1 Diabetes Mellitus

Clinical needs for in vivo monitoring with glucose sensors

1.1 Diabetes Mellitus

Diabetes mellitus is a term applied to a number of conditions or syndromes that in untreated state are characterised by hyperglycaemia. It is a disorder of metabolism of carbohydrate, fat and protein associated with a relative or absolute insufficiency of insulin secretion and with various degrees of insulin resistance. Insulin is a hormone that is produced in the beta cells of the pancreatic islets of Langerhans. Its role is twofold, firstly to enhance the entry of glucose into the liver, muscle and adipose tissue, and secondly to promote storage of energy substrate in the form of glycogen, fat and protein thus resulting in a lowering of the blood glucose concentration. It is very important to keep blood glucose concentrations within a narrow range of 3 to 10 mM both under conditions where the patient has been fed or has been fasting. Blood glucose concentrations under 3 mM (hypoglycaemia) impair brain function, whereas glucose concentrations higher than 10 mM (hyperglycaemia) exceed the renal glucose reabsorption threshold, which results in wasting of glucose. In addition, protracted hyperglycaemia causes degenerative complications in the long-term [2].

Classification, causes and complications of diabetes

Diabetes Mellitus can be subdivided in a number of classes that differ in aetiology and pathogeneses. However, the two most occurring types are type-1- and type-2-diabetes. They both account for 99.9% of all prevalence of diabetes and affect about 135 million people worldwide (WHO).
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Type-1 diabetes is a result of a chronic autoimmune destruction of the pancreatic beta cells resulting in an absolute insulin deficiency. The onset of type-1 diabetes is usually in childhood and early adulthood (< 35 years). The first clinical symptoms are thirst, polyuria, loss of weight and a tendency to keto-acidosis of the patient. At the time diabetes mellitus is diagnosed, 75% of the patients have antibodies against the beta cells and have lost most of their capability to produce insulin. The destruction of beta cells is irreversible. Genetic factors are thought to be important to this autoimmune beta cell destruction. In addition, it has been postulated that environmental factors such as certain viral infections and possibly chemical or nutritional agents may worsen these genetic factors.

Type-2 diabetes occurs in approximately 90% of all diabetic patients in the Western world. The onset of the disease is usually between 50 and 75 years of age. In type-2 diabetic patients, organs are less sensitive for the action of insulin (insulin resistance) and/or the production of insulin by the beta cells is insufficient. The cause of this dysfunction of beta cells is still unclear but there may be some genetic factors playing a part in the onset of this type of diabetes.

Blood glucose levels in the range of 1 to 30 mM may be observed in “treated” type-1 diabetic patients. First priority is to maintain a stable supply of glucose for central nervous function. Too low levels of blood glucose cause at first mental confusion and if sustained coma and death. On the other hand, protracted high glucose concentrations cause damage to small blood vessels (micro-angiopathy) and large blood vessels (macro-angiopathy). The damage to the small blood vessels results in problems with eye (retinopathy), kidney (nephropathy) and peripheral nerves. It was always suspected that regular and sustained hyperglycaemia was responsible for the chronic long-term symptoms of diabetes. Only recently has it been proven that near normoglycaemia will prevent or delay the onset of these long-term symptoms of diabetes [3].

For type-2 diabetic patients, the risk of acute hypoglycaemia is relatively low compared to type-1 diabetic patients. However, type-2 diabetic patients will suffer from the same degenerative long-term complications as type-1 diabetics. In addition, type-2 diabetics have a higher risk on cardiovascular
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Treatment of Type-1 and Type-2 diabetes

Diseases because classical risk factors such as high concentrations of triglycerides in blood, high blood pressure and overweight are more seen in type-2 diabetic patients than with people who have no insulin resistance.

1.2 Treatment of Type-1 and Type-2 diabetes
A diet, oral hypoglycaemic agents and/or the administration of insulin usually manage to regulate the blood glucose concentration in type-2 diabetics. The aim is not only to increase insulin concentrations, but also to reduce the levels of triglycerides and to normalise the level of protecting \( \text{HDL} \)-cholesterol in blood. First priority in the treatment of type-2 diabetics is to reduce chronic hyperglycaemia and the associated long-term degenerative complications.

Type-1 diabetic patients have an absolute insulin deficiency and can only be treated by insulin injections mostly in the subcutaneous tissues of arms, legs or abdomen (\( \text{iddm} \)). Main objective is to normalise the blood glucose concentration in order to reduce long-term complications. An intensive regime of short-acting insulin before meals with an additional injection of intermediate-acting insulin before bedtime mimic the normal insulin profile in blood and improve the metabolic control of the patient. Provided that the patient checks his blood glucose concentration regularly by means of a blood glucose monitor device (finger-prick method) and adjusts the insulin dosage based on the results. Even better glucose regulation can sometimes be obtained by continuous subcutaneous insulin infusion (\( \text{CSII} \)). Insulin delivery to the peritoneal cavity (implantable pumps) can further improve metabolic control for a special group of type-1 patients who are difficult to regulate [4, 5].

1.3 How can the metabolic control of type-1 diabetes patients be improved?
Insulin injections, in combination with frequent self-monitoring of blood glucose (\( \text{smbg} \)), have improved diabetic control. However, it is still difficult to achieve normoglycaemia because subcutaneous insulin injections do not mimic non-diabetic insulin secretion patterns sufficiently closely. High concentrations of peripheral insulin are needed to achieve sufficient insulin
concentration levels in the portal vein where it can slow down the glucose production of the liver. Also the resorption of short-acting insulin from the subcutaneous tissue is much slower in comparison with insulin secretion from the beta cells. Moreover, with injections there is no feedback control of insulin delivery rates according the prevailing glucose level.

Two other important approaches to improve metabolic control in type-1 diabetics are:

- The transplantation of the pancreas or isolated islets of Langerhans.
- The use of continuous glucose monitoring systems (i.e. glucose sensors) preferentially combined with a feedback controlled insulin dosage system.

Transplantation of the pancreas or islands of Langerhans

Replacement of fully functional pancreatic beta cells is the only treatment for type-1 diabetes that will eliminate the need for exogenous insulin, establish insulin independence, and maintain long-standing normoglycaemia [6]. This approach seems the most natural method to regain metabolic control. In principal two methods are available to transplant beta cells: the transplantation of the whole pancreas or the transplantation of isolated islets of Langerhans.

The first pancreas transplantation was reported in 1967 [7]. Due to technical complications, the success rate of pancreas transplantation was limited during the next decade. By the late 1970s the surgical technique was significantly improved and better management of immunosuppression and infection contributed to a higher percentage of successful transplantations. Today, the majority of pancreas transplantations are in combination with kidney transplantation (spk). This combined transplantation has a success rate of approximately 75% [8, 9].

Although the current success rate of a simultaneous pancreas-kidney transplantations is impressive, it remains to be seen whether the majority of type-1 diabetic patients will profit. Patients who have had transplantation must use immunosuppression for the rest of their life and an additional problem is the shortage of suitable donors.
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The American Diabetes Association (ADA) has therefore proposed that pancreas transplantation should only be considered appropriate therapy in the two following circumstances [10]:

1. In type-1 diabetic patients with end-stage renal disease who have or plan to have a kidney transplant.
2. As a therapeutic alternative for patients who exhibit a history of frequent acute and severe metabolic complications.

The success rate of islet-transplantation is much lower in comparison with simultaneous pancreas-kidney transplantation (<10% against 75% for SPK). Mostly, transplanted islet tissue is rejected as a result of an immune response of the acceptor site. Non-specific inflammatory responses, occurring at the time of implantation, may alter islet function.

Isolation of islet tissue is performed by collagenease digestion of pancreatic donor tissue. After purification, a sufficient mass of islet tissue is infused into the hepatic parenchyma via injection through the portal vein. The present immuno rejection of the implanted islet tissue requires the use of immunosuppressiva. To reduce the amount of immunosuppressiva, isolated islets can be encapsulated with a porous material that is permeable for substances such as insulin and glucose but impermeable to leukocytes. The use of encapsulated islet tissue originating from animals might be a solution for the shortage of donor tissue [11]. Although encapsulated islets are less vulnerable to immune reactions, the membrane retards the reaction dynamics of the islets resulting in a delayed insulin secretion. This because encapsulated islands are not directly provided by blood capillaries, which elongates the diffusion pathway of substances like insulin and glucose from and towards the islet tissue. A new development is the use of a solid support for the encapsulated islets [12]. Placed in the peritoneal cavity, this solid support is quickly accommodated with blood capillaries, which shortens the diffusion pathway. Still, improvements in immunosuppressive therapy will likely be required before islet transplantation can be routinely employed [13].
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The use of continuous glucose-monitoring systems (glucose sensors)
Preferably the regulation of blood glucose concentrations should be equivalent to that of non-diabetics. The development of devices for self-monitoring of blood glucose has given new possibilities to improve the metabolic control of the patient [14, 15] and is now recognised as a milestone in the history of insulin therapy. Especially, the occurrence of hyperglycaemic events is better managed by an intensified insulin regime in combination with self-monitoring. A side effect of an intensified regime is, however, the occurrence of hypoglycaemia episodes, which represent an immediate subject of concern for the patient [16]. Its incidence increases with time, and is more frequently seen with tightly metabolic controlled patients. Hypoglycaemic attacks are always unpleasant and can lead to loss of consciousness. Nocturnal hypoglycaemia is especially dangerous because the patient is usually asleep and not aware of his low blood glucose level. Hypoglycaemia is not only restricted to nocturnal attacks. The hypoglycaemic unawareness syndrome, defined by the occurrence of sudden and unpredictable hypoglycaemia without clear warning symptoms, is now a major focus of interest for diabetologists.

Self-monitoring of blood glucose suffers from the fact that it is discontinuous. It is therefore very difficult to prevent a hypoglycaemic attack if only two or three glucose determinations are made per day. The number of determinations that the patient is willing to perform is limited by factors such as pain, the fact that the procedure is boring, and simple dislike because the patient is confronted with the disease each time a measurement is made. Only highly motivated patients are willing to determine their blood glucose frequently (> 6 times per day) but this is certainly not the case for the majority of the patients. A continuous glucose monitoring system would therefore provide an alternative to the present discrete methods of glucose determination.

In principle, a continuous glucose monitoring system (i.e. glucose sensor) provides a basis for insulin administration. In its simplest form, a sensor measures on-line the body glucose concentration and informs the user with the results. On the basis of this information the patient can anticipate and
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take the necessary steps to prevent hyper- or hypoglycaemia. The advantage of this type of glycemic control over conventional blood glucose testing is that measurements with a sensor are continuous where blood glucose testing with fingersticking are intermittent e.g. a “point in time” measurement. Glucose sensors can detect changes in blood glucose concentration continuously. With the traditional finger-prick method, on the other hand, it depends how frequently the blood glucose measurements take place. The use of a continuous glucose measurement system would therefore be an improvement in the self-monitoring of blood glucose and can especially be helpful in the prevention of nocturnal hypoglycaemic attacks (i.e. “hypoglycaemic attacks”). In addition, a physician could record the daily glucose-concentration profiles for consideration.

Figure 1-1. Flowchart of a “Closed-loop” system where the in vivo glucose regulation is controlled by a glucose sensor. Administration of insulin when the blood glucose is too high or signalling at too low blood glucose levels is based on the on-line measurement of in vivo glucose by the sensor.

A technical advanced application is an insulin delivery system that is feedback controlled by a continuous glucose measurement (artificial beta cell). In this closed-loop system, a glucose sensor is integrated with an insulin
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delivery device (Figure 1-1, page 7). Insulin administration is based on the on-line measurement of the glucose sensor without or with minimal interference of the patient. A complicating factor in the regulation can be the delay time between the actual change in blood glucose concentration and the glucose measurement. Preferably this delay time must be as short as possible so that insulin administration based on normal insulin profiles can be applied.

In a healthy person a complex series of events leads to the secretion of insulin prior to the direct stimulus to insulin secretion from a rising blood glucose level. There is evidence of the involvement of gut hormones [17] and neural factors [18] in this mechanism of anticipatory insulin secretion. A more realistic design goal for a start would therefore be a closed-loop glucose sensor for operation in the “non-meal” or basal periods. Additional insulin must be delivered at or just prior to the start of the meal.

The development of self-adaptive fuzzy logic controlled insulin delivery might be the next step in the development of an artificial beta cell [19-22]. A microprocessor is used to calculate and control the pattern of insulin administration using algorithms that predict the glucose concentration based on extrapolation and pattern recognition. At present two closed-loop devices have been developed commercially: the Biostator [23-25] and the Ulm glucose sensor [26, 27], which is based on the Biostator device. Both systems are able to withdraw blood from a peripheral vein via a double lumen catheter and measure on-line the glucose concentration in whole blood. The Biostator uses an integrated computer program to calculate the amount of glucose or insulin that can be infused to maintain a certain predefined blood glucose level. For this purpose several control algorithms have been developed [28-31]. Although the devices have demonstrated their usefulness in glucose clamp studies, in practise these systems are burdened with technical difficulties and considerable costs in comparison to the manual clamp technique [32]. In addition these rather bulky devices can only be used in hospitals under well-controlled conditions. This makes them all together unsuitable for out-clinic use by diabetic patients. It is questionable whether the artificial beta cell will be reality in the near future. First, a reli-
able and miniaturised glucose sensor must be developed able to measure glucose concentrations in vivo for longer periods with a minimal delay.

### Potential in vivo applications of a glucose sensor.

Possible in vivo use of glucose sensors in the glycemic control of type-1 diabetics can be divided in short-term and long-term applications. In hospitalised patients a short-term glucose sensor would be useful in glycemic stabilisation, intensive care or for monitoring before, during and after surgery [33, 34].

Short-term glucose sensors for non-hospitalised patients would be used from several days up to 4 weeks. After this period the system must be replaced. The glucose sensor is used instead of the conventional blood glucose tests where it may improve the patients glycemic control. The ambulatory patient may especially benefit from the sensors function of notifying for hypoglycaemic situations during the night (“hypoglycaemic-alarm”) [35]. For patient compliance the glucose sensor should be small and simple in use without the need of frequent (re)-calibration.

The ultimate goal would be a sensor applicable for long periods. A long-term glucose sensor would be implanted in tissues for at least a half to one year or even longer, together with a telemetric system that transmits data to an external receiver. A long-term sensor could be used as a monitor but its ultimate application would be as a part of a total implantable automatic feedback-controlled insulin delivery system. It goes without saying that the long-term application of a sensor is technically the most difficult.

### 1.4 Glucose monitoring

Since the 1960s, reasonable research effort has been devoted to the development of a glucose sensor. Measuring principles can be classified into two main groups based on the interaction between the patient's body and glucose sensors employed: invasive and non-invasive [36]. Invasive glucose sensors use techniques that have intimate mechanical contact with the biological tissue or fluids. Non-invasive glucose sensors obtain information without mechanical intervention, using characteristic properties (spectral, optical, thermal, etc.) of glucose, which can be detected remotely.
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Non-invasive methods
The near-infrared (nir) spectrum of glucose has been proposed for non-invasive monitoring [37]. Direct spectroscopic measurements of unmodified body fluids or tissue using more traditional ultraviolet, visible and infrared (IR) regions of the spectrum are impractical because of the limited penetration depths, interfering absorption and excessive scattering. In contrast, the weak absorption of nir radiation by most biochemicals makes nir spectroscopy useful because body fluids and soft tissues are relatively transparent at these wavelengths [38-41]. nir-measurements are usually taken at tissue that is relatively well circulated with blood as in the tips of fingers, ear lobes, inner lip or oral mucosa. Just as the finger-prick method these measurements are intermittent but nir has the advantage that it is a painless technique and it can therefore be applied more frequently. A number of commercial devices based on nir-measurement (Dream-beam® device, Diasensor®, Glucocontrol®/Touchtrak®) have been developed and have received considerable attention from the popular press in recent years. However, no scientific in vivo studies regarding these devices have been published at the moment of writing. The main reason for this is that the nir-measurement technique in general has a low accuracy even in the normal physiological range. In addition a subject-dependent concentration bias has been reported [42]. A significant source of error is the base-line variation in the spectra as a result of the temperature sensitivity of water absorption bands in the glucose-measuring region. Moreover sweat and changes in the local blood circulation or absorption by other body chemicals [43] may affect the measurement accuracy substantially. At the moment, these non-invasive nir-devices suffer from low sensitivity and thus low accuracy of measurement. From the analytical point of view this method is at present an estimation technique rather than an exact analytic measurement and it is questionable whether it will leave its “science fiction” status in the near future.

Invasive methods
The fast majority of glucose sensor research has been devoted on methods of invasive glucose sensing. Many researchers have investigated the possibility of continuous in vivo glucose sensing using a wide range of different
approaches (see chapter 2), which are mainly based on analytical techniques that are already widely applied in clinical laboratories.

In nearly all glucose sensor designs, glucose is measured in the subcutaneous tissue [44-58] using a miniature needle-sized sensor that is directly inserted in the subcutaneous tissue to monitor the glucose concentration. The subcutaneous tissue is regarded as the most appropriate site of implantation because of good accessibility for surgery and relative easy replacement of the sensor in case of impaired function.

Sensing of glucose in the vascular compartment has been avoided notwithstanding that at present glycemic control is based on blood glucose concentrations. The risk of thrombosis, embolism and septicaemia is thought to be too great. Nevertheless, some glucose sensors have been developed to operate intravenously [59-62].

Despite this effort, currently no clinical application based on the needle-type of glucose sensor is available to be used routinely in clinical practice. Short-term in vivo studies have demonstrated in principle the feasibility of an implanted needle-type glucose sensor but also the major limitation of this type of sensor: the rapid loss of sensitivity after implantation. This is caused by a number of reasons, which mainly depend on the way these sensors are designed. The different types of glucose sensors, measurement principles and problems are discussed more extensively in chapter 2, “(Minimal)-invasive glucose sensors: an overview” on page 17.

**Glucose monitoring using microdialysis**

A number of systems have been developed which use microdialysis as a basis for continuous glucose sensing [1, 63-69]. The concept involves the use of a hollow fiber inserted in the subcutaneous tissue through which a saline or buffer solution is circulated and returned to an ex vivo glucose sensor. The technique is regarded as minimally invasive when compared to the needle-type sensor because relatively small needles are used for the insertion of the hollow fiber [70]. The use of microdialysis circumvents a number of problems that are seen with needle-type glucose sensors. The microdialysis approach gives in general far better results in comparison to in vivo monitoring with needle sensors.
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Requirements for an implantable glucose sensor
In general, a glucose sensor should have the following requirements for reliable functioning [36, 71, 72]:

1. Measurements with glucose sensors should be specific. The ability to recognise glucose in a complex medium, is the most important quality of a glucose sensor.

2. The detection of glucose should be accurate. Measurements with a sensor should give a value that corresponds to a high degree with the true glucose concentration.

3. The sensitivity of a glucose sensor should be high enough. The signal to noise ratio must be large and small changes in concentration (0.1-0.25 mM) must be detectable.

4. Each glucose sensor has a detection range i.e. an upper and a lower limit where a linear relationship exists between the electrical signal from the sensor and the quantity of glucose. If this detection window is enlarged the sensitivity of a sensor decreases. In literature different opinions about the proper detection range of operation in vivo can be found. Some authors propose that linearity of response in the range from 1 to 15 mM is required for a glucose sensor [73]. It has been argued by other authors that a sensor should respond over the entire concentration range (2 to 30 mM) commonly observed in diabetic patients [74]. In contrast, Kreagen and Chisholm suggest only a response-linearity up to 8 mM, which is the absolute minimum for glycaemic control where no large variations in glucose would be expected [75]. At the very least, a range up to 10 mM seems essential for in vivo monitoring of glucose.

5. A parameter that typifies a sensor is its response time. This is the time that is needed to reach a steady state when there is an instant change in the concentration of the substance under investigation. It is a measure how quick a sensor responds to changes in concentration. In practice, not the real response time is given because the time needed to reach steady state is infinitely long. Instead the $T_{90\%}$ or $T_{95\%}$ are used, which means the time needed to reach respectively 90% or 95% of the steady state condition.
6. The biocompatibility of the glucose sensor implanted into the body is especially important. A good biocompatibility means that the glucose sensor can function in the body without adverse reactions of the host due to toxicity or local foreign body reactions caused by materials used in the construction of the sensor or substances produced by the sensor. Biostability, i.e. the stability of the sensor when used in vivo, is directly related to the biocompatibility. For example, the permeability of sensor membranes may be influenced by reactions of tissue around the site of implantation causing a change in the sensor characteristics. Also diminishing response due to electrode fouling by biosubstances has a negative influence on the biostability.

7. An invasive glucose sensor must be of a size and shape that can be easily inserted and causes minimal discomfort to the patient. Also the glucose sensor should be simple to use, enabling the device to be operated by the patient himself.

In summary, in order to function reliably glucose sensors must have a high specificity, sensitivity, be accurate, have fast response times and a good biocompatibility. In addition, for a good patient compliance sensors should be small and simple to operate.

1.5 Conclusion

In this introduction some basic concepts of diabetes mellitus and the application of glucose sensors in the treatment of diabetes have been described. The long-term study performed by the Diabetes Control and Complications Trial Research Group (dcct group) has conclusively demonstrated that if glucose levels are tightly regulated diabetic complications can be controlled [3]. Main objective is the normalisation of the blood glucose concentration to reduce long-term complications and prevent hypoglycaemic events. Although progress has been made with pancreas- and islet-transplantation, it is not likely that these methods will be implemented on a large scale in the treatment of diabetes in the near future. In the beginning of the 1980s there has been a debate about the clinical need for a glucose sensor in diabetes treatment [76]. As a result of the intensive insulin treatment of diabetic patients, it is necessary to perform several blood glucose measurements
a day to adjust the insulin dosage properly. In practice, however, the number of measurements done with the finger-prick method is limited and will only provide information about blood glucose values at intermittent moments. Continuous in vivo glucose monitoring may therefore be an improvement and may contribute to a more adequate insulin administration. In addition, a glucose sensor could be of use in the early detection of hypoglycaemia. Despite considerable research efforts no glucose sensor is available in clinical practise. At present, non-invasive in vivo glucose-sensing methods are still very immature and are not serious substitutes for standard (invasive) analytical glucose-detection techniques. Various implantable glucose-sensor designs have been brought forward, but in general these sensors show a significant decay in sensitivity over the implantation period and are therefore of limited use. The combination of microdialysis and a standard glucose sensor can avoid a lot of the difficulties associated with sensors that are directly implanted in the subcutaneous tissue. Sensor systems based on the microdialysis technique may therefore be an important alternative for these needle-type glucose sensors.