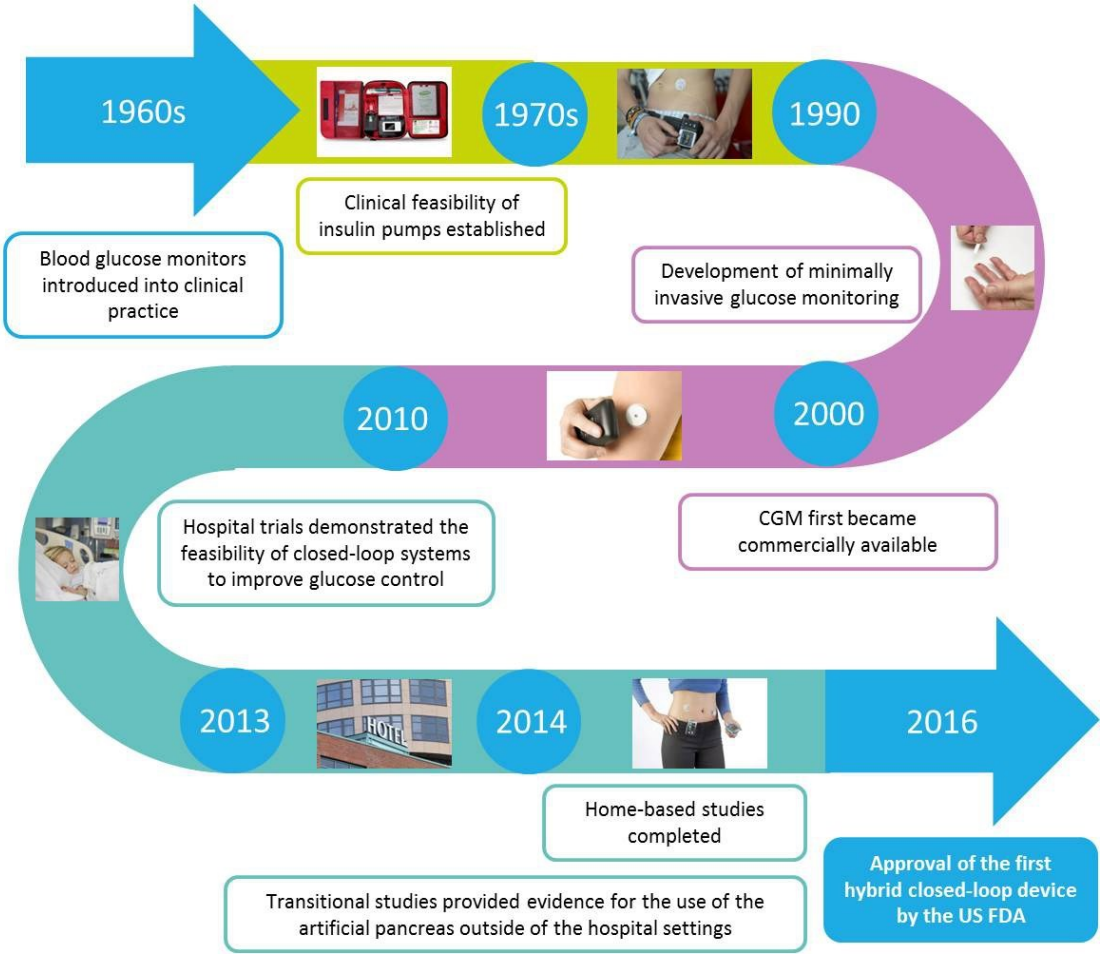

Fig.12 History of diabetes care: from SMBG to AP(54)

7.1 Urinary glucose determination

As it was known for centuries, diabetes is associated with elevated urinary glucose concentrations. Therefore first attempts to assess the extent of hyperglycemia were based on urinary glucose testing.(8,55–57)

Even in established diabetes elevated urinary glucose levels are only present during hyperglycemia when glucose is excreted via the kidney which usually occurs when the kidney threshold of 180 mg/dl is exceeded. During eu- and normoglycemia glucose is not excreted via the kidney. Additionally, the amount of glucosuria does not represent the current blood glucose level and the process is too slow, therefore insulin does adjustments cannot be based on urinary glucose levels. However, urinary glucose determination was the first method available for home-use and still has its value in current medical care since elevated urinary glucose levels can give a first hint towards present diabetes. (8,55)

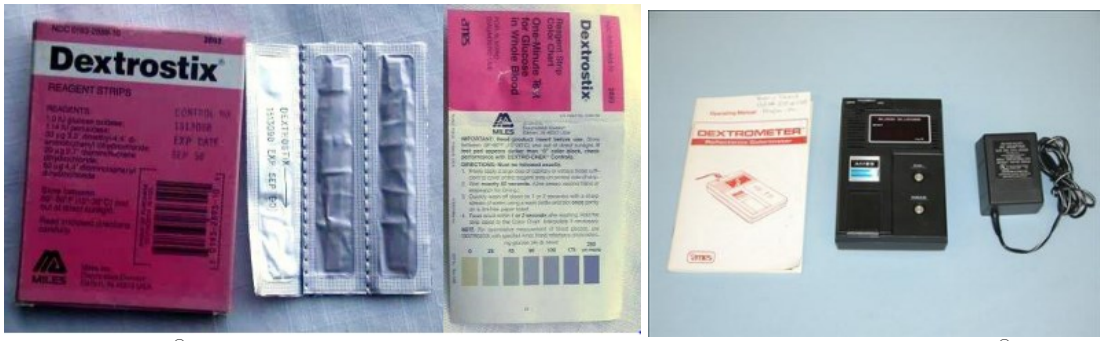

Fig.13 Dextrostix® urinary glucose strips working on the basis of color change (left panel).Dextrometer® electronic device to analyze Dextrostix (right panel).(8)

7.2 Self-monitoring of Blood Glucose

The measurement principle that was first developed for home use were test strips for the assessment of urinary glucose concentrations. The mechanism is based on a biochemical reaction of glucose and glucose oxidase. The resulting hydrogen peroxide induces a change in color of the test strip and can be compared to a color scale which reflects the glucose level. Consecutively, to allow more accurate glucose determination, first devices that converted the color-based result in a glucose value were developed. (5,7,8)

Due to the limitations of urinary based glucose measurements as described above, development of blood glucose monitoring systems was reinforced. Initially the principle used was similar to the measurement of urinary glucose: test strips with color indicators were used. Consecutively first systems for self-monitoring of blood glucose were developed based on the Clark (oxygen) electrode which used the biochemical reaction of

glucose oxidase as measurement principle. These glucose meters no longer used a visual color scale. Instead the test strip was inserted into the meter, and a drop of blood was put onto the measurement are. After a short measurement period the test result was displayed digitally on the display. (5,8)

Meter technology has continued to evolve until today and a vast number of companies has marketed devices. In currently available systems test times are short (seconds) and the required amount of blood is minimal (0.3 microliters). (5,6,8)

Nowadays self-monitoring blood glucose (SMBG) using glucose meters have become state of the art in diabetes care. To obtain a blood drop, patients have to perform a fingerstick using a lancing device. The small drop of blood (3-10 µL) is then used for the detection of the current plasma glucose value using an enzymatic reaction (e.g. glucose oxidase). Depending on the type of diabetes and therapy regimen different measurement strategies are recommended. Patients with diabetes mellitus type 1 should measure their blood glucose at least four times per day (premeal, bedtime). To optimize insulin therapy post-prandial and nocturnal measurements might be necessary. In patients with diabetes mellitus type 2 who are on intensified insulin therapy the measurement frequency is similar to patients with T1D. Patients on basal insulin therapy only need to measure their fasting plasma glucose. Measurement strategies for patients with T2D who are on oral agents only are still under debate.

However, SMBG only provides spot-measurements and does not provide glucose trends. (16,53,58)

SMBG is at present the most frequently used method for glucose determination, however recent publications show that continuous subcutaneous glucose monitoring with its alarm functions and trend analysis has a favorable effect on glycemic control while also reducing hypoglycemia. (59)

Fig.14 Example of a blood glucose meter (Abbott FreeStyle InsuLinx, Abbott Diabetes Care) (60)

7.3 Continuous Glucose Monitoring

In recent years, development of continuous glucose monitoring systems has made enormous progress in terms of longevity and accuracy. CGM systems are classified according to their invasiveness: invasive, minimally-invasive or non-invasive, the physical measurement principles or the use for clinical care (intravascular systems) or home monitoring (subcutaneously inserted sensors). (53) The most frequently used systems are minimally-invasive subcutaneous glucose monitoring systems, which are primarily inserted and managed by the patients themselves. An exception is the Eversense system, an implantable system which needs a minimally invasive surgical procedure for insertion. Continuous subcutaneous glucose monitoring systems are devices that continuously measure glucose values in the subcutaneous adipose tissue. The inserted senor responds to local blood flow and reacts electrochemically with glucose oxidase. Consecutively, the signal is displayed as glucose value in real-time. Glucose values are either displayed in real-time (rtCGM) or are recorded on the transmitter for analysis at a later stage (blinded CGM). In rt-CGM values are displayed in 1-5 minute intervals, show glucose trends and warn patients in case of impending hypo- or hyperglycemia. Recent studies showed that regular use of rt-CGM can improve glycemic control whilst also reducing the risk of hypoglycemia. (15,16,36,37,59,61–63)

In blinded CGM, however, a device is worn over a period (up to 5 weeks) and tracks glucose values continuously but without referring any information about glucose status, even in dangerous hypoglycemia or extreme hyperglycemia. In clinics or research centers, the data is downloaded and evaluated by the investigators. The aim of blinded CGM technique is to find trends or patterns as food or exercise in order to make decisions in diabetes management or to compare treatments in clinical trials.

Application in routine care could have a substantial benefit especially in patients who are at increased risk of hypoglycemia e.g. in diabetic pregnancy, patients with impaired hypoglycemia awareness, physically active patients, children/adolescents with T1D and patients in need of (long term) care with insulin-dependent diabetes. (16,37,47,59)

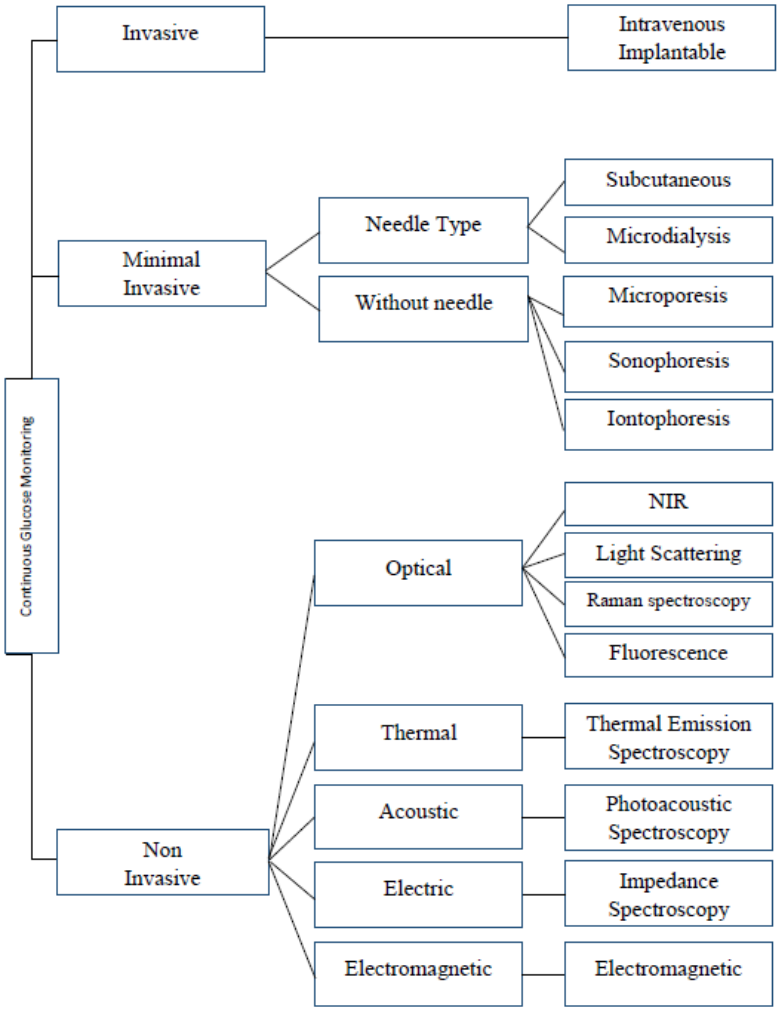

Fig.15 CGM sensor classification

7.3.1 Measurement principles

- Glucose-oxidase reaction

The glucose sensor works by an enzymatic reaction of glucose oxidase. Glucose-oxidase (GOx) is an oxido-reductase. The enzyme catalyses the oxidation and hydrolyzation of glucose to hydrogen peroxide and gluconic acids which is directly linked to the glucose concentration. Since oxygen is limited, and for every glucose molecule at least one oxygen molecule is required, mediators are donating electrons, generating a measurable electrical signal. (53,64,65)

- Glucose-sensitive hydrogel

When glucose is bound to the hydrogel, a measureable change in the hydrogel impedance can be detected, which correlates to the current glucose value. (53)

- Fluorescence

Fluorescence-based sensors measure fluorescence emitted by the involved tissue, when it is irradiated by light at specific wavelengths. The emitted light links the intensity of fluorescence to a defined glucose level. For example, elevated glucose concentrations are associated with a decrease of emitted fluorescence light. (16,64,66,67)

7.3.2 Invasive Continuous Glucose Monitoring

Invasive continuous glucose monitoring systems that are either intravascular systems or implantable sensors.

7.3.2.1 Implantable Glucose Sensors

At present, the only commercially available implantable glucose monitoring system is the Eversense® system. It consists of an implantable wired sensor containing fluorescent, glucose-sensing enzymes and a removable and rechargeable transmitter. (68) Glucose concentration is measured in the adipose tissue by using a fluorescent glucose-sensing polymer technology Currently the sensor can be used for up to 90 days; it is planned that in the near future the wear-time can be extended to 180-360 days.
The sensor is inserted in the upper arm during a minimally-invasive surgical procedure under the skin in the subcutaneous tissue after local anesthesia. Thereafter the sensor

wirelessly sends glucose data to a transmitter which is placed on the upper arm directly above the insertion site. The transmitter processes data and calculates the current glucose value which is then sent to a Smartphone app, which displays the current glucose value every 5 minutes. The app incorporates predictive alarming prior to hypo- or hyperglycemia. The sensor needs to be calibrated twice daily by using a capillary fingerstick measurement. Currently the system is not approved for guiding treatment decisions but is considered an adjunctive device. The transmitter can be worn 23.5 hours a day and only requires 30min of charging. Even if the smart phone is not available, the transmitter directly alarms the patient in case of hypo- or hyperglycemia by a vibrating signal.

After the 90-day wear-period the sensor needs to be explanted and a new one implanted by a physician.

In a 180-day prospective, multicenter, pivotal study the Eversense® system was evaluated. Results showed a mean absolute relative difference (MARD) of 11.1% as compared to plasma reference. Illustrated on the Clarke Error Grid analysis, 99.2% of the measured values fell within Zones A and B. 81% of hypoglycemic events were detected within 30 minutes. (63)

MARD and Clarke Error Grid analysis are discussed in more detail in chapter 7.6, “Criteria for accuracy of CGM”.

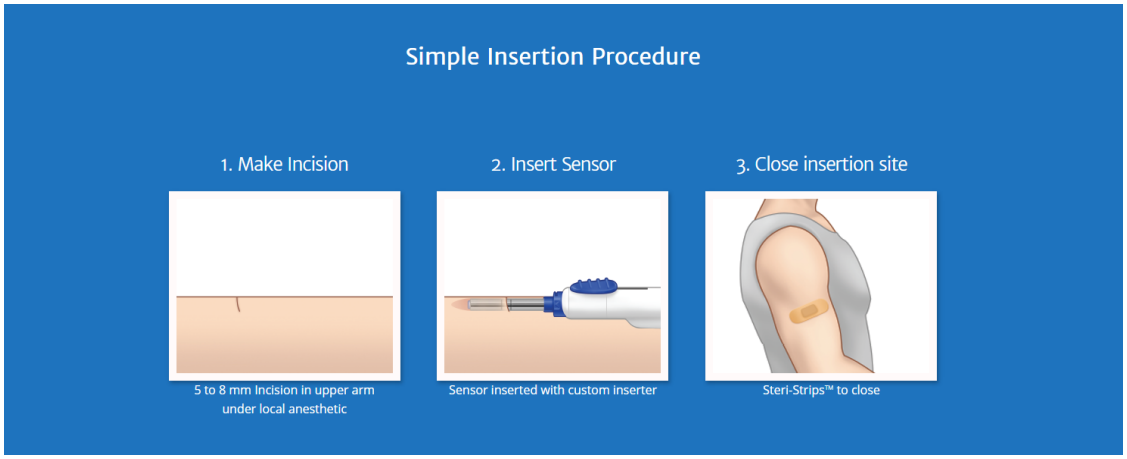

Fig.16 Insertion procedure of the Eversense® System(68)

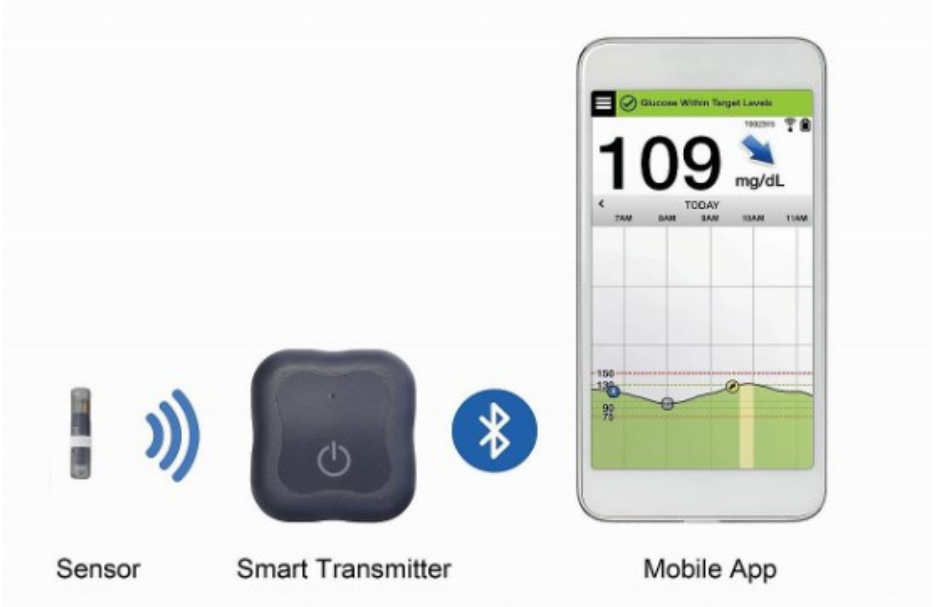

Fig.17 Schematic overview of the Eversense® System(68)

7.3.2.2 Intravascular Glucose Monitoring

Intravascular glucose monitoring was designed for in hospital, especially for intensive care unit (ICU) and ambulatory usage as alternative to invasive strip glucose measurements or laboratory analysis. The developed sensors using different detection techniques (e.g. electrochemical glucose oxidase, NIR, MIR, fluorescence) through a vascular access (central venous via CVC, peripheral venous or arterial) monitoring glucose values. Currently, the only commercially on market available intravascular glucose monitoring systems are GlucoScout® (Europe, United States), GlucoClear® (Europe) and OptiScanner 5000® (Europe) and in development, GlucoSet®. The development of intravascular glucose sensors is facing many technical challenges as difficulties in regulatory approval, thrombus formation, interferences with metabolic patterns and immune system and drugs, as heparin. (69–71)

Fig.18 The GlucoScout® accesses blood from a peripheral vein, using glucose oxidase for determination. It first samples about 1 mL of blood and after electrochemical analysis, it returns the collected sample with a non-heparinized calibration solution. (72)

7.3.3 Minimally-Invasive Glucose Monitoring

7.3.3.1 Microdialysis based CGM

Microdialysis mimics the function of a capillary blood vessel using a catheter with a semipermeable membrane which is perfused with an isotonic perfusion solution. Because of the concentration gradient from interstitial fluid to perfusate, glucose freely diffuses, across the semipermeable membrane, into the catheter, where it is pumped outside of the body. It can then be either directly measured with a connected glucose sensor or sampled in vials and measured offline in the laboratory. The amount of molecules crossing the membrane depends on tissue characteristics such as volume, pressure, temperature, and hydration but also on flow rate, size and cut-off the membrane and the concentration gradient.(53,73,74)

Currently no commercially available microdialysis based CGM system is used in routine care. The two main manufacturers of these systems are CMA Microdialyis (Sweden) and A. Menarini Diagnostics (Italy), but both systems are mainly applied in research.

7.3.3.2 Subcutaneous needle-type CGM

The most commonly used CGM systems are subcutaneous needle–type, amperometric systems. These systems sense glucose levels based on an electrochemical reaction of glucose oxidase with the enzyme immobilized on the inserted subcutaneous electrode. The

catheters are inserted into the subcutaneous adipose tissue and constantly measure the current of the interstitial fluid which is after calibration representative of the glucose level. Currently four systems are available on the market; details of the individual systems are described below. Depending on the individual properties of the systems, sensors can be worn between 5-14 days and calibrations to blood are required in most cases twice daily. The only system that is currently working calibration-free is the Abbott FreeStyle Libre system. For commercially available systems MARDs of 10-15% are deemed safe for application in routine care. Of note, signal accuracy largely depends on glucose fluctuations, variable tissues oxygen tension, applied pressure and wound response. (53,75,76)

7.3.3.2.1 Dexcom G4 Platinum

Dexcom G4 Platinum (DG4P) measures glucose continuously every 5 minutes, using a thin electrochemical sensor, which is placed subcutaneously by an injector. The current glucose readings are displayed wirelessly in real time to a transmitter which can be located up 6 meters apart. The readings can be stored for up to 7 days.
The sensor requires to be calibrated every 12 hours. According to the manufacturer accuracy is sufficient for directly basing insulin dosing decisions on the sensor readings: 97% of the measurements are in the clinically accurate zones A and B of Clarke Error Clark Grid. DG4P alarms in case of hypo and hyperglycemia. The alarm thresholds can be programmed individually according to patient requirements. The default alarm settings are 11.4 mmol/L and 4.4 mmol/L. These alarms can be muted. However, if glucose levels fall below 3.1mmol/l this alarm is non-deactivatable.
DG4P sensor can used for up to seven days.(51,77,78)

Fig. 19 Dexcom® G4 Platinum – set(79)

7.3.3.2.2 Medtronic MiniMed 640G

The Medtronic MiniMed 640G systems is integrated in the MiniMed insulin pump. The CGM component is working similarly to the Dexcom system: an electrochemical sensor (Enlite®) is inserted into the subcutaneous adipose tissue with an insertion device and is connected to a transmitter. The MiniMed 640G system enables the tracking of glucose values in real time and incorporates an alarm function in case of hyper- and hypoglycemia. As a first step towards artificial pancreas, this system can proactively shut down basal insulin delivery for up to 2 hours in case of impending hypoglycemia when used in combination with the MiniMed insulin pump (predictive low glucose suspend function, SmartGuard® technology).

The sensor wear-time is 6 days and requires to be calibrated at least twice daily. For calibration, a Contour® Next Link blood glucose meter from Ascensia is used that automatically transmits the SMBG value to the CGM system.(80,81)

Fig. 20 Medtronic® MiniMed 640G(82)

7.3.3.2.3 Medtronic iPro®2 CGM

The iPro®2 professional continuous glucose monitoring by Medtronic is a small, waterproof subcutaneously inserted device, which tracks glucose values continuously and enables analysing a patient's diet, medications and glycaemic ranges during daily activities. It contains the iPro®2 recorder and the Enlite® sensor, that tracks glucose values every five minutes and memorizes up to 288 glucose values per 24 hours. Via iPro-Software, data can be uploaded to CareLink™ and be evaluated, making it easier to control patient's lifestyle and if necessary adapt therapy to lead to a glycaemic improvement. Also, blinded CGM can be used in clinical research, in which data are used to compare treatment or determine quality of diabetic control. (83–85)

Fig. 21. Medtronic® iPro®2 CGM(86)

7.3.3.2.4 Flash Glucose Monitoring System

Flash glucose monitoring (FGM) is an alternative to conventional CGM. The only system of its kind is manufactured by Abbott Diabetes Care and it is on the market since 2014. It consists of a subcutaneous sensor placed on the back- or outside of the upper arm. The sensors also measure glucose in the interstitial fluid based on an electrochemical reaction every minute. The sensor can be worn for up to 14 days. In contrast sensor data are transmitted to the reader by near-field communication (NFC) only when the user proactively scans the sensor. Glucose data are stored on the sensor in 15 minute intervals and can be stored on the sensor for up to 8 hours. Values older than 8 hours will be overwritten if they are not transferred to the reader. The system does not require calibration but is factory-calibrated. The reader also incorporates a built-in glucose and ketone meter. The reader can memorize data for up to 90 days.
Due to NFC, FGM does not alarm when glucose levels approach hypo- or hyperglycemia. (9,87)

Fig. 22 From Abbott® recommended placement of Abbott FreeStyle Libre sensor unit on the upper arm. The reader uses near field communication. To show glucose trend arrow, data are transmitted when reader is brought to the sensor. (9)

7.3.3.2.5 *Experimental approaches Single-port Systems*

Research also focuses on novel methods of minimally glucose monitoring to relive the burden of disease of patients.

Single Port Systems

Different to on market available CGMs, single-port systems such as the Spidiman approach aim to combine the insertion site of the insulin infusion cannula with the glucose sensor to reduce invasiveness. The Spidiman sensor is based on phosphorescence oxygen sensors, which are not influenced by electrochemical parameters (e.g. acetaminophen, ascorbate…(88)). (10,48,88) Therefore a fluorescent dye is applied onto the outside of an insulin infusion cannula. The Spidiman sensor unit comprises two phosphorescence based sensors coated onto the cannula of the insulin infusion set. The optical read-out unit consists of miniaturized two channel phase fluorimeters which can be attached to the sensor unit, where the signal is processed. The glucose sensor utilizes an oxygen sensitive fluorescent dye coated with glucose oxidase as a sensing element that converts oxygen measurement into a glucose concentration. At current stage, to log raw data and set measuring parameters, the control unit is USB connected to a notebook.

The main advantage of single port systems is that insulin can be delivered directly at the site of the glucose measurement, but does not influence sensor glucose readings and by using only one insertion site increases patient comfort.

To date, no single-port system is commercially available and they are used in clinical trials only. (10,48) Other research groups aim to combine commercially available electrochemical sensors with insulin infusion cannulas in proof-of-concept studies. Regittnig et al have been the first to demonstrate that basal insulin delivery does not influence sensor glucose readings when the sensor is applied directly at the infusion site. Only during bolus insulin administration due to the dilution of the glucose concentrations readings are erroneously low.(89)

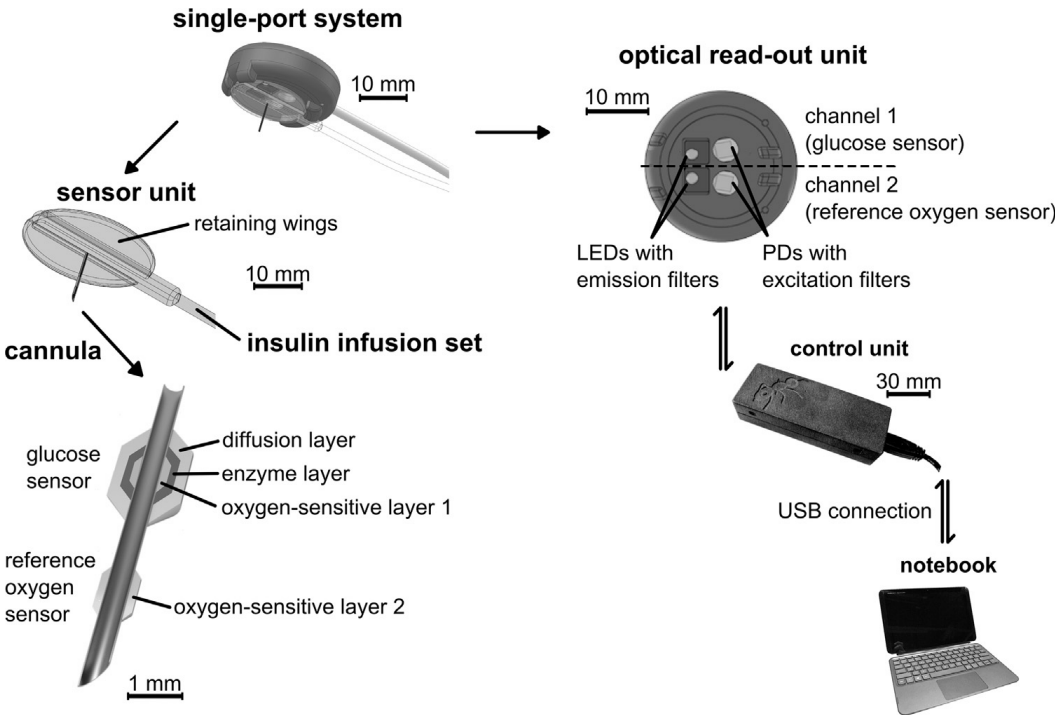

Fig. 23 Schematic diagram of the Spidiman single-port system. It consists of two units: The sensor unit and the optical read-out unit. The sensor unit comprises two phosphorescence based sensors coated onto the cannula of the insulin infusion set. The optical read-out unit is a miniaturized two channel phase fluorimeter which can be attached to the sensor unit. The optical read-out unit is connected via a thin, flexible cable to the control unit, where the signal is processed. To log the raw data and set the measuring parameters, the control unit is USB connected to a notebook.(10)

7.3.3.3 Transdermal techniques

Transdermal techniques are minimally–invasive. They do not require the insertion of a needle-type sensor into the subcutaneous adipose but are based on the application of physical energy to access the interstitial fluid by a device attached to the surface of the skin. A interstitial fluid (ISF) sample is extracted and analyzed for its glucose concentrations.(8,64,90) Various methods have been described by research groups:

- Micropores

Transdermal glucose sensing using pulsed laser beam to create micro-pathways by thermal ablation to perforate the stratum corneum of the skin and thus enabelling the collection of ISF by applying a vacuum to the skin. The glucose level is determined by an externally worn glucose sensor using colorimetry.(64,91)

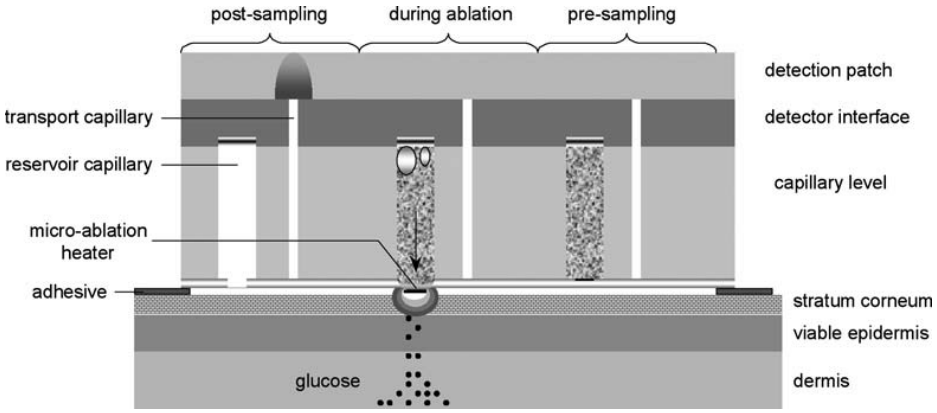

Fig.24 A Polydimethylsiloxane-based patch device is fixed to skin surface. The stratum corneum is thermally ablated, allowing glucose to diffuse from the dermis into the capillaries of the device. The molecules reach the detection layer by capillary forces, where they are quantified colorimetrically.(64)

- Sonophoresis

Sonophoresis based glucose monitoring uses low-frequency ultrasound that generates microscopic holes into the stratum corneum in order to collect ISF for glucose extraction. Glucose concentration is then measured using electrochemical or optical methods. The sensors are directly placed in contact with skin. (53,64,90)

- Reverse Iontophoresis

Reverse iontophoresis uses electric current to drive charged compounds, which also are high polar, across the skin. Electromigration (movement of small ions through skin under influence of induced electric field) and electroosmosis are the two main transport mechanisms. Electrode reactions transform the electron fluxes into ions whose transport proceeds electroneutrally through the skin.

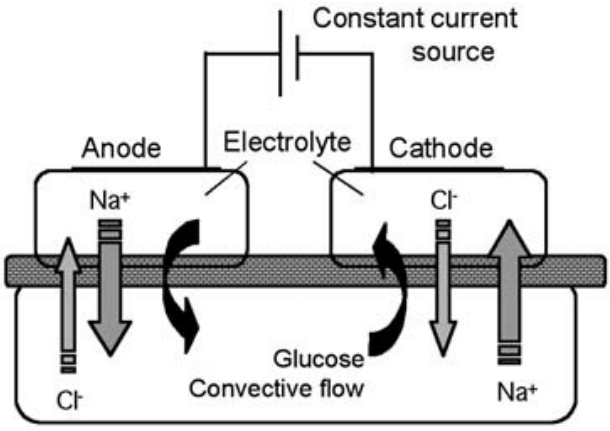

Fig. 25 Scheme of reverse iontophoresis:
Preferential Na^+ migration across the permselective membrane includes convective solvent flow, which carries glucose towards the cathodal chamber.(64)

- Skin suction blister technique

This reportedly painless and well tolerated technique uses vacuum to form blisters at the epidermal-dermal junction. Once a blister is formed due to the introduction of negative pressure, vacuum is released and samples of blister fluid are collected. The ISF of the blisters is consecutively analyzed biochemically. Qualitatively it is reported to have similar characteristics to serum patterns, but contains less protein. In literature, it is also described as the mirror of the interstitial fluid.(64)

7.3.4 Non-Invasive Glucose Monitoring

These technologies are also still very experimental and none of these methods have been CE-marked or FDA approved. No commercially system is available on the market. Whether in the future these technologies will replace currently available technologies is not clear yet.
Non-invasive glucose monitoring (NI-CGM) measures glucose using optical, thermal, acoustic and electrical techniques through skin without the need to extract blood or to collect ISF.(59,64)

7.3.4.1 Optical techniques

Truly non-invasive techniques for glucose monitoring are based on spectroscopy. A light beam is emitted into the tissue, where it interacts by absorption, reflections and/or scattering.

- Near-infrared absorption

The near–infrared optical absorption is described by the Lambert-Beer law as a spectroscopic method that uses the near–infrared electromagnetic spectrum for medical and physiological diagnostics e.g. the determination of blood glucose, pulse oximetry and more. The method is based on excitation of molecular vibrations by electromagnetic radiation.
The overtone and combinations of the fundamental vibrations of molecular bonds (C-H, N-H and O-H) define the frequency band of light-absorption. The attenuated re-emerging light is measured, statistical evaluated and so quantities analyzed. (8,53,64,90)

$$E_\lambda = -\lg\left(\frac{I}{I_0}\right) = \epsilon_\lambda \cdot c \cdot d$$

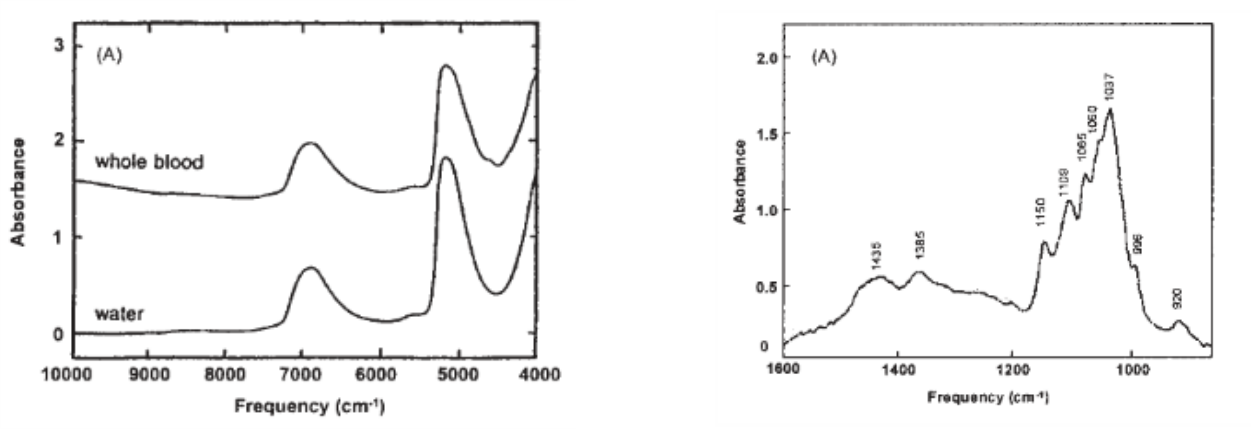

Fig. 26 Near- infrared absorbance of interfering substances of glucose measurements (left) and optical absorption spectra for glucose (right.)(64)

- Light Scattering

Light interaction with tissue, wherever it is directed to, leads to absorption, reflection and scattering. The amount of scattering depends on the density of the medium to be penetrated and the scattering particle. If the scattering particle is greater than the medium, a strong scatter results; if the medium and the particle are of equal size, the scattering suspension is transparent. As soon as the glucose concentration increases, refraction of blood and intestinal fluid remain elevated as an optical correction, while the refracting index of particles is unchanged in skin, which means a decrease of overall scattering. (8,53,64,90)

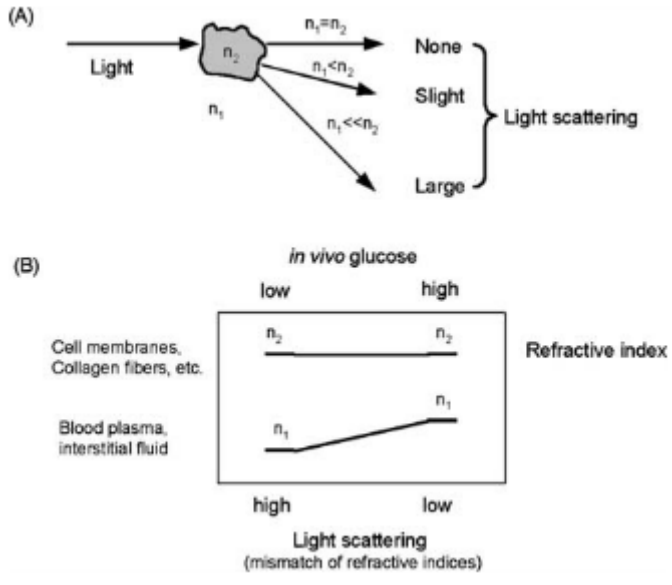

Fig.27 Glucose measurements by light scattering. (A)
Scattering in turbid media depends on the ratio of refractive indices of the solvent (n1) and the scattering particles (n2). Scattering increases with a rising mismatch of refractive indices. (64)

(B)
An increase in glucose concentration in vivo increases the refractive index of blood and ISF (n1), while the refractive index of the scattering particles in the skin (n2) is not affected. Because of the lower mismatchof refractive indices, light scattering is therefore decreased. (64)

- Raman spectroscopy

Raman spectroscopy is an analytical method, applied especially in biochemistry, which uses the inelastic scattering of molecules. Complementary to infrared, it observes vibrations, rotations and scattering of monochromatic light irradiation. In the spectrum of the light scattered on the sample, frequencies are recorded. The difference in frequency to the frequency of irradiated light is matched to the energies of optical-rotation or vibration processes which allows the description of certain molecules.
Raman signal from human skin is known for being weak and overlapped by fluorescence signals. However due to high-pass filter, the reduction of fluorescence light is possible and so, an accurate measurement of blood glucose might be feasible. (8,64,90,92)

- Fluorescence

Fluorescence based glucose monitoring system is explained above in chapter 7.3.2, "Invasive Continuous Glucose Monitoring". Of note, fluorescence based monitoring can also applied transcutaneous.

7.3.4.2 Thermal Emission Spectroscopy

Thermal emission spectroscopy uses the thermal-optical response of the skin to mid-infrared spectroscopy that is emitted by human body. As already mentioned, mid-infrared obtains spectral information from blood, which can be transferred directly to blood glucose values, which influence the cutaneous microcirculation. The dependence of the infrared signal on temperature presents a source for reflectance measurements. Thermal spectral characteristic is influenced by human tissue composition and concentration of molecules. Especially the tympanic membrane is a good temperature measurement spot due to its direct blood-sharing with the hypothalamus (temperature regulatory center). (53,64,90)

7.3.4.3 Impedance Spectroscopy

Electro radio-wave spectroscopy is used to measure dielectric properties of biological tissues in three basic methods: bridge method, voltage method and current method. A generator uses constant current applied to the tissue and its impedance measured. For glucose measuring, higher frequencies up to 200 MHz are used to interfacial polarization of erythrocytes and their interaction with glucose patterns. (53,64,90)

7.4 Criteria for the Assessment of CGM Accuracy

Development in technology requires evaluation of accuracy of the new CGM systems (agreement between novel technology and reference method), performance and safety of the monitoring systems. Recent studies confirm that overall CGM performance has improved in accuracy and reliability over time. This advances in CGM accuracy has promoted these systems from being supportive/adjunctive tools to stand-alone systems based on which patients can steer their diabetes therapy.(93–95)

Performance of CGM systems can be evaluated by the following criteria:

7.4.1 Bias

Systematic error in measurement can be caused by inexpert or missing calibration or the usage of an inaccurate reference system. Built-in errors can lead to erroneous measurements so that they cannot be used for therapy. Bias can typically be observed during hypoglycemia or/and hyperglycemia, as a (percentage of) difference between sensor and reference under- or overestimating the true glucose concentration. The more accurate a CGM, the less is the bias (ideal: 0.0% deviation).(94)

7.4.2 ISO Criteria

ISO criteria are applied to describe glucose meter performance and upon these criteria, approval of a system can be granted or denied. The errors are classified according to the relative or absolute size of the error. ISO 15197: 2013 requires that 95% of the meter values are within 15 mg/dL of the reference value at glucose concentrations <100 mg/dL and within 15% at glucose concentrations ≥100 mg/dL.(61,94,96)

7.4.3 Precision Absolute Relative Difference

The reproducibility of measurements evaluated by precision are assessed by repeating the measurements and comparing the results.
The Precision Absolute Relative Difference was specially developed for the assessment of continuous glucose monitoring systems. It expresses and compares data pairs of at least two CGM systems instead of blood glucose. Since Mean Absolute Relative Difference (MARD) is directly affected by outliers, PARD is rather robust due to the large number of data.

$$PARD = 100\frac{|y_{CGM1} - y_{CGM2}|}{\text{mean}(y_{CGM1}, y_{CGM2})} (0.1)$$

The difference of the two data pairs, are divided by the arithmetic mean of both values (mean y_{CGM1}, mean y_{CGM2}).
Data of Median Absolute Relative Difference (med ARD) and MARD can be generally interpreted as PARD, and vice versa median and mean PARD values can be converted and interpreted as MARD. (61,94,97)

7.4.4 Arithmetic deviation

For each sensor-reference pair, arithmetic deviation can be calculated by calculating the difference between sensor and reference values. The closer to zero the difference, the more accurate is the glucose reading. The arithmetic deviation is because of its precision a very popular method to assess sensor accuracy.
The absolute value of the arithmetic deviation is called absolute deviation and thus is not influenced by positive or negative deviations which might add up to zero difference. (94)

7.4.5 Absolute Relative Difference (ARD)

The ARD is the most common method to express the absolute difference from sensor to reference glucose values. Data from blood glucose and sensor glucose are paired and difference is calculated as described below. (61,94,97)

$$ARD = 100\frac{|y_{CGM} - y_{RBG}|}{y_{RBG}}$$

The Mean Absolute Relative Difference (MARD) is a precise measure for the evaluation of CGM performance. MARD calculates the difference between by CGM and reference values as described below.
Limitations of MARD are dependence of rate of change of the glucose, the number of paired values and the comparison with the reference glucose, which may also incorporate an error (e.g. if the reference measurement is not taken properly, is diluted etc.).(61,94,97)

$$\mathrm{MARD} = \frac{1}{N}\sum_{k=1}^{N} \mathrm{ARD}_k$$

In contrast to MARD where the mean value of the ARDs is used, the median ARD (medARD) is calculated by computing the median of all ARDs. The median ARD is usually less affected by outliers.(94,97)

7.4.6 The Clarke Error Grid

The Clarke Error Grid is used to quantify the clinical significance sensor accuracy as compared to reference. A Cartesian diagram illustrates the reference values on x-axis and on the sensor values on the y-axis. Data are then displayed in one of five possible regions:

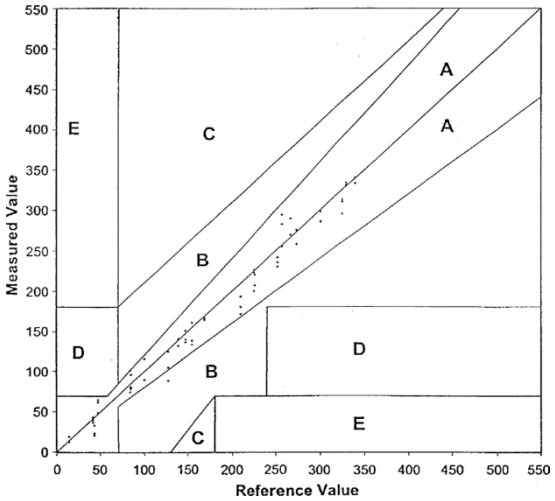

Fig.28 Clarke Error Grid(98)

Zone A: data with a maximal deviation of 20%, no effect on clinical decision

Zone B: benign error; data pairs with a deviation more than 20% but with no effect on therapeutic decision

Zone C: glucose values, which may affect clinical decision/outcome

Zone D: altered clinical action; potentially dangerous error by over- or underestimating of hypo- or hyperglycemia

Zone E: altered clinical action; may lead to dangerous consequences

The Clarke Error Grid was first presented in 1987. Limitations of this type of grid are the regions A to E, which are arbitrarily chosen and the "time-delay" of CGM which is not taken into account. (94,97–99)

To overcome this problem, in 2000 a modification of the Clarke Error Grid was presented by Park et al., called Parkes Error Grid. (100)

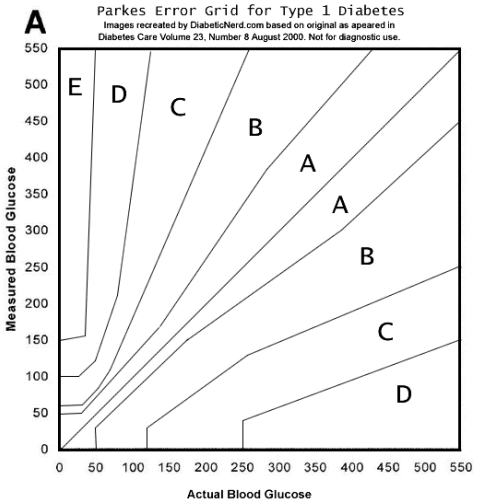

Fig.29 Parkes Error Grid (101)

The Parkes Error Grid also is composed of 5 zones with similar clinical consequences as described for the Clarke Error Grid but also incorporates the glucose rate of change (rate–time –relation).(100)

MARD, medARD and Clarke Error Grid are preferentially used in head-to-head comparisons of CGM systems. In the subsequent part of the Master Thesis, as main part of the Thesis, a monocentric single-center study to compare the performance of three different glucose monitoring systems (Abbott, Dexcom and Medtronic) will be described.

8 Clinical Study

In the present trial, the performance of three commercially available glucose monitoring (GM) systems, namely Abbott FreeStyle libre, Dexcom G4 Platinum and Medtronic MiniMed 640G, were tested under standardized real-life conditions in a head-to head comparison. Concomitantly the performance of a novel optical glucose sensor developed by Joanneum Research in collaboration with the Medial University of Graz and the University of Technology was used for the first time in humans.(10,48)

8.1 Methods

8.1.1 Study Design

The trial was approved by the local ethics committee of Medical University of Graz (EC number 27-393 ex 14/15) and the Austrian Agency for Health and Food Safety (AGES, reference number INS-621000- 0734-005) and is listed at the National Institutes of Health (http://www. clinicaltrials.gov, reg. no. NCT02614768). The study was conducted according to the principles of Good Clinical Practice (GCP) and following the Declaration of Helsinki. The study nature was explained to each participant and a written informed consent was obtained before any study-related activities were started.
This open, single-center, non-controlled clinical study, was performed at the Clinical Research Center (CRC) of Medical University of Graz. Twelve patients with diabetes mellitus type 1 on continuous insulin infusion (CSII) or multiple daily insulin injections (MDI) participated in this study.
The aim of the study was to assay accuracy and reliability of GM systems over 12 hours at the CRC under standardized real-life conditions. Secondary objectives included evaluation of sensor performance with regard to varying levels of metabolic parameters (non-esterified fatty acids (NEFA), glucagon, lactate, betahydroxybutyrate).

Main *inclusion criteria* were the following:
- known diagnosis of diabetes mellitus type 1 for at least 6 months
- insulin treatment for at least 3 months
- age >18 years
- body mass index <35 kg/m²
- glycated hemoglobin <86 mmol/mol

Main *exclusion criteria* included:

- pregnancy
- medical condition that might influence glucose metabolism other than T1D
- use of medication that impacts glucose metabolism other than insulin therapy

The study consisted of three visits:

1. Screening Visit (Visit 1) to assess patients' eligibility
2. Sensor Insertion Visit (Visit 2) 24 hours prior to the study visit
3. Study Visit (Visit 3) including 12 hours at the CRC

Visit 1:

After signing the informed consent, in- and exclusion criteria were evaluated. A physical examination including assessment of vital signs was performed. Height and weight were measured and the BMI was calculated. A blood sample was taken for assessment of blood count, HbA1c, clinical chemistry (including renal function, liver function, and electrolyte status), infectious parameters (HIV, HCV) and coagulation parameters. The study physician recorded the medical history including diabetes history as well as concomitant medication (including diabetes-specific medication). Subjects who fulfilled all of the inclusion and none of the exclusion criteria were invited to Visit 2.

Visit 2:

Visit 2 was allowed to take place between 1 and 21 days after the screening visit and needed to be performed 24 hours prior to the study visit (Visit 3).
During visit 2, the sensors of the Abbott FreeStyle libe, Dexcom G4 and Medtronic 60G were inserted into the subcutaneous adipose tissue (SAT) as specified by the manufacturer and using the respective insertion gadget. Patients were instructed in the use of the devices and consecutively all sensors were calibrated by the patients according to manufacturers' instruction. Additionally, 2 Spidiman sensors were into the SAT of the abdominal region located 2-4 mm beneath the skin surface. In contrast to the insertion of the commercially available sensors, specific procedures were required for Spidiman insertion. Prior to

insertion, the skin was punctured with a Sterican® cannula (23 G) to minimize the shear forces during insertion on the sensor unit and to facilitate the insertion procedure. The respective cannulas of the sensor units were oriented in a 90° angle to the surface of the skin. The sensor units were covered with wound dressing films immediately after insertion. Each Spidiman sensor was allocated a sensor number which was documented onto the wound dressing films and the position of the respective sensors were documented in the source documentation form. No further measures with regard to the Spidiman sensors were required by the patients since calibration of the Spidiman sensors was planned to be retrospective.

During Visit 2 the participants received a subject diary where they were asked to document glycemia, food intake, insulin doses and any problems with the sensors. Thereafter, the participants left the CRC for 24 hours and continued their regular lifestyle with the limitation that bathing, showering and sports were not allowed whilst wearing the sensors.

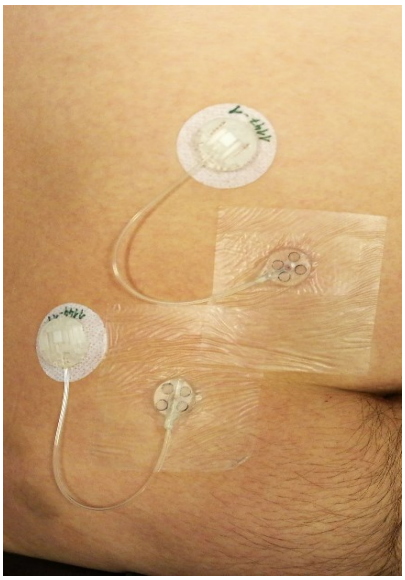

Fig. 30 Spidiman sensors implanted into SAT during Visit 2

Visit 3:

Subjects attended the CRC at 7:30 am under fasting conditions at the beginning of the study visit. After a physical examination of the insertion sites and check that all CGM systems were working the wound dressing films of Spidiman sensors were removed and the attachment optical read out units were attached to the Spidiman sensors. The raw data set included the actual phase shift and signal intensity of watch channel and was logged with the Oxygen logger software 3.204 from Pyro Science GmbH (Aachen, Germay).

Thereafter, the continuous glucose monitoring session started and reference venous plasma glucose samples were withdrawn every five minutes for a period of twelve hours and measured using the Super GL Glucose Analyzer (Freital, Germany). Continuous subcutaneous glucose monitoring was performed using the 3 commercially GM systems as well as the SPIDIMAN sensors in parallel.
Additionally, sampling of 3-hydroxybutyrate, lactate, NEFA and glucagon was performed over the 12-hour study period at an interval of every 30 minutes. The samples were stored and analyzed according to standard procedures at the central laboratory of the University and Joanneum Research.
Insulin therapy was performed by the patients themselves as under daily life conditions. To evaluate sensor performance under conditions mimicking real-live meal/insulin as well as exercise experiments were performed to provoke hypo- and hyperglycemia. During the three meal experiments (breakfast, lunch, dinner) the subjects injected an increased insulin dose to induce hypoglycemia as described in more detail below.

At 8:15 am, subjects received a standardized breakfast with 60g carbohydrates, followed by an increased bolus insulin dose (180% of subjects' calculated mealtime dose), and scheduled to be injected at 8:30 am to cause mild postprandial hyperglycemia with subsequent hypoglycemia. In case of a steep drop in glycemia or occurrence of symptoms of hypoglycemia, subjects received snacks and/or orange juice.

Lunch (60g carbohydrates) was scheduled for 1:00 pm followed by an increased bolus insulin dose (also 180% of subjects' calculated mealtime dose), to trigger the same effect again.

In the evening at 6:30 pm the exercise test was performed. The exercise test consisted of two 15minutes sessions of cycling at 50% of individual VO_2 max separated by five minutes of rest. To normalize glycemia prior to exercise, snacks and/or juice up to 30g carbohydrates were given.
At 7:30 pm participants received a standardized dinner consisting of 40g carbohydrates, with subjects' regular insulin bolus dose.
Glucose monitoring continued until 8pm. After collecting the last sample, the venous line and all sensors were removed and the insertion site were investigated. (10,48)

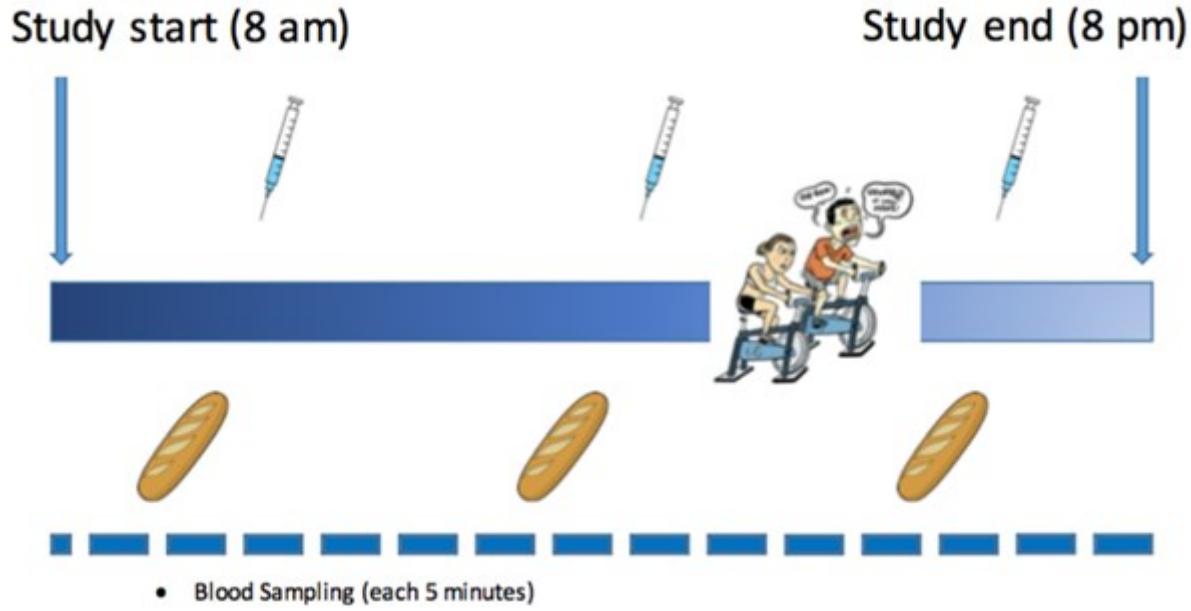

Fig. 31 Study scheme:
Timeline of the study visit (Visit 3). The experiment took place from 8am to 8 pm and consisted of three meal/insulin challenges as well as one exercise test. Reference blood sampling was performed at 5minutes intervals throughout the study.

8.1.2 Data analysis

For data analysis, sensor glucose values were matched with the corresponding venous reference glucose values. Overall sensor accuracy was determined using ISO 15197:2013 (percentage of sensor values that are within ±15mg/ dL of the reference value at glucose concentrations <100 mg/dL and within ±15% at glucose concentrations ≥100 mg/dL). Overall sensor accuracy and sensor accuracy during hypoglycemia (<70 mg/dL), euglycemia (70 - 180 mg/dL) and hyperglycemia (>180 mg/dL) as well as during exercise were assessed by calculating the MARD between sensor and venous glucose measurements. For analyzing the single-port sensor, instead of the MARD the median

ARD was used, because of sensor raw data were neither corrected for time lag, nor smoothed or erroneous readings removed before glucose calculation.

Real-time continuous glucose sensor values and venous glucose values were compared by Bland-Altman analysis. Clinical relevance of discrepancies between sensor and reference values was illustrated by Clarke Error Grid analysis, overall and during exercise. The influence of glucagon, NEFA, betahydroxybutyrate and lactate levels on sensor performance was tested via Spearman correlation analysis. Results are presented as mean ± standard deviation, if not indicated otherwise. (10,48)

8.2 Results

8.2.1 Primary Outcome

Twelve subjects (5 women, 7 men) with a mean age of 33 ± 11 years, BMI of 22.5 ± 2.4 kg/m^2, diabetes duration of 17 ± 12 years and a mean $HbA1_C$ of 7.6 ± 1.1% participated in the trial.
For the 3 commercially available GM devices (Abbott, Dexcom and Medtronic) 462, 540 and 502 reference pairs were available, respectively. The systems fulfilled the ISO 15197 2013 criteria in 73.2% (Abbott), 56.1% (Dexcom) and 56.1% (Medtronic).

MARD data over the entire glycemic range as well as during eu-, hypo- and hyperglycemia are indicated in Table 3.1. Additionally, MARD during exercise and in the postprandial phase were calculated (Table 3.1).

	Abbott	Dexcom	Medtronic
Data Pairs	462	540	502
MARD (total)	13.2 (10.9)	16.8 (12.3)	21.4 (17.6)
MARD (70 – 180 mg/dl)	13.7 (11.3)	16.3 (11.6)	20.9 (15.3)
MARD (< 70 mg/dl)	14.6 (10.2)	23.8 (15.7)	26.9 (20.0)
MARD (> 180 mg/dl)	10.1 (7.9)	11.6 (7.2)	17.1 (21.9)
MARD (Ergometer)	8.7 (5.9)	15.7 (14.6)	19.4 (13.5)
MARD (postprandial)	11.7 (10.5)	15.1 (12.5)	20.5 (17.9)

Tab. 2 MARD – Mean Absolute Relative Difference

During all glycemic ranges, as well as during exercise and in the postprandial phase, Abbott exhibited the lowest and Medtronic the highest MARD. All sensors shown showed superior performance during exercise.(48)

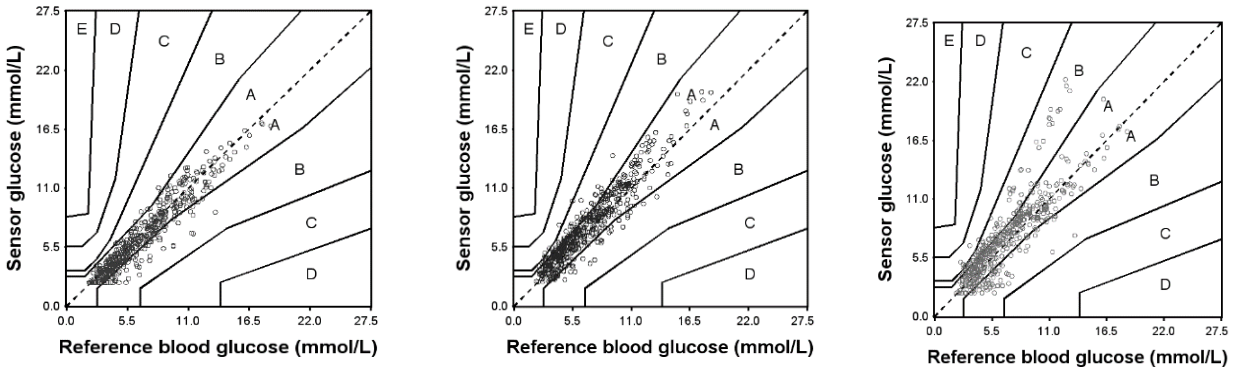

Fig.32 Parkes Error Grid Analysis of paired references of Abbott, Dexcom and Medtronic

Lactate, glucagon, NEFA and betahydroxybutyrate were actively influenced by exercise and food intake but had not strong correlation on sensor performance. A positive correlation indicates that a higher level of metabolic parameter was associated with worse sensor performance, leading to higher ARD. A negative correlation indicates that a higher

level of a metabolic parameter was associated with improved sensor performance (lower ARD).

Parameter	n	Mean (mmol/L)	SD	Abbott		Dexcom		Medtronic	
				Correlation coefficient	*p*	*Correlation coefficient*	*p*	*Correlation coefficient*	*p*
Lactate	298	1.34	0.87	-0.03	0.49	0.06	0.16	-0.08	0.08
Betahydroxy- butyrate	298	0.09	0.10	-0.13	0.06	-0.15	0.01	-0.12	0.06
Glucagon	298	4.7	2.98	-0.09	0.18	-0.08	0.20	-0.17	<0.01
NEFA	298	0.25	0.35	-0.16	0.02	-0.20	<0.01	-0.09	0.16

Tab.3: Metabolic parameters and statistic correlation to sensor performance

8.2.2 Secondary Outcome

Spidiman data were evaluated by using Parkes Error Grid Analysis and the median ARD, because of sensor raw data were not corrected for time lag, were not smoothed and erroneous readings were not removed before glucose calculation. Per subject 2 sensors were implanted and used for glucose monitoring. Data from all Spidiman sensors were pooled. One sensors/data sets had to be excluded from analysis due to total sensor failure. In total, 23 Spidiman sensors were evaluable. 87.2% of the resulting data pairs were located in the clinically accurate zones A and B of the Parkes Error Grid. Zones C and D contained 12.8% all data pairs. The median ARD of all Spidiman sensors was 22.5%.(10) Parkes Error Grid for all values is displayed in Figure 33.

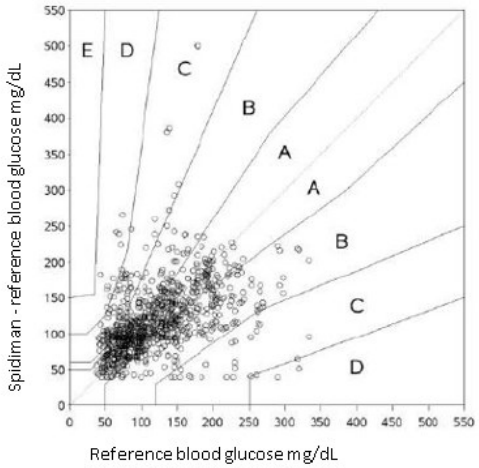

Fig.33 Parkes Error Grid analysis for paired Spidiman – reference glucose values.

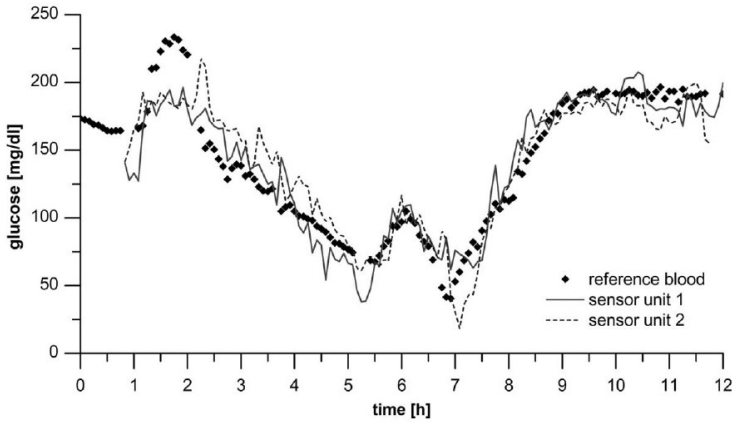

Fig. 34: Exemplary glucose profile indictaing reference glucose and 2 Spidiman sensors (sensor unit 1 and sensor unit 2) during the 12 hour experiment

8.3 Discussion and Conclusion

Previous studies have assessed and compared the accuracy of different generations of the two most frequently used sensors but not comparing three on market available sensors, to a simultaneously in one subject under real life conditions including exercise periods. Few studies exist that performed a head-to-head comparison of two sensor systems. Although standards for blood glucose meter accuracy exist (e.g. ISO 15197:2013) comparison of the performance of different CGM systems is still troublesome since no widely-accepted assessment standard exists. This is the first head-to-head analysis of the currently most widely used GM systems compared for investigation only biosensor Spidiman in parallel under standardized conditions including meals and exercise at a research center. Findings from this study offer an important overview of sensor performance during different levels of glycaemia as well as during exercise and in the fasting, prandial and postprandial state. This is of importance since reliable and accurate sensor performance plays a substantial role for the establishment of GM systems as non-adjunctive tools in diabetes care.
One limitation of our study is that sensor performance was only evaluated under artificial conditions at the research center and therefore might not reflect sensor performance during routine use under daily life conditions (59). Furthermore, we collected data only during a short period of 12 hours 24 hours after sensor insertion. As it is known that sensor performance is worst on the first day of use, a 24-hour run-in period was chosen to mitigate the risk of misinterpreting sensor accuracy. Previous studies showed that sensor performance was not weakened during the remaining period of designated time of use. One limitation of the
study is that sensor performance was not evaluated during sleep which could have influenced the overall results in two ways: on the one side, less glucose fluctuations could have resulted in slightly superior sensor performance during sleep; on the other side pressure artifacts caused e.g. by sleeping on the sensor - so called PISA (pressure-induced sensor attenuations) events - could have resulted in worse sensor performance. Although the overall performance could change numerically in a 24-hour study setting, the major aim of our study was the comparison of the 3 devices used simultaneously in various non-steady state settings. In our study, we observed superior sensor accuracy for the factory-calibrated Abbott using standardized metrics such as ISO 15197 2013 criteria and MARD. This outcome is comparable to available data1 and proves the feasibility of factory-calibration. Factory calibrated systems might benefit from the implementation of a stable

calibration factor which is independent of disruptive factors such as using an unsuitable point in time for calibration (e.g. unstable glycemia), incorrect reference measurement due to failure of the blood glucose meter or erroneous measurement caused by contaminated skin.

The Medtronic sensor which was tested in this trial, is normally used in combination with the MiniMed® 640G insulin pump with SmartGuard® technology automatically shutting down insulin delivery when hypoglycemia is impeding. Since this is the only currently available sensor system which actively communicates with an insulin pump and which interrupts insulin delivery, higher rates of sensor failure requiring new sensor insertion and consecutively reduced sensor runtime might be expected. This might be due to a more precautious underlying sensor algorithm which rather shuts off the sensor than accepting a potentially erroneous sensor signal.

Glucose swings during/after exercise tests were less pronounced than during/after meals, what might partly explain better sensor performance since it is more difficult to closely follow larger glycemic excursions than smaller ones. Interestingly superior performance of Abbott was even pronounced during exercise when compared to the other GM systems. In contrast to

our data performance of the Dexcom G4 Platinum and the Medtronic Paradigm Veo system in a publication by Taleb et al showed significantly better performance for both systems during rest in comparison to exercise. Data from the Abbott Freestyle Navigator which uses similar sensor technology as Abbott are in line with our data, showing superior performance during exercise. Even if the exercise period was only short in our study this finding is of interest especially for athletes. Larger studies including both continuous and interval exercise tests are needed to address this finding in more detail.

Meanwhile newer versions of two of the tested three systems have been presented (Enlite 3, Medtronic; Dexcom G5, Dexcom) reporting MARDs of <10% according to manufacturers, but to date no direct standardized comparison is available and only Dexcom G5 has been marketed yet. (102,103)

The biosensors showed several improvements compared to a previous trial by Rumpler et al; e.g. the miniaturization of the whole biosensor system improves the usability of the system to become comparable system regarding size, weight and wear comfort. Also, the sensor chemistry was adapted and all patient safety tests according to EN60601 were passed. Furthermore the construction of the sensor was changed to realize oxygen compensation with a reference oxygen sensor. (10)

Results also show that single-port may have potential to improve precision of the artificial pancreas, due to successful combination with an insulin infusion set. Compared to reference blood values, the sensor sensitivity shows a positive relation but still needs more improvement with a goal of a MARD of <10%. The average measuring time was delayed and lasted about 10 hours because of a technical error. An example of two retrospectively calibrated glucose traces of Spidiman sensors is displayed above in Figure 31.
Next steps towards combination of artificial pancreas and single-port, is sensor improvement and integration of an insulin dosing algorithm, which will calculate the currently needed insulin dose, back-coupling to the measurement. Single-port systems show a great potential to become the central element of an artificial pancreas system.(10)

References

1. Bilous R, Donnelly R. Handbook of Diabetes: Fourth Edition. Handbook of Diabetes: Fourth Edition. 2010. 13 - 15, 22-43, 53-100 p.
2. Homeostasis - NCEA Biology [Internet]. [cited 2017 Mar 10]. Available from: http://www.passbiology.co.nz/biology-level-3/homeostasis
3. Post-translational Modification Of Preproinsulin T... | Chegg.com [Internet]. [cited 2017 Mar 10]. Available from: http://www.chegg.com/homework-help/questions-and-answers/post-translational-modification-preproinsulin-biologically-active-insulin-preproinsulin-in-q9647139
4. MECANISMO DEL HI – AFHICO [Internet]. [cited 2017 Mar 10]. Available from: http://afhico.org/mecanismo-del-hiperinsulinismo-congenito/
5. What Are Proper Insulin Injection Sites? | New Health Advisor [Internet]. [cited 2017 Mar 10]. Available from: http://www.newhealthadvisor.com/insulin-injection-sites.html
6. Insulin Pens: How to Give a Shot [Internet]. [cited 2017 Mar 9]. Available from: http://www.upmc.com/patients-visitors/education/diabetes/Pages/insulin-pens-how-to-give-a-shot.aspx
7. Diabetics Lack Backup Plans for Insulin Pump Problems [Internet]. [cited 2017 Mar 11]. Available from: http://www.mdmag.com/medical-news/diabetics-lack-backup-plans-for-insulin-pump-problems
8. Smith JL. The Pursuit of Noninvasive Glucose : " Hunting the Deceitful By John L . Smith Fourth Edition : Revised and Expanded Preface to the Fourth Edition. 2015;
9. FreeStyle Libre | Abbott Diabetes Care [Internet]. [cited 2017 Mar 14]. Available from: https://www.abbott-diabetes-care.at/freestyle_libre.html
10. Rumpler M, Mader JK, Fischer JP, Thar R, Granger JM, Deliane F, et al. First application of a transcutaneous optical single-port glucose monitoring device in patients with type 1 diabetes mellitus. Biosens Bioelectron [Internet]. 2017 Feb [cited 2017 Mar 14];88:240–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0956566316307989
11. Thomas N. A practical guide to diabetes mellitus. 7th Edition. 2016. 133-154, 159-170 p.
12. Poretsky L. Principles of Diabetes Mellitus. Springer Verlag; 2010. 3-14 p.
13. History of Diabetes [Internet]. [cited 2017 Mar 10]. Available from: https://en.wikipedia.org/wiki/History_of_diabetes#cite_note-5
14. Diabetes History - Defeat Diabetes Foundation [Internet]. [cited 2017 Mar 10]. Available from: http://www.defeatdiabetes.org/diabetes-history/
15. History of Diabetes: American Diabetes Association® [Internet]. [cited 2017 Mar 10]. Available from: http://www.diabetes.org/research-and-practice/student-resources/history-of-diabetes.html
16. Jameson JL. Harrison's endocrinology 4th edition. 2016. 280-328 p.
17. Horn F, Biochemie des Menschen. 2015. 398-406 p.
18. Diabetes Mellitus [Internet]. [cited 2017 Mar 10]. Available from: http://flexikon.doccheck.com/de/Diabetes_mellitus?utm_source=www.doccheck.com&utm_medium=web&utm_campaign=DC%2BSearch
19. GLOBAL REPORT ON DIABETES WHO Library Cataloguing-in-Publication Data. ISBN [Internet]. [cited 2017 Mar 10];978:92–4. Available from: http://www.who.int/about/licensing/
20. Gruber S, Blanck S. BASICS Gynäkologie und Geburtshilfe. Elsevier, Urban et Fischer; 2014.107 p.

21. Hyperosmolar Hyperglycemic State: Background, Pathophysiology, Etiology [Internet]. [cited 2017 Mar 9]. Available from: http://emedicine.medscape.com/article/1914705-overview
22. Hypoglycemia: Causes, Diagnosis, and Treatment - Medical News Today [Internet]. [cited 2017 Mar 9]. Available from: http://www.medicalnewstoday.com/articles/166815.php
23. Hypoglycemia - Low Blood Glucose (Blood Sugar): American Diabetes Association® [Internet]. [cited 2017 Mar 9]. Available from: http://www.diabetes.org/living-with-diabetes/treatment-and-care/blood-glucose-control/hypoglycemia-low-blood.html
24. Mader JK, Birngruber T, Korsatko S, Deller S, Köhler G, Boysen S, et al. Enhanced absorption of insulin aspart as the result of a dispersed injection strategy tested in a randomized trial in type 1 diabetic patients. Diabetes Care. 2013;36(4):780–5.
25. Drouin P, Blickle JF, Charbonnel B, Eschwege E, Guillausseau PJ, Plouin PF, et al. Diagnosis and classification of diabetes mellitus. Porte D, Sherwin RS, Baron A, editors. Diabetes Care [Internet]. American Diabetes Association; 2009;32(Supplement_1):S62–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2797383&tool=pmcentrez&rendertype=abstract
26. Fasting Plasma Glucose Test [Internet]. [cited 2017 Mar 9]. Available from: http://www.diabetes.co.uk/fasting-plasma-glucose-test.html
27. Glucose Tolerance Test [Internet]. [cited 2017 Mar 9]. Available from: http://www.diabetes.co.uk/oral-glucose-tolerance-test.html
28. The Effect of Intensive Treatment of Diabetes on the Development and Progression of Long-Term Complications in Insulin-Dependent Diabetes Mellitus. N Engl J Med [Internet]. 1993 Sep 30 [cited 2017 Mar 9];329(14):977–86. Available from: http://www.nejm.org/doi/abs/10.1056/NEJM199309303291401
29. Insulin Basics: American Diabetes Association® [Internet]. [cited 2017 Mar 10]. Available from: http://www.diabetes.org/living-with-diabetes/treatment-and-care/medication/insulin/insulin-basics.html
30. Insulin Types and Information [Internet]. [cited 2017 Mar 10]. Available from: http://www.diabetes.co.uk/insulin/insulin-types.html
31. Different Types of Insulin | Joslin Diabetes Center [Internet]. [cited 2017 Mar 9]. Available from: http://www.joslin.org/info/insulin_a_to_z_a_guide_on_different_types_of_insulin.html
32. Brands and Types of Insulin: Rapid-Acting, Long-Acting, and More [Internet]. [cited 2017 Mar 10]. Available from: http://www.webmd.com/diabetes/guide/diabetes-types-insulin#1
33. Pre-Mixed Insulin :: Diabetes Education Online [Internet]. [cited 2017 Mar 9]. Available from: https://dtc.ucsf.edu/types-of-diabetes/type2/treatment-of-type-2-diabetes/medications-and-therapies/type-2-insulin-rx/types-of-insulin/pre-mixed-insulin/
34. Trevitt S, Simpson S, Wood A. Artificial Pancreas Device Systems for the Closed-Loop Control of Type 1 Diabetes What Systems Are in Development? J Diabetes Sci Technol [Internet]. 2015;1932296815617968. Available from: http://dst.sagepub.com/lookup/doi/10.1177/1932296815617968
35. Bergenstal RM, Garg S, Weinzimer SA, Buckingham BA, Bode BW, Tamborlane W V., et al. Safety of a Hybrid Closed-Loop Insulin Delivery System in Patients With Type 1 Diabetes. JAMA [Internet]. 2016;316(13):1407. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27629148%5Cnhttp://jama.jamanetwork.com/

article.aspx?doi=10.1001/jama.2016.11708
36. Garg SK. The Future of Glucose Monitoring. Diabetes Technol Ther [Internet]. Mary Ann Liebert, Inc.; 2016 Feb [cited 2017 Mar 9];18 Suppl 2(Suppl 2):S2iv–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26784132
37. Kropff J, DeVries JH. Continuous Glucose Monitoring, Future Products, and Update on Worldwide Artificial Pancreas Projects. Diabetes Technol Ther [Internet]. 2016;18 Suppl 2:S253-63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4717501&tool=pmcentrez&rendertype=abstract
38. Lind M, Polonsky W, Hirsch IB, Heise T, Bolinder J, Dahlqvist S, et al. Continuous Glucose Monitoring vs Conventional Therapy for Glycemic Control in Adults With Type 1 Diabetes Treated With Multiple Daily Insulin Injections. JAMA [Internet]. American Medical Association; 2017 Jan 24 [cited 2017 Apr 5];317(4):379. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2016.19976
39. Bergenstal RM, Tamborlane W V, Ahmann A, Buse JB, Dailey G, Davis SN, et al. Sensor-augmented pump therapy for A1C reduction (STAR 3) study: results from the 6-month continuation phase. Diabetes Care [Internet]. American Diabetes Association; 2011 Nov [cited 2017 Apr 5];34(11):2403–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21933908
40. Bolinder J, Antuna R, Geelhoed-Duijvestijn P, Kröger J, Weitgasser R. Novel glucose-sensing technology and hypoglycaemia in type 1 diabetes: a multicentre, non-masked, randomised controlled trial. Lancet [Internet]. 2016 Nov 5 [cited 2017 Apr 5];388(10057):2254–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27634581
41. Beck RW, Riddlesworth T, Ruedy K, Ahmann A, Bergenstal R, Haller S, et al. Effect of Continuous Glucose Monitoring on Glycemic Control in Adults With Type 1 Diabetes Using Insulin Injections. JAMA [Internet]. American Medical Association; 2017 Jan 24 [cited 2017 Apr 5];317(4):371. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2016.19975
42. Gómez AM, Marín Carrillo LF, Muñoz Velandia OM, Rondón Sepúlveda MA, Arévalo Correa CM, Mora Garzón E, et al. Long-Term Efficacy and Safety of Sensor Augmented Insulin Pump Therapy with Low-Glucose Suspend Feature in Patients with Type 1 Diabetes. Diabetes Technol Ther [Internet]. 2017 Feb [cited 2017 Apr 5];19(2):109–14. Available from: http://online.liebertpub.com/doi/10.1089/dia.2016.0332
43. Buckingham BA, Bailey TS, Christiansen M, Garg S, Weinzimer S, Bode B, et al. Evaluation of a Predictive Low-Glucose Management System In-Clinic. Diabetes Technol Ther [Internet]. 2017 Feb 16 [cited 2017 Apr 5];dia.2016.0319. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28221823
44. Bally L, Thabit H, Hovorka R. Closed-loop for type 1 diabetes - an introduction and appraisal for the generalist. BMC Med [Internet]. BioMed Central; 2017 Jan 23 [cited 2017 Mar 14];15(1):14. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28114938
45. Trevitt S, Simpson S, Wood A. Artificial Pancreas Device Systems for the Closed-Loop Control of Type 1 Diabetes: What Systems Are in Development? J Diabetes Sci Technol [Internet]. Diabetes Technology Society; 2016 May [cited 2017 Mar 14];10(3):714–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26589628
46. Artificial Pancreas Project - JDRF [Internet]. [cited 2017 Mar 15]. Available from: http://www.jdrf.ca/our-research/treat/artificial-pancreas-project/
47. Garg SK. The Future of Glucose Monitoring. Diabetes Technol Ther [Internet].

2016;18(S2):S2-NaN-S2-2. Available from: http://online.liebertpub.com/doi/10.1089/dia.2015.0421
48. Aberer F, Hajnsek M, Rumpler M, Zenz S, Baumann P, Elsayed H, et al. Evaluation of Subcutaneous Glucose Monitoring Systems under Routine Environmental Conditions in Patients with Type 1 Diabetes. Diabetes, Obes Metab [Internet]. 2017; Available from: http://doi.wiley.com/10.1111/dom.12907
49. Modified iPhone Helps Regulate Blood Sugar in Diabetes Patients: Study | NDTV Gadgets360.com [Internet]. [cited 2017 Mar 15]. Available from: http://gadgets.ndtv.com/science/news/modified-iphone-helps-regulate-blood-sugar-in-diabetes-patients-study-543769
50. Rodbard D. Continuous Glucose Monitoring: A Review of Successes, Challenges, and Opportunities. Diabetes Technol Ther [Internet]. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA ; 2016 Feb [cited 2017 Mar 14];18(S2):S2-3-S2-13. Available from: http://online.liebertpub.com/doi/10.1089/dia.2015.0417
51. Vashist S. Continuous Glucose Monitoring Systems: A Review. Diagnostics [Internet]. 2013;3(4):385–412. Available from: http://www.mdpi.com/2075-4418/3/4/385/htm
52. Bolinder J, Antuna R, Geelhoed-Duijvestijn P, Kröger J, Weitgasser R. Novel glucose-sensing technology and hypoglycaemia in type 1 diabetes: a multicentre, non-masked, randomised controlled trial. Artic 2254 www.thelancet.com [Internet]. 2016 [cited 2017 Mar 14];388. Available from: http://ac.els-cdn.com/S0140673616315355/1-s2.0-S0140673616315355-main.pdf?_tid=4eb5fb98-08c6-11e7-941a-00000aab0f6c&acdnat=1489503527_39ff77001e2f985aaf3168c9a78ed89a
53. Zanon M, Sparacino PG. Non-Invasive Continuous Glucose Monitoring: Identification of Models for Multi-Sensor Systems. Dep Inf Eng [Internet]. 2013;PhD. Available from: http://www.dart-europe.eu/full.php?id=809207%5Cnhttp://paduaresearch.cab.unipd.it/5684/1/Zanon_Mattia_tesi.pdf
54. Milestone of CGM evolution [Internet]. [cited 2017 Mar 15]. Available from: https://diabetes.medicinematters.com/artificial-pancreas-systems/the-artificial-pancreas-potential-to-transform-diabetes-care/12111508
55. The History and Future of Blood Glucose Monitoring - Diabetes Self-Management [Internet]. [cited 2017 Mar 14]. Available from: https://www.diabetesselfmanagement.com/blog/history-future-blood-glucose-monitoring/
56. History of Blood Glucose Meters [Internet]. [cited 2017 Mar 14]. Available from: http://www.mendosa.com/history.htm
57. Clarke SF, Foster JR. A history of blood glucose meters and their role in self-monitoring of diabetes mellitus. British Journal of Biomedical Science. 2012.
58. Garg SK. Standardization of Self-Monitoring of Blood Glucose and Continuous Glucose Monitoring Reporting.
59. Vashist SK. Continuous Glucose Monitoring Systems: A Review. Diagnostics (Basel, Switzerland) [Internet]. Multidisciplinary Digital Publishing Institute (MDPI); 2013 Oct 29 [cited 2017 Mar 14];3(4):385–412. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26824930
60. abbott freestyle insulinx - Google-Suche [Internet]. [cited 2017 Mar 14]. Available from: https://www.google.at/search?q=abbott+freestyle+insulinx&source=lnms&tbm=isch&sa=X&ved=0ahUKEwi_j_qy_tbSAhWEuBoKHc68AM0Q_AUIBygC&biw=933

&bih=346#imgrc=9BlQkjJ-lPADBM:
61. Wentholt IME, Hart AAM, Hoekstra JBL, Devries JH. How to Assess and Compare the Accuracy of Continuous Glucose Monitors? DIABETES Technol Ther [Internet]. 2008 [cited 2017 Mar 14];10(2). Available from: http://online.liebertpub.com/doi/pdf/10.1089/dia.2007.0216
62. Pandit K. Continuous glucose monitoring. Indian J Endocrinol Metab [Internet]. Medknow Publications; 2012 Dec [cited 2017 Mar 14];16(Suppl 2):S263-6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23565395
63. Kropff J, Choudhary P, Neupane S, Barnard K, Bain SC, Kapitza C, et al. Accuracy and Longevity of an Implantable Continuous Glucose Sensor in the PRECISE Study: A 180-Day, Prospective, Multicenter, Pivotal Trial. Diabetes Care [Internet]. 2017 Jan [cited 2017 Mar 14];40(1):63–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27815290
64. Sieg A, Guy RH, Begoña Delgado-Charro M. Noninvasive and Minimally Invasive Methods for Transdermal Glucose Monitoring. DIABETES Technol Ther [Internet]. 2005 [cited 2017 Mar 14];7(1). Available from: http://online.liebertpub.com/doi/pdf/10.1089/dia.2005.7.174
65. Romey M, Jovanovič L, Bevier W, Markova K, Strasma P, Zisser H. Use of an intravascular fluorescent continuous glucose sensor in subjects with type 1 diabetes mellitus. J Diabetes Sci Technol [Internet]. 2012;6(6):1260–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23294770
66. Peyser T, Zisser HC, Khan U, Jovanovič L, Bevier W, Romey M, et al. Use of a novel fluorescent glucose sensor in volunteer subjects with type 1 diabetes mellitus. J Diabetes Sci Technol [Internet]. 2011;5(3):687–93. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3192635&tool=pmcentrez&rendertype=abstract
67. Pickup JC, Hussain F, Evans ND, Rolinski OJ, Birch DJS. Fluorescence-based glucose sensors. Vol. 20, Biosensors and Bioelectronics. 2005. p. 2555–65.
68. Eversense CGM System - Eversense [Internet]. [cited 2017 Mar 14]. Available from: http://eversensediabetes.com/products/
69. Smith JL, Rice MJ. Why Have So Many Intravascular Glucose Monitoring Devices Failed? J Diabetes Sci Technol [Internet]. Diabetes Technology Society; 2015 Jul [cited 2017 Apr 14];9(4):782–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26129733
70. Beier B, Musick K, Matsumoto A, Panitch A, Nauman E, Irazoqui P. Toward a continuous intravascular glucose monitoring system. Sensors (Basel) [Internet]. Multidisciplinary Digital Publishing Institute (MDPI); 2011 [cited 2017 Apr 14];11(1):409–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22344366
71. Crane BC, Barwell NP, Gopal P, Gopichand M, Higgs T, James TD, et al. The Development of a Continuous Intravascular Glucose Monitoring Sensor. J Diabetes Sci Technol [Internet]. 2015 [cited 2017 Apr 14];9(4):751–61. Available from: http://journals.sagepub.com/doi/pdf/10.1177/1932296815587937
72. Continuous blood glucose meter - GlucoScout™ - International Biomedical [Internet]. [cited 2017 Apr 14]. Available from: http://www.medicalexpo.com/prod/international-biomedical/product-68893-435321.html
73. Heise HM, Kondepati VR, Damm U, Licht M, Feichtner F, Mader JK, et al. Microdialysis based monitoring of subcutaneous interstitial and venous blood glucose in type 1 diabetic subjects by mid-infrared spectrometry for intensive insulin therapy - art. no. 686308. Opt Diagnostics Sens Viii. 2008;6863:86308.
74. Mader_DiabResClinPract_2012_Microdialysis.pdf.

75. Maran A, Crepaldi C, Tiengo A, Grassi G, Vitali E, Pagano G, et al. Continuous subcutaneous glucose monitoring in diabetic patients: A multicenter analysis. Diabetes Care. 2002;25(2):347–52.
76. Renard E. Implantable continuous glucose sensors. Curr Diabetes Rev [Internet]. 2008 Aug [cited 2017 Mar 14];4(3):169–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18690897
77. Nakamura K, Balo A. The Accuracy and Efficacy of the Dexcom G4 Platinum Continuous Glucose Monitoring System. J Diabetes Sci Technol [Internet]. 2015;9(5):1021–6. Available from: http://dst.sagepub.com/content/9/5/1021%5Cnhttp://dst.sagepub.com/content/9/5/1021.full.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25802469
78. Peyser T a., Nakamura K, Price D, Bohnett LC, Hirsch IB, Balo A. Hypoglycemic Accuracy and Improved Low Glucose Alerts of the Latest Dexcom G4 Platinum Continuous Glucose Monitoring System. Diabetes Technol Ther [Internet]. 2015;17(8):548–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25961446%5Cnhttp://online.liebertpub.com/doi/10.1089/dia.2014.0415%5Cnhttp://online.liebertpub.com/doi/10.1089/dia.2014.0415
79. Dexcom G4 Platinum [Internet]. [cited 2017 Mar 14]. Available from: https://www.nintamed.eu/p/shop/catalog?id=40
80. The MiniMed 640G Insulin Pump - Medtronic Diabetes UK [Internet]. [cited 2017 Mar 14]. Available from: https://www.medtronic-diabetes.co.uk/minimed-system/minimed-640g-insulin-pump
81. McGowan K, Thomas W, Moran A. Spurious reporting of nocturnal hypoglycemia by CGMS in patients with tightly controlled type 1 diabetes. Diabetes Care. 2002;25(9):1499–503.
82. Pompe à Insuline Système 640G 3 ml violet + kit transmetteur Guardian Enlite + Contour Next Link 2.4 BAYER [Internet]. [cited 2017 Mar 14]. Available from: https://www.ugap.fr/achat-public/pompe-a-insuline-systeme-640g-3-ml-violet-kit-transmetteur-guardian-enlite-contour-next-link-2.4-bayer_1696903.html#
83. iPro2 Professional CGM | Medtronic [Internet]. [cited 2017 Apr 14]. Available from: http://www.professional.medtronicdiabetes.com/ipro2-professional-cgm
84. iPro®2 Professional Continuous Glucose Monitoring (CGM) Evaluation | Medtronic Diabetes [Internet]. [cited 2017 Apr 14]. Available from: https://www.medtronicdiabetes.com/products/i-pro-evaluation
85. NEUES SYSTEM ZUR KONTINUIERLICHEN GLUKOSEAUFZEICHNUNG IN DER DIABETESBEHANDLUNG IST VERFÜGBAR [Internet]. [cited 2017 Apr 14]. Available from: http://wwwp.medtronic.com/Newsroom/NewsReleaseDetails.do?itemId=1277303161623&lang=de_DE
86. iPro®2 Professional Continuous Glucose Monitoring – Diabetes Education Network [Internet]. [cited 2017 Apr 14]. Available from: http://denbahamas.com/medical-care/continuous-glucose-monitoring/glucose-monitoring/ipro2-professional-continuous-glucose-monitoring/
87. Bailey T, Bode BW, Christiansen MP, Klaff LJ, Alva S. The Performance and Usability of a Factory-Calibrated Flash Glucose Monitoring System. Diabetes Technol Ther [Internet]. 2015;17(11):150714062940004. Available from: http://online.liebertpub.com/doi/10.1089/dia.2014.0378
88. Basu A, Veettil S, Dyer R, Peyser T, Basu R. Direct Evidence of Acetaminophen Interference with Subcutaneous Glucose Sensing in Humans: A Pilot Study. Diabetes Technol Ther [Internet]. 2016;18 Suppl 2:S243-7. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4717519&tool=pmcentrez&rendertype=abstract
89. Regittnig W, Lindpointner S, Korsatko S, Tutkur D, Bodenlenz M, Pieber TR. Periodic Extraction of Interstitial Fluid from the Site of Subcutaneous Insulin Infusion for the Measurement of Glucose: A Novel Single-Port Technique for the Treatment of Type 1 Diabetes Patients. Diabetes Technol Ther [Internet]. 2013 Jan [cited 2017 Apr 14];15(1):50–9. Available from: http://online.liebertpub.com/doi/abs/10.1089/dia.2012.0173
90. Osiecka I. OVERVIEW OF SOME NON-INVASIVE SPECTROSCOPIC METHODS OF GLUCOSE LEVEL MONITORING PRZEGLĄD WYBRANYCH N IEINWAZYJNYCH. 22(1):1–8.
91. Vaddiraju S, Burgess DJ, Tomazos I, Jain FC, Papadimitrakopoulos F. Technologies for continuous glucose monitoring: current problems and future promises. J Diabetes Sci Technol [Internet]. Diabetes Technology Society; 2010 Nov 1 [cited 2017 Mar 15];4(6):1540–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21129353
92. Pandey R, Paidi SK, Valdez TA, Zhang C, Spegazzini N, Dasari RR, et al. Noninvasive Monitoring of Blood Glucose with Raman Spectroscopy. Acc Chem Res [Internet]. 2017 Feb 21 [cited 2017 Mar 15];50(2):264–72. Available from: http://pubs.acs.org/doi/abs/10.1021/acs.accounts.6b00472
93. Wentholt IME, Hart a a M, Hoekstra JBL, Devries JH. How to assess and compare the accuracy of continuous glucose monitors? Diabetes Technol Ther [Internet]. 2008;10(2):57–68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18260769
94. Bailey TS, Grunberger G, Bode BW, Handelsman Y, Hirsch IB, Jovanovič L, et al. American Association of Clinical Endocrinologists and American College of Endocrinology 2016 Outpatient Glucose Monitoring Consensus Statement. Endocr Pract [Internet]. 2016;22(2):231–61. Available from: http://journals.aace.com/doi/10.4158/EP151124.CS
95. Rodbard D. Continuous Glucose Monitoring: A Review of Successes, Challenges, and Opportunities. Diabetes Technol Ther [Internet]. 2016;18 Suppl 2:S23–213. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4717493&tool=pmcentrez&rendertype=abstract
96. Overview of CGM Standards. [Internet]. [cited 2017 Mar 15]. Available from: http://www.itl.nist.gov/div897/ctg/graphics/cgm_std.htm
97. Obermaier K, Schmelzeisen-Redeker G, Schoemaker M, Klötzer H-M, Kirchsteiger H, Eikmeier H, et al. Performance evaluations of continuous glucose monitoring systems: precision absolute relative deviation is part of the assessment. J Diabetes Sci Technol [Internet]. Diabetes Technology Society; 2013 Jul 1 [cited 2017 Mar 15];7(4):824–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23911163
98. Clarkeerrorgrid - Clarke Error Grid - Wikipedia [Internet]. [cited 2017 Mar 15]. Available from: https://en.wikipedia.org/wiki/Clarke_Error_Grid#/media/File:Clarkeerrorgrid.gif
99. Clarke WL. The Original Clarke Error Grid Analysis (EGA). Diabetes Technol Ther [Internet]. 2005 Oct [cited 2017 Mar 15];7(5):776–9. Available from: http://online.liebertpub.com/doi/abs/10.1089/dia.2005.7.776
100. Pfützner A, Klonoff DC, Pardo S, Parkes JL. Technical aspects of the Parkes error grid. J Diabetes Sci Technol [Internet]. Diabetes Technology Society; 2013 Sep 1 [cited 2017 Mar 15];7(5):1275–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24124954
101. Parkes Error Grid for Type 1 Diabetes [Internet]. Parkes Error Grid for Type 1

Diabetes. [cited 2017 Mar 15]. Available from: http://www.diabeticnerd.com/img_library/Parkes-Error-Grid-A.gif
102. RESEARCH Forschungsgesellschaft mbH J. prmpos14042 hth single port system A0.indd – Combining Continuous Glucose Monitoring with Simultaneous Insulin Infusion in a Transcutaneous Optical Single-Port System. [cited 2017 Apr 8]; Available from: https://www.joanneum.at/uploads/tx_publicationlibrary/IDF2015_Hajnsek_Spidiman_Single-Port_Glukose_Messung_und_Insulin-Infusion_in_einem_Geraet.pdf
103. Hajnsek M, Rumpler M, Mader J, Pieber T. Optical Glucose Sensor for Single-Port Glucose Monitoring. [cited 2017 Apr 8]; Available from: https://www.joanneum.at/uploads/tx_publicationlibrary/ADA2016_Hanjsek_Optical_Glucose_Sensor_for_Single-Port_Glucose_Monitoring.pdf