NEW METHODS FOR GLUCOSE MONITORING IN HUMANS

Roland Schaller

DOCTORAL THESIS

GRAZ UNIVERSITY OF TECHNOLOGY
INSTITUTE FOR GENOMICS AND BIOINFORMATICS
PETERSGASSE 14, 8010 GRAZ, AUSTRIA

JOANNEUM RESEARCH FORSCHUNGSGESELLSCHAFT GMBH
INSTITUTE OF MEDICAL TECHNOLOGIES AND HEALTH MANAGEMENT
ELISABETHSTRASSE 11 A, 8010, GRAZ, AUSTRIA

Graz, April 2010

EIDESSTATTLICHE ERKLÄRUNG

Ich erkläre an Eides statt, dass ich die vorliegende Arbeit selbstständig verfasst, andere als die angegebenen Quellen / Hilfsmittel nicht benutzt und die den benutzten Quellen wörtliche und inhaltlich entnommene Stellen als solche kenntlich gemacht habe.

Graz, am …… ……..

(Unterschrift)

For scientific leadership give me Scott; for swift and efficient travel Amundsen; but when you are in a hopeless situation, when there seems no way out, get down on your knees and pray for Shackleton.

Sir Raymond Priestley

FIRST EXAMINER: Univ. Prof. Dipl. Ing. Dr. techn. Zlatko Trajanoski
Graz University of Technology
Institute for Genomics and Bioinformatics
Petersgasse 14, 8010 Graz, Austria
zlatko.trajanoski@tugraz.at

SECOND EXAMINER: Univ. Prof. Dr. Thomas Rudolf Pieber
Medical University of Graz
Department of Internal Medicine
Division of Endocrinology and Nuclear Medicine
Auenbruggerplatz 15, 8036 Graz, Austria
thomas.pieber@joanneum.at

ADVISOR: Dipl. Ing. Dr. techn. Lukas Schaupp
Medical University of Graz
Department of Internal Medicine
Division of Endocrinology and Nuclear Medicine
Auenbruggerplatz 15, 8036 Graz, Austria
lukas.schaupp@medunigraz.at

NEW METHODS FOR GLUCOSE MONITORING IN HUMANS

Roland Schaller

DOCTORAL THESIS

GRAZ UNIVERSITY OF TECHNOLOGY
INSTITUTE FOR GENOMICS AND BIOINFORMATICS
PETERSGASSE 14, 8010 GRAZ, AUSTRIA

Abstract:

Glyceamic management of critically ill patients reduces mortality and morbidity but imposes great demands on medical staff who must take frequent blood samples for the determination of glucose levels. Automated glucose monitoring systems, which measure glucose in interstitial fluid (ISF), overcome this resourcing problem but struggle with sensitivity loss, time delay and the discrepancy between glucose concentrations in plasma and ISF. To overcome these drawbacks, the aim of the present work was to develop two automated glucose monitoring systems extracting blood from a peripheral vein and to evaluate them technically and in human clinical studies.
The first system comprises an intermittent automatic blood sampling system (IABS) linked to a glucose biosensor (IAGM). Glucose concentrations obtained from manually drawn reference samples and those taken with the IABS system exhibited excellent correlation (Pearson's correlation coefficient R=0.982 and 0.976, system error 1.7±9.0% and -3.3±5.5% for 12h and 30h studies, respectively). This system can, in addition, be used to "feed" a blood gas analyser (e.g. Roche OMNI) as system errors < ±15% were achieved for most metabolites, ions, blood gases and CO-oximetry. Only the values for pO_2 and HHb were elevated. 1-point calibrated glucose values, obtained fully automatically with the IAGM system, also exhibited a very high degree of correlation (R=0.950, system error 1.0±6.6%). Clinical evaluation using Clark's Error Grid analysis (EGA) revealed, in 100% of cases, an accurate and acceptable treatment for the 12h and 30h studies. Automated blood sampling from a peripheral vein coupled with automatic glucose determination thus has the potential to provide a viable alternative to frequent manual blood sampling.
The second system continuously heparinises blood at the tip of a double lumen catheter and pumps this through a membrane-based microfluidic device to generate protein-free microdialysed blood samples (CMDS). Glucose recovery, as an indicator of long term stability, was studied in vitro with heparinised bovine blood and remained stable for 72h. 1-point calibrated glucose concentrations of the CMDS system and the reference method correlated to a very high level (R=0.960, system error 1.9±11.2%). EGA revealed that the glucose concentrations obtained were accurate and clinically acceptable in 99.6% of all cases. The CMDS thus delivers microdialysate samples suitable for accurate and long term stable continuous glucose monitoring in blood.

Key words:

Automated, blood, clinical trial, ex vivo, extra-vascular, glucose, in-vitro, microdialysis, monitoring, sampling, sensor

PUBLICATIONS

This thesis is based on the following publications and on the authors' non-published observations

JOURNALS

1. Schaller R., Feichtner F., Köhler H., Bodenlenz M., Plank J., Wutte A., Mader J., Ellmerer M., Hainisch R., Pieber T.R., Schaupp L.A.; **"Automated discontinuous venous blood sampling system for ex vivo glucose determination in humans"**; *Journal of diabetes science and technology, Vol. 3 (1), 2009*

2. Schaller, R., Feichtner F., Köhler H., Bodenlenz M., Plank J., Wutte A., Mader J.K., Ellmerer M., Hellmich R., Wedig H., Hainisch R., Pieber T.R., Schaupp L.A.; **"A novel automated discontinuous venous blood monitoring system for ex vivo glucose determination in humans"***; Biosens Bioelectron. Vol. 24 (7), 2009*

3. Feichtner F., Schaller R., Fercher A., Ratzer M., Ellmerer M., Plank J., Krause B., Pieber T.R., Schaupp L.A.; **"Microdialysis based device for continuous extravascular monitoring of blood glucose"**; *Biomedical Microdevices, published online 26th of January 2010; DOI 10.1007/s10544-010-9396-3 [in press]*

4. Lindpointner S., Korsatko S., Köhler G., Köhler H., Schaller R., Kaidar R., Yodfat O., Schaupp L.A., Ellmerer M., Pieber T.R., Regittnig W.; **"Use of the Site of Subcutaneous Insulin Administration for the Measurement of Glucose in Patients with Type 1 Diabetes"**; *Diabetes Care Vol. 33 (3), 2010*

5. Lindpointner S., Korsatko S., Köhler G., Köhler H., Schaller R., Ellmerer M., Pieber T.R., Regittnig W.; **"Glucose Levels at the Site of Subcutaneous Insulin Administration and Their Relationship to Plasma Levels"**; *Diabetes Care Vol. 33 (4), 2010*

CONFERENCE PROCEEDINGS

1. Schaller R., Schaupp L., Ellmerer M., Koller D., Eberharter R., Telalbasics S., Toller W. G., Plank J., Pieber T. R., Obermaier K., Schoemaker M.; **"Intensive Insulin Therapy in the ICU: Assessment by Interstitial Fluid Sampling"**; *Diabetalogia, Vol. 48: A300-A300, 2005*

2. Feichtner F., Schaller R., Fercher A., Schaupp L.A., Bodenlenz M., Köhler H., Plank J., Wutte A., Ellmerer M., Pieber T. R.; **"Kontinuierliches Glukosemonitoring bei Intensivpatienten mit Hilfe der Mikrodialyse in Vollblut"**; *Wiener Klinische Wochenschrift 118 (4), 2006; Annual Meeting of the Austrian Diabetes Society, Innsbruck, Austria, November 2006*

3. Schaller R., Feichtner F., Fercher A., Schaupp L.A., Bodenlenz M., Koehler H., Plank J., Wutte A., Ellmerer M.; **"Automatisches diskontinuierliches System zur venösen Blutabnahme für die Blutzuckerbestimmung bei Intensivpatienten"**; *Wiener Klinische Wochenschrift 118 (4), 2006; 34th Annual Meeting of the Austrian Diabetes Society, Innsbruck, Austria, November 2006*

4. Feichtner F., Schaller R., Fercher A., Schaupp L.A., Bodenlenz M., Köhler H., Plank J., Wutte A., Ellmerer M., Pieber T.R.; **"Manuelle Blutentnahme bei klinischen Studien in der Diabetesforschung: Ist die herkömmliche Referenzmethode zuverlässig?"**; *Wiener Klinische Wochenschrift 118 (4), p8 2006; 34th Annual Meeting of the Austrian Diabetes Society, Innsbruck, Austria, November 2006*

5. Schaller R., Feichtner. F, Fercher A., Schaupp L.A., Bodenlenz M., Köhler H., Plank J., Wutte A., Hainisch R., Pieber T. R.; **"System for automated discontinuous venous blood withdrawal for glucose determination of patients at the ICU"**; Critical Care 11 *(2), March 2007), 51-52. 2007; 27th International Symposium on Intensive Care and Emergency Medicine, Brussels, Belgium, March 2007.*

6. Feichtner, F., Schaller, R., Fercher, A., Schaupp L.A., Plank, J, Wutte, A., Ellmerer, M, and Pieber T.R.; **"Continuous glucose monitoring for intensive care patients using whole blood microdialysis"**; *Crit Care 11 (2), 57-58, 2007; 27th International Symposium on Intensive Care and Emergency Medicine, Brussels, Belgium, March 2007*

7. Schaller R., Feichtner F., Schaupp L.A., Köhler H., Bodenlenz M., Korsatko S., Wutte A., Doll W., Ellmerer M., Hainisch R., Pieber, T. R.; **"Automated Discontinuous Venous Blood Sampling System for Glucose Determination in ICU Patients"**; *7th Diabetes and Technology Meeting; San Francisco, USA, October 2007*

8. Feichtner F., Schaller R., Koehler H., Korsatko S., Wutte A., Hellmich R., Wedig H., Gruendig B., Ellmerer M., Pieber T.R.; **"Continuous Glucose Monitoring Based on Whole Blood Microdialysis and Online Sensors"**; *7th Annual Meeting of the Diabetes Technology Society, San Francisco, USA, October 2007*

9. Feichtner F., Schaller R., Krause B., Schaupp L., Ellmerer M., Pieber T.R.; **"Extravascular Microdialysis as a Promissing Tool for Systemic Long Term Monitoring of Glucose in Humans"**; *4th International Conference on Clinical Microdialysis, Cambridge, UK, November 2007*

10. Schaller R., Kulnik R., Feichtner F., Schaupp L.A., Bodenlenz M., Pickl K., Doll W., Sinner F., Ellmerer M.; **"Development of a Novel Clinical Diagnosis System – Legal Requirements for Product Certification"**; *2nd ZMF Day, November 2007, Graz, Austria*

11. Schaller R., Feichtner F., Schaupp L.A., Köhler H., Bodenlenz M., Korsatko S., Wutte A., Doll W., Ellmerer M., Hainisch R., Pieber T. R.; **"Methode zur Automatischen Blutprobenentnahme"**; *35th Annual Meeting of the Austrian Diabetes Society, Innsbruck, Austria, November 2007*

12. Feichtner F., Schaller R., Koehler H., Korsatko S., Wutte A., Hellmich R., Wedig H., Gruendig B., Damm U., Kondepati V.R., Heise M., Ellmerer M., Pieber T.R.; **"Kombination aus Vollblut-Mikrodialyse und Online-Sensoren fuer kontinuierliches Glukosemonitoring"**; *35th Annual Meeting of the Austrian Diabetes Society, Innsbruck, Austria, November 2007*

13. Köhler H., Pasic A., Bauer G., Bodenlenz M., Feichtner F., Schaller R., Mader J., Klimat I., Schaupp L., Pieber T.R.; **"Fibreoptic hybrid sensor for continuous glucose monitoring"**; *27th Workshop of the AIDPIT Study Group, Igls, Austria, January 2008*

14. Mader J.K., Feichtner F., Korsatko S., Köhler G., Schaller R., Plank J., Pieber T.P., Ellmerer M.; **"Evaluation of microdialysis-based glucose monitoring in blood and subcutaneous adipose tissue for ICU application"**; *Annual meeting of the European Society of Intensive Care Medicine, Lisbon, Portugal, September 2008*

15. Mader J.K., Feichtner F., Korsatko S., Köhler G., Schaller R., Plank J., Pieber T.R., Ellmerer M.; **"Evaluation of microdialysis-based glucose monitoring in blood and subcutaneous adipose tissue in type 1 diabetic patients";** *44th Annual Meeting of the European Association for the Study of Diabetes (EASD), Rome, Italy, September 2008*

16. Mader J.K., Feichtner F., Korsatko S., Köhler G., Schaller R., Plank J, Pieber T.R. Ellmerer M.; **"Evaluation of microdialysis-based glucose monitoring in blood and subcutaneous adipose tissue in type-1-diabetic patients"**; *Abstract book of the 2nd International Conference on Advanced Technologies & Treatments for Diabetes (ATTD), Athens, Greece, February 2009*

17. Mader J.K., Feichtner F., Bock G., Köhler G., Schaller R., Plank J., Pieber T.R., Ellmerer M.; **"Evaluation of microdialysis-based glucose monitoring in blood and subcutaneous adipose tissue in type 1 diabetic patients"**; *Diabetes Technology Meeting, San Francisco, November 2009; Diabetes Technology Peterson Student Research Bronze Prize*

AWARDS

Winner of the Stefan-Schuy-Preis 2009

CONTENTS

ABBREVIATIONS

ADA	American Diabetes Association
APTT	Activated Partial Thromboplastin Time
ARD	Absolute Relative Difference
BF	Body Floating
BMI	Body Mass Index
Ca^{++}	Calcium
CGMS	Continuous Glucose Monitoring System
Cl^{-}	Chloride
CLINICIP	Closed Loop Insulin Infusion for Critically Ill Patients
CMDS	Continuous Microdialysate Sampling
COHb	Carboxyhaemoglobin
CRF	Case Report Forms
DLC	Double Lumen Catheter
ECF	Extra Cellular Fluid
EGA	Error Grid Analysis
EtO	Ethylene Oxide
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GOD	Glucose Oxidase
GUI	Graphical User Interface
Hct	Haematocrit
HHb	Deoxyhaemoglobin
IABS	Intermittent Automated Blood Sampling
ICU	Intensive Care Unit
ID	Inner Diameter
ISF	Interstitial Fluid
ITEGA	Insulin Titration Error Grid Analysis
IV	Intravenous
K^{+}	Potassium
LAN	Local Area Network
MAD	Mean Absolute Difference
MARD	Mean Absolute Relative Difference
MD	Mean Difference
µD	Micro Dialysis
MetHb	Methaemoglobin
MRD	Mean Relative Difference
Na^{+}	Sodium
NC	Normal Condition
O_2Hb	Oxyhaemoglobin
OD	Outer Diameter
OGTT	Oral Glucose Tolerance Test
pCO_2	Partial pressure of Carbon dioxide
PFTMD	Planar Flow Through Microdialysis Device
pH	pH value
pO_2	Partial pressure of Oxygen
POC	Point of Care
R	Pearson’s coefficient of correlation
SD	Standard Deviation
SE	System Error
SELV	Safety Extra Low Voltage
SFC	Single Fault Condition
SMBG	Self Monitoring of Blood Glucose
SO_2	Oxygen saturation of haemoglobin
SOP	Standard Operating Procedure
SOTA	State Of The Art
TGC	Tight Glycaemic Control
tHb	Total Haemoglobin
WHO	World Health Organization

1. INTRODUCTION

1.1. Diabetes and its complications

In healthy individuals, blood glucose concentration is maintained by the pancreas between 72-126mg/dl (4 and 7mmol/l). This entails balancing the amount of glucose entering the blood stream (e.g. from carbohydrate food consumption) with its uptake from the blood stream by cells. After glucose ingestion, this balance is disrupted and its re-establishment is predominantly effected by insulin release in a homeostatic process. Insulin, which is produced and secreted by the pancreatic β-cells situated in the islets of Langerhans, has extensive effects on metabolism and other bodily functions. It causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle and stops the use of fat as an energy source.

1.1.1. Type 1 and Type 2 diabetes

Diabetes mellitus is a chronic disease that occurs when the pancreas does not produce enough insulin (Type 1, previously known as insulin-dependent, juvenile or childhood-onset), or when the body cannot effectively use the insulin it does produce (Type 2, formerly called non-insulin-dependent or adult-onset). Type 2 diabetes is much more common than Type 1 diabetes, and accounts for about 90% of all diabetes worldwide. Diabetes, if left untreated, will often result in blood glucose levels in the range of 270-450mg/dl (15-25mmol/l) and even considerably higher [1]. Chronically elevated blood sugar, or hyperglycaemia, leads to serious damage to many of the body's systems, especially the heart, blood vessels, eyes, kidneys, and nerves. Diabetes causes about 5% of all deaths globally each year: WHO [2] projects that diabetes deaths will double between 2005 (1.1 million) and 2030. 171 million people in the world had diabetes in the year 2000 and this number will increase to 366 million by 2030 [3].

1.1.2. “Stress diabetes”

Recent medical results have revealed that patients brought to intensive care units (ICU) are at risk of hyperglycaemia due to trauma or stress, even without a clinical history of diabetes [4, 5, 6]. The stress of critical illness induces glucose counter regulatory hormones and alterations in carbohydrate metabolism, resulting in peripheral glucose demands, enhanced hepatic glucose production, insulin resistance and relative insulin deficiency [7]. Additionally, clinical interventions such as corticosteroids, vasopressors and enteral or parenteral nutrition, elevate blood glucose levels [8]. Hyperglycaemia is associated with adverse clinical outcomes such as myocardial infarctions, polyneuropathy and multiorgan failure [9, 10]. Stress diabetes must thus be treated to improve ICU patient outcome.

1.2. Management of diabetes

All forms of diabetes have been treatable since insulin was isolated by Sir Frederick Grant Banting and became medically available in 1921. Both Type 1 and Type 2 diabetes presently remain incurable however. The aim of the current approach to the treatment of Type 1 diabetes is to keep blood sugar levels as close as possible to normal ("euglyceamia") by external glucose regulation through insulin substitution, (Figure 1), i.e. by mimicking the function of the healthy pancreas (“artificial pancreas”). Exogenous insulin is administered to the diabetic patient several times per day by subcutaneous or intramuscular injection (syringe or insulin pen) or continuously (insulin pump using a catheter placed in the subcutaneous adipose tissue) at dosages that are determined by blood glucose levels, carbohydrate intake and level of physical exercise. To reduce the risk of acute hypoglycaemic

shock, a life-threatening consequence of low blood sugar level caused by excessive insulin administration, blood glucose levels must be monitored continuously or at least several times a day before insulin injection. Spot measurements, which are normally performed manually by the diabetic patient, are analogous to still camera pictures. A continuous signal, normally generated by an online glucose sensor, in contrast, is comparable to video, and provides additional information about the direction and rate of change of the blood glucose level. Critically, the more limited information provided by spot measurements compared to continuous monitoring, increases the risk of undetected hypo- or hyperglycaemic episodes. Since such episodes influence the severity of the complications suffered by diabetic patients, an increased frequency of blood glucose measurement is essential to improve the treatment of diabetes.

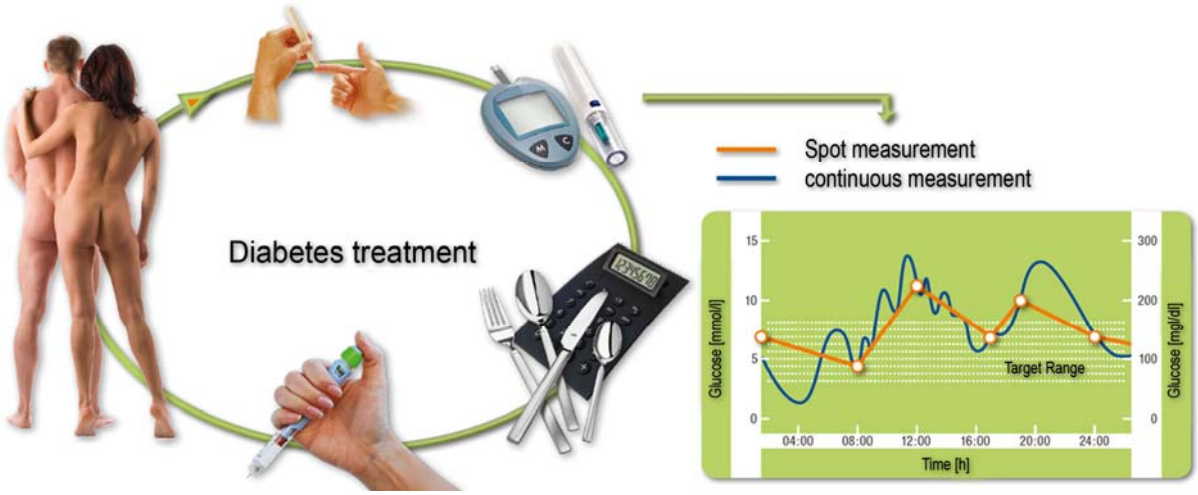


Figure 1: Treatment of diabetes mellitus: Insulin substitution therapy; continuous vs. discontinuous measurements

Euglyceamic glucose levels are maintained in Type 2 diabetics by dietary management, exercise and the use of orally administered anti-hyperglyceamic agents. Insulin substitution therapy is only required for the very few cases in which a combination of different anti-hyperglyceamic agents does not normalize blood sugar level.

1.2.1. Management of Type 1 diabetes

As there is currently no artificial pancreas available, diabetic patients must monitor their blood glucose levels and self-administer insulin subcutaneously or intramuscularly according to their carbohydrate intake. Since the diabetic patient can inject insulin intermittently using syringes or pens or even continuously with well established insulin pumps, further development of diabetes management is focused mainly on improved glucose determination (see Figure 2). In the past, intermittent glucose determination was performed with urine test strips. Nowadays, two main types of devices are used: Intermittent self-monitoring of blood glucose (SMBG**)** devices and continuous glucose monitoring systems (CGMS):

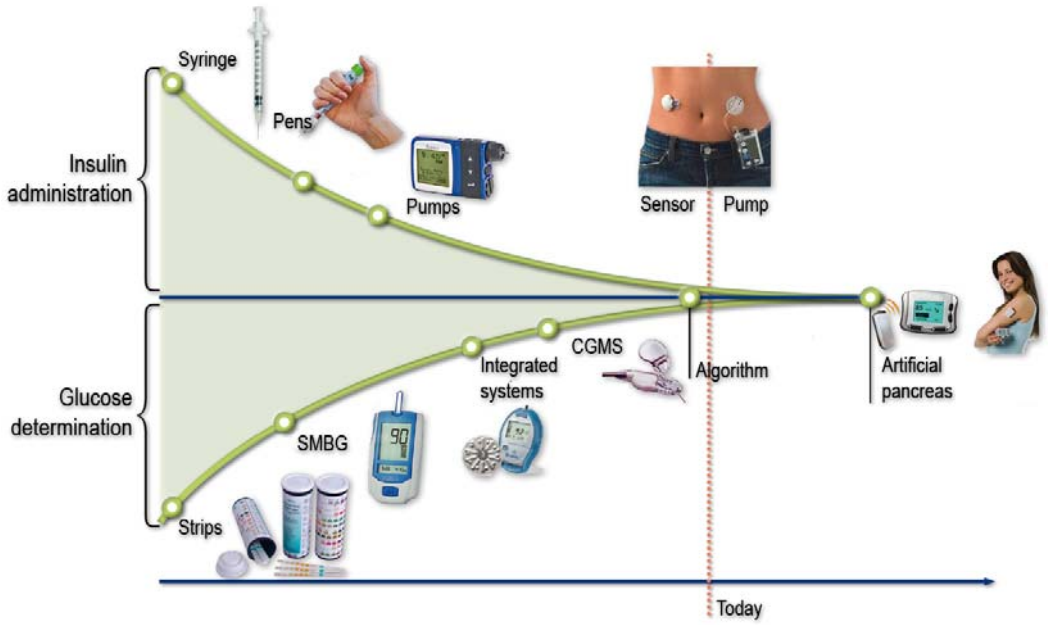


Figure 2: Milestones towards the development of an artificial pancreas for Type 1 diabetes

SMBG devices (Figure 2), which are easy-to-use hand-held systems for intermittent, real time, point of care glucose measurement, are used predominantly for the treatment of Type 1 diabetic patients. A range of SMBG devices are available, usually in the form of strips or meters. These differ in the amount of blood necessary for each test (at least 0.3µl), the measuring principle (colorimetric, photometric or electrochemical principle), the measurement time (at least 5s), the size of the device and their cost. Most SMBG devices have been approved by the US Food and Drug Administration (FDA). Blood to feed the SMBG device can be taken very simply by finger pricking using lancets which are available with antibacterial properties [11]. The American Diabetes Association (ADA) recommends that SMBG should be carried out by the diabetic patient at least three times a day to effectively monitor blood glucose over time and determine the need for insulin administration [12]. The reality is however starkly different: a survey published in 2001 documented that 39% of diabetic patients taking insulin monitored their blood glucose only once a day and 29% did so less than once a month [13].

CGMS which automatically measure glucose concentrations in the subcutaneous adipose tissue represent the latest alternatives to SMBG devices. Both minimally invasive and non-invasive CGMS are available. The following continuous or near continuous sensor approaches are amongst those used: implantable needle-type sensors, minimally invasive sensors based on microdialysis and non-invasive optical spectroscopic sensors. These are the "Continuous Glucose Monitoring System Gold", "Guardian Telemetered Glucose Monitoring System" and "Guardian RT" from Medtronic Minimed and the "STS Continuous Glucose Monitoring System" from Dexcom Inc. The non-invasive system, the "GlucoWatch G2 Biographer" from Johnson and Johnson (formerly Cygnus) which operates by reverse iontophoresis was withdrawn from the market. For the remaining systems, gradients ranging from 20% [14] to110% [15] were found between whole blood and extra cellular fluid (ECF) requiring that these systems be calibrated using conventional SMBG devices.
Closing the loop of diabetes management using automated glucose determination followed by automated insulin infusion is today relatively uncomfortable: To use CGMS the diabetic patient must place a minimally invasive or needle type sensor in his or her subcutaneous adipose tissue using a special inserter. A transmitter, that sends

the information to the insulin pump, must then be attached and carried around. Additionally, a catheter must be placed in the subcutaneous adipose tissue which is then attached to the insulin pump via tubing.
One of the aims of this work [16] was, therefore, to address these issues by introducing a “single port” system using just one catheter. This would require that only one device, incorporating the sensor and pump in a single housing, need be carried around. Additionally the size of this device should be reduced to as few components as necessary (e.g. transmitter, receiver, one power supply, etc)

1.2.2. Management of “Stress diabetes”

“Stress diabetes” could be managed in a manner similar to the way in which Type 1 diabetes is treated, i.e through application of tight glyceamic control (TGC) by external regulation of glucose supported by insulin substitution therapy. Exogenous insulin would be administered by intravenous infusions according to blood glucose levels and carbohydrate intake from enteral and parenteral nutrition and medication. An “artificial pancreas” would address the two main reasons why TGC has not been successfully implemented in European ICUs, namely high workloads and the fear of hypoglycaemia. The idea of replacing lost endocrine function with an artificial pancreas is more than 45 years old and was introduced in 1963 by Dr. Arnold Kadish [17]. The artificial pancreas (Figure 3) consists in principle of four components: a “body interface” to access any body fluid containing glucose information (e.g. ECF or blood), a sensor that continuously monitors glucose levels in this fluid, an algorithm that calculates the correct insulin dose based on the sensor readings and an insulin infusion system. As appropriate insulin pumps are available for ICUs and algorithms suitable to titrate insulin have been reported [18], we focused principally on the glucose monitoring component.

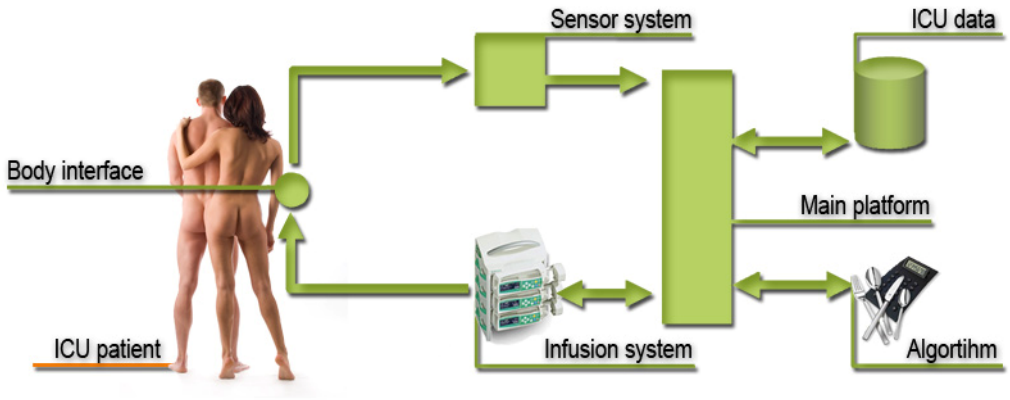


Figure 3: Artificial pancreas

The field of stress diabetes management is complicated by the current lack of a broad consensus regarding the optimum glucose level target in ICU patients.
Greete van den Berghe reported a substantial reduction of mortality and morbidity in critically ill patients [19] in a clinical study conducted in 2001, involving 1548 surgical ICU patients. Blood glucose was maintained between 80-110mg/dl (4.4-6.1mmol/l, intensive insulin group) and between 180-200mg/dl (10-11.1mmol/l, conventional insulin group). Furthermore a substantial reduction in medical care cost was also an outcome [20].
In contrast, in 2009 the Nice sugar study, a large, international, randomized trial involving 6104 patients, reported increased mortality among adults in the ICU undergoing intensive glucose control. A blood glucose target of 180mg/dl (10mmol/l) or less resulted in lower mortality than a target of 81 to 108mg/dl (4.5 to 6) [21].

A Meta analysis involving 26 trials and a total of 13 567 patients could not exclude the possibility that some patients may benefit from intensive insulin therapy. Surgical ICU patients for instance appear to benefit [22]. Opinion leaders argue that intensive insulin therapy may not always describe the same intervention. In some settings, such therapy may have induced large fluctuations in blood glucose, possibly with undetected hypoglycaemia alternating with hyperglycaemia. The result is a reduction of average blood glucose level, accompanied by fluctuation in blood glucose that may be worse for the patient than constant moderate hyperglycaemia [23]. As a result, glycaemic disturbances, including severe hypoglycaemia that occurs during the day [24] before symptoms are recognized, or during the night without recognition of symptoms [25] often go undetected.

Whatever the ideal glucose target levels turn out to be, the minimum measurement frequency remains to be defined. It is generally agreed that continuous glucose measurement as a part of TGC has the potential to provide affordable improvements in the management of glycaemia in the ICU, in particular by filling current glucose "data holes". The sampling intervals in the Greete van den Berghe and Nice Sugar studies were between 1 and 4h. More frequent intermittent or continuous measurements, possibly with a bedside device, are however necessary to avoid undetected hypoglycaemia. Bremer et al [26] showed that a simple linear model can be used to obtain 10min predictions from data representing a variety of glycaemic states. In certain circumstances, 20 or 30min predictions may also be acceptable. It is therefore clear that strict glyceamic control using intensive insulin therapy protocols requires frequent and accurate glucose monitoring.

This cannot be achieved with hand held glucose meters such as the Roche Accu Chek Inform, which is similar to SMBG devices, even if they are specially designed for use in ICUs. The accuracy of SMBG devices may, moreover, be affected by skin temperature, measurement site, whether the patient is severely dehydrated, hypotensive, in shock, in a hyperosmolar state or when the blood circulation is reduced [27]. Point of Care (POC) solutions, such as the Cobas b 221 system (formerly Omni S6), are available in most ICUs, enabling the most important critical care blood parameters to be determined within minutes. The Cobas b 221 system is a multi-parameter analyser for metabolites (e.g. glucose and lactate), blood gas, electrolytes and CO-oximetry. The crux of the problem is however that, whatever device is used, frequent manual glucose monitoring is labour-intensive and thus expensive. An automated system would provide clear advantages.

At face value, CGMS**,** as used for Type 1 diabetic patients, who continuously measure glucose within subcutaneous adipose tissue, could provide a solution. Discrepancies between glucose concentrations in blood and ECF have however been reported in critically ill patients [28] and in critically ill children [29]. CGMS that sense glucose in the ECF of the subcutaneous adipose tissue face the disadvantage of a time lag between blood and ECF ranging from 5min [30] to up to 30min [31]. Whilst this may be acceptable for Type 1 diabetic patients, it is certainly not for ICU patients who are critically ill. Additionally, since insulin is administered to ICU patients intravenously, blood glucose fluctuates more than in Type 1 diabetic patients. This has been confirmed by Klonoff [32] who recently reviewed CGMS approved by the FDA for use in the US or Europe and reported that values provided by CGMS during hypoglycaemia or periods of rapid fluctuation may be inaccurate unless they are repeatedly recalibrated. In addition, a study on CGMS guided insulin adjustment [33] suggested that CGMS are no more useful than intermittent finger pricking and the use of SMBG devices. Since blood from an arterial or venous line is anyway available in ICU patients, it is obvious from a therapeutic point of view that the gold standard, whole blood should be accessed for continuous glucose monitoring in ICU patients.

Since blood tends to clot outside the body and/or if it contacts artificial surfaces [34], short contact times or heparinisation are obvious. Because of the need to avoid systemic heparinisation of ICU patients, especially after surgery, systems for extracorporeal heparinisation using a "Double lumen catheter" (DLC) were introduced in the 1960's [35].The outer lumen of the DLC is used to deliver a NaCl-Heparin solution to the tip of the catheter to prevent coagulation whilst anti-coagulated diluted blood is withdrawn simultaneously through the inner lumen. The first commercial product using this technology was the Biostator produced by the Miles Laboratory in 1977. Next

generation models were the Glucostator (mtb Zier GmbH, Lonsee, Germany) and the Nikkiso STG-22 which is similar to the Glucostator but only available in Japan. These devices were mostly used in clamp studies and not designed for ICU use. Via medical [36] in contrast, introduced a non-blood consuming, real time blood glucose analyzer using a single lumen catheter to withdraw undiluted whole blood and an ex vivo glucose sensor. This device is, to the best of our knowledge, no longer available. Systems using extracorporeal enzyme-based electrochemical glucose sensors are vulnerable to a loss of activity and sensitivity due to effects on signal stability and enzyme function caused by the settling of high molecular weight proteins onto the sensor surface. Such sensor activity loss can be minimized by the use of special polymers as protective coatings for implantable sensors. An alternative to this is the use of microdialysis (μD) [37,38] to extract blood components via a semi-permeable membrane. High molecular weight compounds are filtered out, before sensing small molecules such as glucose. An analyte-free aqueous solution (perfusate) is pumped through the microdialyser and enriched by diffusion with molecules smaller than the membrane's molecular weight cut-off. The analyte concentration in dialysate and blood, processed through the microdialyser, are linked up and depend in extracts on flow rates, temperature, charge and membrane surface. A critical issue is clot formation around the membrane [39], which decreases microdialysis efficacy and necessitates frequent re-calibration. To minimise the time delay before a glucose measurement result is available, an intravenous μD catheter rather than an extracorporeal dialyser can be used. Intravenous μD was introduced in the 1990's [40] but the use of this technique in humans has rarely been reported [41,42,43,44,45,46,47]. An explanation for this small number of studies might be the lack of commercially available probes and thus the risk of embolism associated with implanting a fragile handmade membrane into the vascular system. This technique moreover also exhibited decreasing membrane μD efficiencies during in vitro and in vivo studies [48,49,50], possibly due to clot formation [51]. Whilst CMA Microdialysis AB has recently launched a product for intravenous microdialysis, the IView, published data are as yet unavailable.

Having taken into consideration the advantages and disadvantages of the different state-of-the-art methods, our aim within the present work was to simplify the management of stress diabetes through the design, construction and evaluation of two systems that automatically extract whole blood either intermittently or continuously from a peripheral vein. A further aim was to directly process the extracted blood, either directly in a polymer protected online glucose sensor, with a SOTA blood gas analyser or through the removal of proteins by extravascular microdialysis to improve the sensor's useful lifetime.

1.3. Objectives

To improve the management of stress diabetes, the main objectives of this thesis were to develop and evaluate two robust, reliable and simple bedside devices for glucose monitoring in ICU patients during different glycaemic conditions. Laboratory analyses and clinical experiments were performed in order to examine the behaviour of these systems:

1.) The “intermittent automated blood sampling system” (subsequently called “IABS system”) intermittently extracts whole blood from a peripheral vein using a single lumen catheter. This allows glucose determination either with an integrated glucose online sensor using special polymers as protective coatings, (subsequently called “IAGM system”) or by coupling to a SOTA glucose analyser.

The results are summarized in the publications entitled:
“A novel automated discontinuous venous blood monitoring system for ex vivo glucose determination in humans”
and
“An automated discontinuous venous blood sampling system for ex vivo glucose determination in humans”.

2.) The “continuous microdialysate sampling system” (subsequently called “CMDS system”) continuously extracts diluted whole blood from a peripheral vein using a double lumen catheter and heparin. This mixture is subsequently processed by a newly developed extravascular planar flow-through microdialysis device (PFTMD) incorporating a new membrane to gain a protein free matrix.

The results are summarized in the publication entitled:
“Microdialysis based device for continuous extravascular monitoring of blood glucose”

Furthermore, additional studies were performed in order to improve the management of Type 1 diabetes. The objective was to develop a single port treatment approach, which allows both variable and controlled insulin delivery and simultaneous glucose sampling at a single subcutaneous adipose tissue site in Type 1 diabetic patients.

These results are summarized in the publications entitled:
“Use of the Site of Subcutaneous Insulin Administration for the Measurement of Glucose in Patients with Type 1 Diabetes”
and
“Glucose Levels at the Site of Subcutaneous Insulin Administration and Their Relationship to Plasma Levels”

2. RESEARCH DESIGN AND METHODS

2.1. Manual state of the art blood sampling

The SOTA manual blood sampling system (Figure 4) comprises a conventional single lumen catheter (a) to access a peripheral vein in the body, an extension line (b) to minimise patient discomfort, two 3-way stop cocks (c, d) and two syringes (f, g) attached to the extension line. In addition, sterile 0.9% saline solution (e) is connected via a standard fluid administration set to guarantee patency of the system. With the distal 3-way stop cock (d) and syringe (g), a predefined amount (normally 5ml) is withdrawn to guarantee withdrawal of undiluted blood at the proximal 3-way stop cock (c) and syringe (f) which then contains the actual blood sample. After withdrawing an undiluted blood sample with the syringe (f) the blood remaining in the distal syringe is reinfused to minimise patient blood loss. Clotting is prevented by flushing the system with physiological saline solution. To ensure that saline-free, undiluted blood is sampled, flushing must be stopped prior to each sampling. The dead volume of the system from the catheter tip to the proximal 3-way stop cock is 0.7ml.

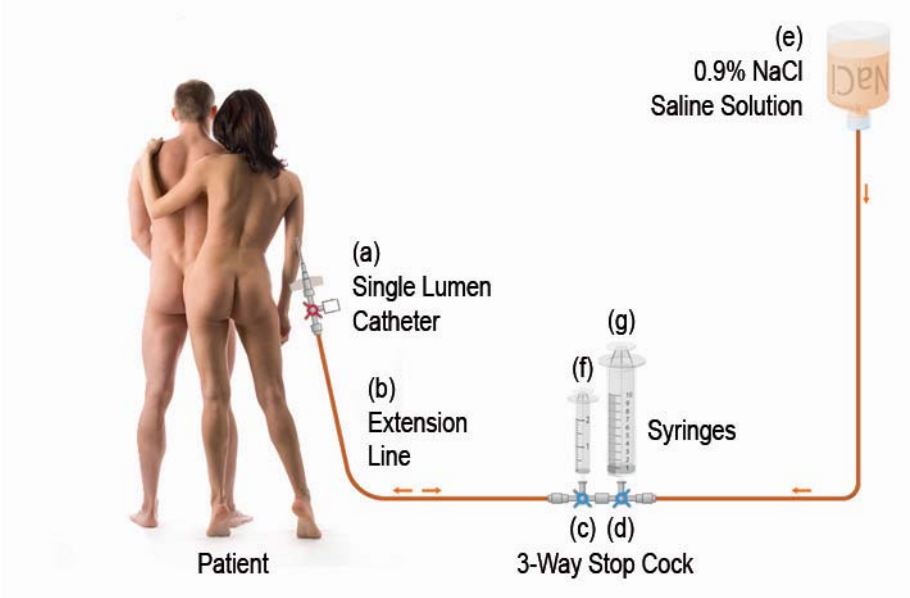


Figure 4: Manual state of the art blood sampling

Materials: Catheter (20 G Tricath, Codan, Germany), two extension lines with a length of 15cm (Extension line, Codan, Germany), two 3-way Stop cocks (Smiths), administration set (Com-Flow®-P, HSO Pharma GmbH) and physiological saline solution (Fresenius 500ml, Fresenius Kabi, Austria), 5ml syringe as reservoir (Kendall Monoject) and a 2ml syringe for sample withdrawal (Kendall Monoject).

2.2. Intermittent systems

2.2.1. Working principle - Intermittent automated blood sampling system (IABS)

The IABS system (Figure 5) mimics the manual SOTA blood sampling process (see 2.1). Before connecting it to the subject via a single lumen catheter, it is flushed with sterile 0.9% saline solution (Fresenius, Fresenius Kabi, Graz, Austria) to remove air. For each sampling, a blood volume of 4ml is aspirated by a peristaltic pump (Minipuls MP3, Gilson, Cedex, France) at a flow rate of 10ml/min and collected in a buffer loop. Saline solution within the system is disposed of directly into the waste container via pinch valve 2. Approximately 1.7ml of this drawn blood (actual blood consumption of the IABS system) is then immediately branched off and ~1ml is directly pumped into the waste container (partly diluted fluid tail) before collecting undiluted samples of ~0.3ml in a probe container of the same type as used for manually drawn reference blood for analysis. The remaining partly diluted blood is afterwards also transferred to the waste container. The blood remaining in the buffer loop (approximately ~2.3ml) is then immediately reinfused into the subject. Following sampling with the IABS system, ~9ml of flushing fluid is pumped through the patient line to clean it and to avoid clotting. The whole procedure is finished after at most 93s, avoiding coagulation by exposing whole blood to artificial surfaces for an unreasonably long time. The in-house assembled tubing system (TYGON S-50-HL, Saint-Gobain Performance Plastics, Beaverton, France, sterilized before initial use with ethylene oxide) is afterwards completely flushed using sterile 0.9% saline solution to prevent clotting. The sterile saline solution is attached to the system using a standard fluid administration set (Infusiomatleitung, B. Braun, Melsungen, Germany), and its fluid level is monitored via a dripping sensor (Type: 3450578 A, B. Braun, Melsungen, Germany). Flow is controlled using pinch valves (PM-0815W, Takasago Electric, Inc., Japan) compatible with the TYGON tubing used.
A check valve (Part No. 11582, Qosina, Edgewood, NY, USA) prevents reflux and maintains the sterility of the saline solution. The dead space of the system from the IV catheter to the sampling site is 0.75ml. For safety reasons, pressure changes within the accessed vessel are monitored with a reusable pressure transducer and a disposable pressure dome (SP854, Memscap, Norway). The maximum allowed pressure is set at 150kPa, which is comparable to the maximum pressure applied by infusion pumps. As well as this, an air-bubble sensor (customized sensor, Zevex, Salt Lake City, UT) is used to avoid accidental air infusion. The system's housing was designed by Solid Works (Solid Works, Concord, MA) and rapidly prototyped (FH OOE, Linz, Austria). All materials used (e.g. tubing) had been previously approved for in vivo use in humans.

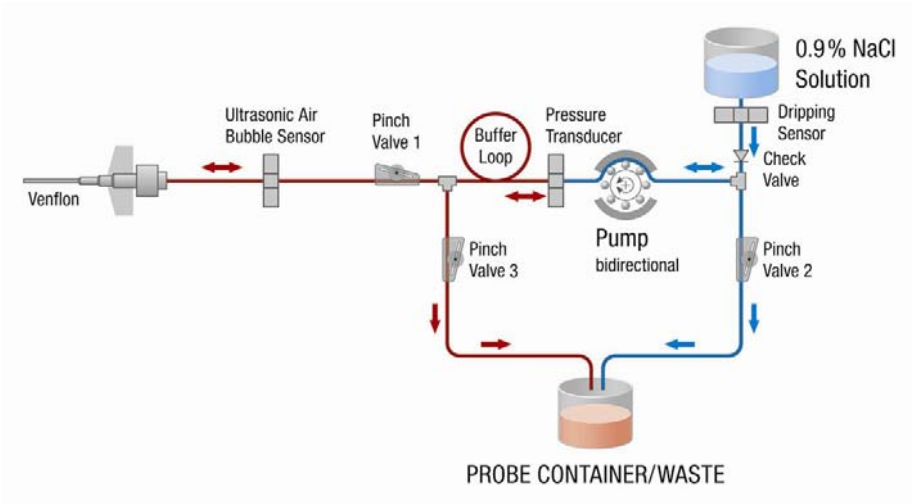


Figure 5: Schematic of the IABS system

2.2.2. Working principle - Intermittent automated glucose monitoring system (IAGM)

The IAGM system (Figure 6) incorporates two additional components into the IABS system. A 2nd Gilson pump extracorporeally heparinises the extracted blood sample to a final concentration of 50IU/ml and stores it in a 2nd buffer loop until the patient line is flushed. The stored and heparinised blood sample is then uni-directionally pumped through the in-house assembled biosensor from SensLab for 10min at 20µl/min and is afterwards immediately discarded. The biosensor current is recorded every 10s. The in-house assembled tubing system is again afterwards completely flushed using sterile 0.9% saline solution to prevent clotting and to restore the initial state of the system.

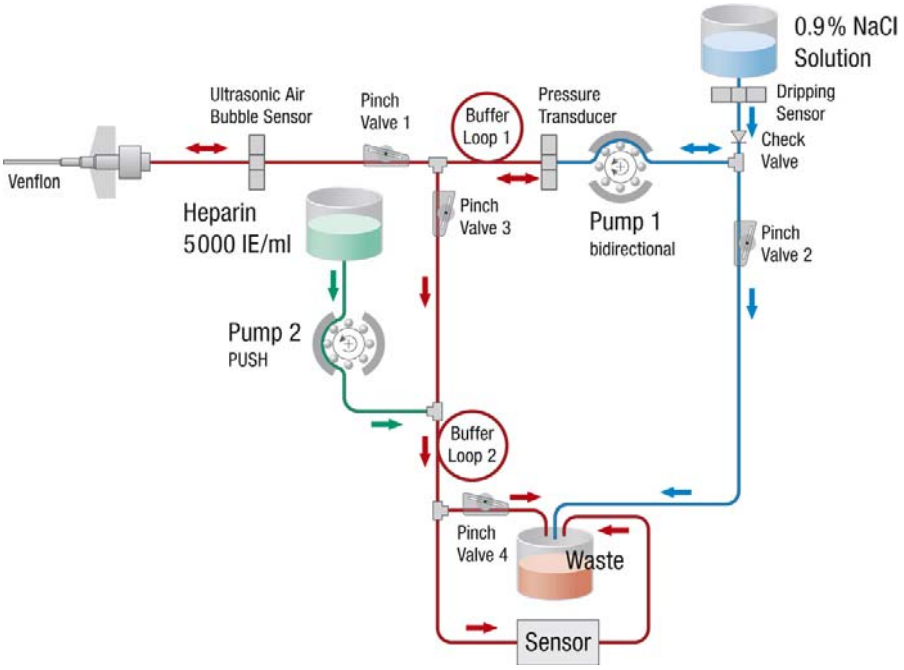


Figure 6: Schematic of the IAGM system with ex vivo heparinisation

2.2.2.1. SensLab glucose sensor

The SensLab glucose biosensor (Figure 7) consists of a micro-structured planar flow-through cell (Polycarbonate, Thyssen Krupp Schulte, Düsseldorf, Germany) with outer dimensions of 10mm×10mm×2mm (internal volume of the measuring chamber: 0.5µl) and a screen-printed amperometric thick film sensor coated with glucose oxidase (GOD). The biosensor comprises a plastic support on which three different polymeric carbon paste conducting paths are printed. In the area of the indicating window, formed later by the dielectric layer, a platinised carbon paste is overprinted on two of these three conducting paths to create respectively working and counter electrodes, The third electrode is covered by a silver/silver chloride paste to create the reference electrode. In a final screen printing step, an isolating (dielectric) paste is overprinted to define the indicating window of the sensor. The three electrode system consists of the working, counter and reference electrodes according to the potentiostatic principle. Approximately 31 units (0.5µg) of GOD (Serva, Heidelberg, Germany) are immobilised on the surface of the working electrode using polycarbamoyl sulfonate (PCS; prepolymer of polycarbomoyl sulfonate, SensLab, Leipzig, Germany) as an entrapping matrix, after which siloprene (Fluka, Schnelldorf, Germany) is deposited at the GOD-PCS matrix layer to form a diffusion barrier and protective layer to enable a diffusion-controlled reaction.

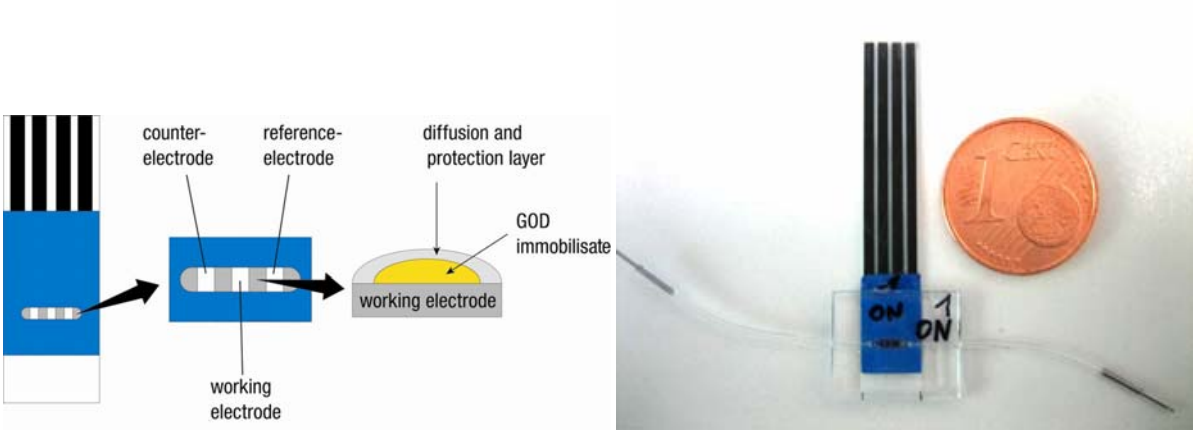


Figure 7: SensLab Biosensor

Glucose is detected by a primary indicating reaction mediated by immobilised GOD, which catalyses the oxidation of glucose to glucono delta-lactone and hydrogen peroxide according to Equation 1:

$$\text{D-glucose} + O_2 + H_2O \xrightarrow{\textit{Glucose oxidase}} \text{D-glucono-1.5-lactone} + H_2O_2 \qquad \text{Equation 1}$$

The hydrogen peroxide generated is then quantified by a secondary electrochemical reaction. Oxidation of hydrogen peroxide according to Equation 2 at the surface of the working electrode caused by the application of a polarisation voltage of +400mV with respect to the internal silver/silver chloride reference electrode generates an anodic electron flow. This electron flow is directly proportional to the glucose content of the sample, which is measured by an ampere meter as part of the potentiostat.

$$2\,H_2O_2 \xrightarrow{\textit{Working electrode (anode)}} O_2 + 2H^+ + 2\,H_20 + 2e^- \qquad \text{Equation 2}$$

2.2.2.2. Pressure Transducer

For safety reasons, pressure changes within the accessed vessel are monitored using a reusable non-invasive pressure transducer and a disposable pressure dome (SP854, Memscap, Norway) in the patient line. The electrical parts of the sensor, including the piezo-resistive transducer, are completely isolated from the housing. The disposable pressure dome is dry coupled to the sensor by a silicone diaphragm. The maximum allowed pressure is set at 150 kPa, which is comparable to the maximal allowed pressure in case of occlusion of a SOTA infusion pump. Signals are acquired using a 2-Channel, Full Bridge module (SCC-SG04, National Instruments, Inc.). The sensor inside the tubing system was calibrated using a pressure/ vacuum transducer module (Rigel 422, Rigel Medical, Durham, UK) as reference method and a linear correlation of y=1.0329x-2.8791 was found.

2.2.2.3. Air bubble sensor

For safety reasons, an air bubble sensor was integrated to avoid accidental air infusion into the patient and consequent embolism. The customised sensor (Zevex, Salt Lake City, UT, USA) was used as there was no sensor available suitable for the tubing used (TYGON®, S-50-HL; ID=0.8mm). The sensor is equipped with a two element ultrasonic sensor that detects the air in the patient line or any air bubbles occurring in blood, saline, and other common solutions. The air bubble detection threshold is 20µl and its response time less than 4ms. The

sensor is non-invasive, meaning that it does not contact the liquid within the tubing. The sensor is dry-coupled, and does not require ultrasonic gel for operation. Contact is maintained between the ultrasonic transducer elements and the tubing by a housing, which positions the tubing in the sensor. The output will be a logic level high (> 4.0 VDC) with fluid in the tubing and a logic level low (< 0.2 VDC) with air in the tubing. Signals were acquired with optically isolated digital I/O modules (SC-2345, National Instruments, Inc.). The air volume was calculated by taking into consideration the inner diameter of the pump tubing, the speed of the pump and the time air bubbles were present at the sensor. The maximal allowed total volume of accidentally infused air was set at 0.5ml for each subject.

2.2.2.4. Dripping sensor

A dripping sensor (3450578 A; BBraun, Melsungen, Germany) was integrated into the system to automatically detect exhaustion of the flushing fluid and thereby to avoid the entry of air into the disposable tubing system. The sensor is designed to be clipped onto a standard administration set for infusion of liquids incorporating a dripping chamber. In general the sensor comprises an infrared diode and a phototransistor. The diode was pulsed with a frequency of 1kHz (square wave signal with a 50µs high and 950 µs low time). When flushing fluid is present, the falling drops behave like an optical barrier between the infrared diode and the photo transistor and thus the received frequency is smaller than the sent frequency of 1kHz. When the flushing fluid is finished and drops no longer interrupt the signals sent to the photo transistor, the detected frequency increases to 1kHz and an alarm message is shown.

2.2.2.5. Data acquisition

The data acquisition system of the IABS system is based on NI – LAB VIEW® and comprises a notebook computer, a 16-bit PCMCIA card (DAQ-Card 6036-E, National Instruments, Inc., Austin, TX), a configurable connector (SC-2345, National Instruments, Inc.) and Lab VIEW® 7.0 (National Instruments, Inc.). Data are stored in Microsoft Excel worksheet files (Microsoft, Inc., Redmond, WA). The graphical user interface (GUI, Figure 8) of the IABS system is designed to be operated by physicians. During standard routine working the blood withdrawal procedure can be started, either by pressing the button “start blood withdrawal” or by programming time and date. All parts of the IABS system are schematically depicted, giving additionally information about the pressure within the system, the position of the valves and remaining time of each action. In case of an emergency, physicians are able to interrupt the program at any time point. Both visual and audible alarms are triggered should a system failure occur. Parameters necessary for the running of the system (e.g. calibration of sensors) can be managed by the technician via a second hidden and more advanced GUI.

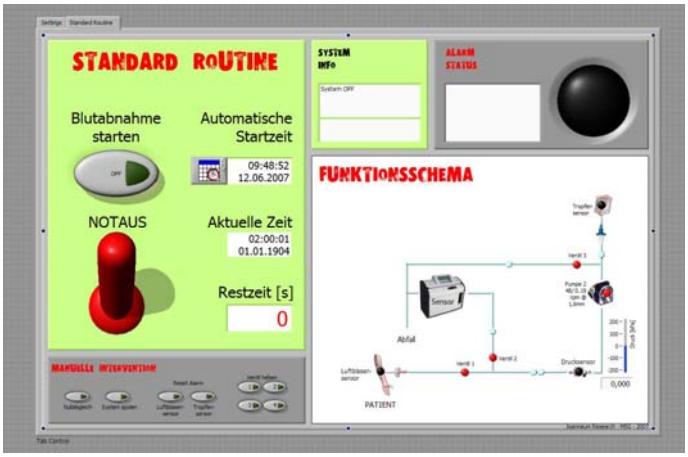


Figure 8: Graphical user interface of the IABS system

The biosensor current (IAGM system, 1-100nA) is acquired at 10s intervals and stored after signal processing using a custom made data logger containing an amperometric detection of the potentiostat and 12-bit analogue/digital converter (Disetronic, Burgdorf, Switzerland). Data are transmitted to a personal computer via an infrared interface using PC software (Disetronic, Burgdorf, Switzerland) and stored in an ASCII format.

2.2.3. Safety considerations

The following chapter describes the safety considerations, derived from a risk assessment, which had to be applied to allow safe operation and evaluation of the newly developed system during human studies. Critical issues address in essence the general requirements for medical electrical devices, classification rules, biocompatibility, packaging and sterilisation.

2.2.3.1. Standards and guidelines

The following standards and guidelines were considered:

Number	Title
EN ISO 14971	Application of risk management to medical devices
MEDDEV 2.4/1	Guidelines for the classification of medical devices
93/42/EEC	Medical devices directive
IEC 60601-1	Medical electrical equipment, general requirements for safety and essential performance
IEC 60601-1-1	Medical electrical equipment, general requirements for safety, - Collateral standard: Safety requirements for medical electrical systems
EN 60601-2-24	Particular requirements for basic safety and essential performance of infusion pumps and controllers
ÖVE/ÖNORM, E 8751-1	Recurrent test and test after repair of medical electrical devices
ICH Q2B	Guidance for Industry, Validation of Analytical Procedures: Methodology Definitions, November 1996
ISO 15197:2003	In vitro diagnostic test systems - Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus
MEDDEV 2.1/5	Medical devices with a measuring function
EN ISO 10993-1	Biological evaluation of medical devices - Part 1: Evaluation and testing
EN ISO 17665-1	Sterilization of health care products - Moist heat
EN ISO 11135	Sterilization of health care products - Ethylene oxide
ISO 11607-1	Packaging for terminally sterilized medical devices

2.2.3.2. Electrical safety

The electrical safety of the devices was guaranteed according to IEC 60601. The following measurements were performed with a safety tester (UNIMET 1100 ST, Bender, Grünberg, Germany) for normal (NC) and single fault condition (SFC) and stored in a database (UniData 1100, v1) prior to clinical investigations:

- Power consumption
- PE resistance
- Insulation resistance

- Device leakage current (NC, SFC)
- Earth leakage current (NC, SFC)
- Patient leakage current (NC, SFC)
- Patient auxiliary current (NC, SFC)

Blood sampling systems are classified as class BF medical devices with one applied part (patient connection). This is achieved by isolating the patient connections from the earthed parts. All electrical connectors and interfaces were non-interchangeable. The data acquisition system based on NI components was supplied with a SELV (Safety Extra-Low Voltage) power source and extra safety features as built into Class I and Class II appliances are therefore not required. As the data acquisition systems are attached to other equipment (e.g. Gilson pump, Laptop computer) and inter-connected by multiple socket outlets, the IABS system is treated as a Class I medical electrical system. Power protection during the clinical investigations was achieved using a non-interruptible power supply (Back-UPS® RS/XS 1500, APC, USA) with a maximum load of 1500 VA - 865 W.
An insulated power supply (IPS-1400R3-8K, DeMeTec, Langgöns, Germany) was used in order to run the medical systems with a galvanically insulated ground-free power supply, which limits any leakage current. By connecting a medical device or system to a network (LAN), the electrical security, accomplished by an insulated power supply, is neutralized. A CE-marked, medical network insulation (NwI v1.2, DeMeTec, Langgöns, Germany) with attenuation < 0.4dB was therefore used.

2.2.3.3. Biocompatibility and sterility of disposable parts

Biocompatibility of disposable tubing systems was guaranteed by choosing products (tubing, glue, connectors, etc.) certified according to the EN ISO 10993 standard or USP Class VI criteria. The disposable tubing systems were assembled in a laminar flow (Clean Air DLF 360, Clean Air Techniek B.V., Woerden, NL) to reduce contamination. The vertical airflow and positive pressure inside the workstation ensure product protection by preventing the ingress of contaminated room air in the work area. All surfaces were furthermore decontaminated before use and assembly was carried out using laboratory gloves. The assembled tubing systems were packed twice using sterilisation packaging (MEG 302550, GLP medical, Hamburg, Germany) and a package sealing device (Melaseal-101, MELAG, Berlin, Germany).
The sterility of the disposable tubing systems was finally guaranteed by ethylene oxide (EtO) gas sterilisation performed at the local hospital according to “Good Laboratory Practice” (GLP) [52].

2.2.4. Technical investigations

Prior to integration of the biosensor into the IABS system, its precision, accuracy and long-term stability was evaluated using diluted bovine serum spiked with glucose. The online biosensor was calibrated before the start of each experiment using 5, 10, 15 and 20mmol/l standard aqueous glucose solutions prepared by spiking ELO-MEL (ELO-MEL, Fresenius Kabi, Graz, Austria) with glucose (D-glucose, 108337,Merck KGaA, Darmstadt, Germany).

2.2.5. Clinical investigations

All studies were single centre trials conducted at the Medical University of Graz (MUG), approved by the local ethics committee and with written informed consent obtained from all participants before the commencement of any trial related activities. Eligible subjects were between 19 and 60 years of age. Exclusion criteria were the presence of severe acute and chronic diseases, mental illness, lack of cooperation or language barriers precluding adequate understanding or cooperation, prescription of vasoactive substances or medication for

anticoagulation or medication that interferes with coagulation, blood donation within the previous 4 weeks, skin diseases that could interfere with catheter placement, pregnancy and breastfeeding. Patients were treated according to the "Declaration of Helsinki" [53] with "Good Clinical Practice" (GCP) [54,55] and samples were analysed according to GLP. Standard operating procedures (SOP) were compiled in order to ensure safe operation of the devices. All patient related data were recorded using case report forms (CRF).

2.2.5.1. Evaluation of the SOTA manual blood sampling procedure

For this open, single centre trial at the MUG, 4 healthy male volunteers (age: 26.34±4.61 years with a body mass index of 24.99±2.78kg/m^2) that passed the inclusion and exclusion criteria were recruited and attended the trial centre in a fasting state. To connect the SOTA manual blood sampling system, a catheter was placed in a peripheral vein of each subject. To prevent clotting within the system and in the catheter tip the system was flushed with 10ml saline solution after the withdrawal of each blood sample.
The aim of this study was to investigate two parameters influencing sample dilution and thus to guarantee the withdrawal of undiluted blood samples with the SOTA manual blood sampling procedure. Firstly, the minimum required reservoir volume was determined using a fixed time interval of 10min between the withdrawn blood samples and a variable reservoir volume of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5ml. Secondly, the minimal interval between withdrawal of blood samples was determined using a 5ml syringe as reservoir, a syringe to withdraw 0.5ml blood samples and intervals of 1, 2, 3, 4, 5, 7 and 10min. Additionally, for reference purposes, three individual blood samples were taken at the beginning, middle and end of the experiment by vein puncture. The glucose concentrations of the withdrawn blood samples derived by vein puncture and by the SOTA manual blood sampling system were used to calculate the dilution. The Beckman Glucose Analyser 2 was used as reference method.

2.2.5.2. 12h study in healthy subjects including OGTT

For the 12h open single centre trial at the MUG, 8 healthy volunteers (7 males and 1 female aged 28.88±3.52 years with a BMI of 25.11±1.49kg/m^2) that passed the inclusion and exclusion criteria were recruited and attended the trial centre at 8:00am in a fasting state. For SOTA manual blood sampling, a catheter was inserted into a peripheral vein in the left forearm with a controlled saline infusion to prevent clotting. A second catheter was then placed in the same forearm and connected to the IABS system. Blood samples were generally withdrawn at 30min intervals. Following oral administration of 75g of glucose (Glucoral; Unipack, Wr.-Neustadt, Austria) at 1:00pm to modify the subjects' glucose concentration profiles, the rate of sampling was increased to 4 samples per hour for 3 hours. Manually and automatically drawn blood samples were bedside analysed using a laboratory glucose analyser (Omni S6, Roche, Basel, Switzerland). The study comprised of two parts. The objective of the first part (subjects 1–6) was to validate the IABS system through a comparison with the SOTA manual blood sampling procedure. In the second part (subjects 7 and 8), the complete IAGM system was validated. Sample processing was carried out directly in the subject room and exclusively by qualified study assistants, trained according to GLP.

2.2.5.3. 30h study in healthy subjects including 4 meal ingestions

For the 30h open, single centre trial at the MUG, 6 healthy male volunteers (age: 28.17±2.04 years; BMI of 23.00±2.41kg/m^2) that passed the inclusion and exclusion criteria were recruited. The study subjects attended the trial centre at 11:00am on Day 1, having fasted from at least midnight. Catheters for SOTA manual blood sampling and the IABS system were again placed within the left forearm of the subjects. The subjects received 4 meals at 5:00pm (dinner), 9:00pm (snack), 8:00 am (breakfast) and 12:00pm (lunch) to enable the observation of

their pre- and postprandial glucose concentration profiles. Blood samples were generally taken at hourly intervals. After each meal, however, the frequency of sampling (manual and automatic) was increased to 4 samples per hour for 3 hours. Thereafter, 2 samples were taken at 30min intervals before resumption of hourly monitoring. A further 7 blood samples were taken throughout the study for the determination of activated partial thromboplastin times (APTT) and analysed in the central laboratory of the MUG. Manually and automatically drawn blood samples were analyzed using a laboratory glucose analyzer (Glucose Analyzers 2, Beckman Coulter, Brea, CA). In order to be able to perform a valid comparison of the results obtained with manually and automatically drawn samples, pre-analytical variables were controlled as far as possible. Thus, undiluted samples were collected in parallel into the same type of vials, in both cases without the use of additives (e.g., anticoagulants or clot activators), and immediately centrifuged to prepare plasma prior to analysis using the Beckmann glucose analyser. This process was completed for each sample pair within 5min. Sample processing was carried out directly in the subject room and exclusively by qualified study assistants, trained according to GLP.

2.2.6. Data analysis

Technical evaluation was performed by using glucose data pairs derived from samples generated with the SOTA manual blood sampling procedure and the IABS system or with the IAGM system. Calculated evaluation parameters included the Pearson's coefficient of correlation (R), mean difference (MAD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria, system error over time, success rate, real blood consumption, the amount of flushing fluid administered, set-up time and improvement of system patency by introducing a Keep Vein Open (KVO) rate. Clinical evaluation was performed by "Clark Error Grid analysis" and by "Insulin Titration Error Grid Analysis". Data analysis was performed using Microsoft Excel (Microsoft, Inc., Redmond, WA, USA).

2.2.7. Reference Methods

2.2.7.1. Roche OMNI S6

The "Omni S6" Point of Care analyser (now cobas b 221 system) from Roche, Basel, Switzerland measures metabolites (glucose and lactate), blood gases (pH, pCO_2, pO_2), electrolytes (sodium Na^+, potassium K^+, chloride Cl^-, calcium Ca^{++}, haematocrit (Hct), haemoglobin (tHb module, SO_2) and CO-oximetry (O_2Hb, HHb, COHb, MetHb and tHb) in whole blood, serum or plasma using a maximal sample volume of 172µl.
Samples can be directly transferred to the analyser or via heparinised capillaries. System and 2-point calibration are performed automatically with internal standards at least once a day.

2.2.7.2. Beckman Glucose 2 Analyser

The "Beckman Glucose 2 Analyser" (Beckman Coulter, Inc., Brea, CA, USA) determines glucose concentrations enzymatically using glucose oxidase. Around 60 samples per hour can be measured using just 10µl of serum, plasma or urine. The device was calibrated prior to each measurement series using a 150mg/dl aqueous standard in accordance with an SOP stipulating that five subsequent measurements must have an accuracy of <3%. The injection of the sample into the device is a crucial step for the determination of valid glucose readings and was therefore only carried out by skilled medical staff. All measurements were performed in duplicate.

2.3. Continuous Microdialysate Sampling (CMDS)

2.3.1. Working principle

The CMDS system (Figure 9) uses a double lumen catheter (DLC; mtb GmbH, Lonsee, Germany) to continuously withdraw blood from a peripheral vein with a peristaltic pump (Minipuls MP3, Gilson, Cedex, France). To prevent coagulation, a NaCl-Heparin solution (50IU/ml) is delivered to the tip of the catheter via the outer lumen of the DLC using a second peristaltic pump (also Gilson Minipuls MP3) at a flow rate of 2ml/h. Anti-coagulated blood is withdrawn simultaneously through the inner lumen of the DLC at a flow rate of 4ml/h. Backflux of potentially contaminated blood from the waste compartment is prevented by using peristaltic pumps uni-directionally.
The anti-coagulated blood is pumped during the planar flow through microdialysis device (PFTMD) and 30µl samples are collected for off-line glucose analysis. All pumps are PC controlled using Lab VIEW® 7.0 software on a notebook and a NI 9263 4-Channel 16-Bit DAQ module (all from National Instruments, Inc., Austin, TX, USA).

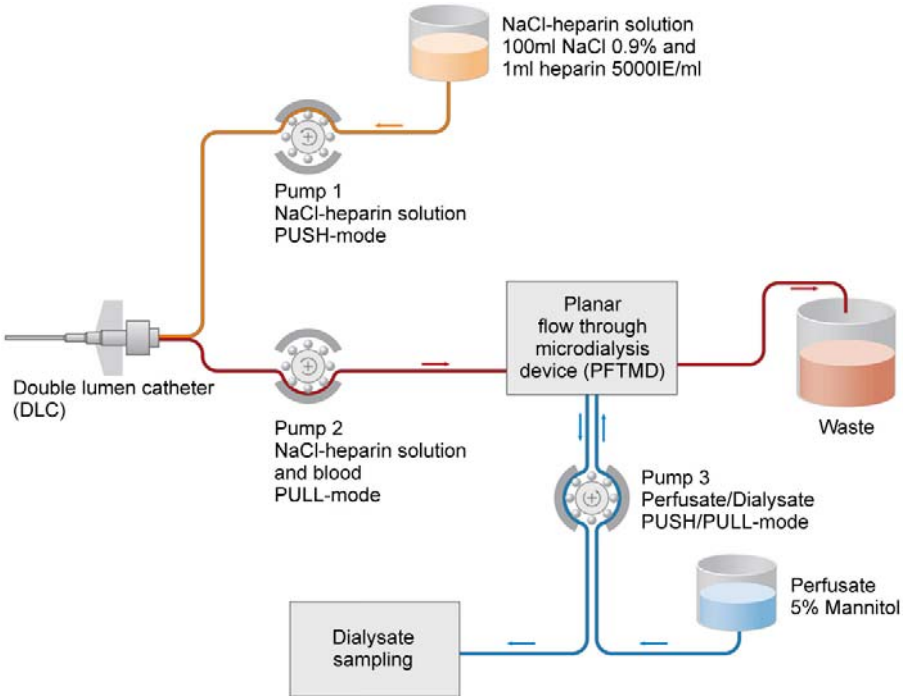


Figure 9: Schematic of CMDS system with extracorporeal heparinisation

The PFTMD (Figure 10a) consists of a semi-permeable membrane sandwiched between two polycarbonate plates (37×33×8mm, Makrolon Rx-1805 from Bayer AG, Leverkusen, Germany). Meandering microfluidic channels, each with a volume of 8µl, are engraved on the plates' surfaces. The first plate is connected to the DLC with TYGON tubing (S-50-HL, ID=0.25mm, OD=0.75mm, l=1.5m, Saint-Gobain Performance Plastics, Beaverton, France) using re-usable, custom-made HPLC connectors and perfused with heparinised blood. The second plate is connected to a 5% Mannitol solution (Fresenius Kabi, Graz, Austria) with TYGON tubing (R-3606, ID=0.19mm, OD=2.01mm) and is perfused counter-currently. The highly porous hydrophilic PAES flat sheet membrane (Figure 10b) Gambro, Hechingen, Germany, molecular weight cut-off=10kDa, thickness=60µm, liquid permeability =2–3×10^{-4}cm³/(cm² sec bar)) is custom made (Figure 10b) and combines rapid diffusive and controlled convective transport characteristics. Its selective layer on the blood contacting side (selective pore diameters ranging from 2–10nm) provides an extremely “smooth” blood contacting surface, which reduces cell and protein adhesion to the membrane’s surface and thus prevents a loss of dialysis efficiency.

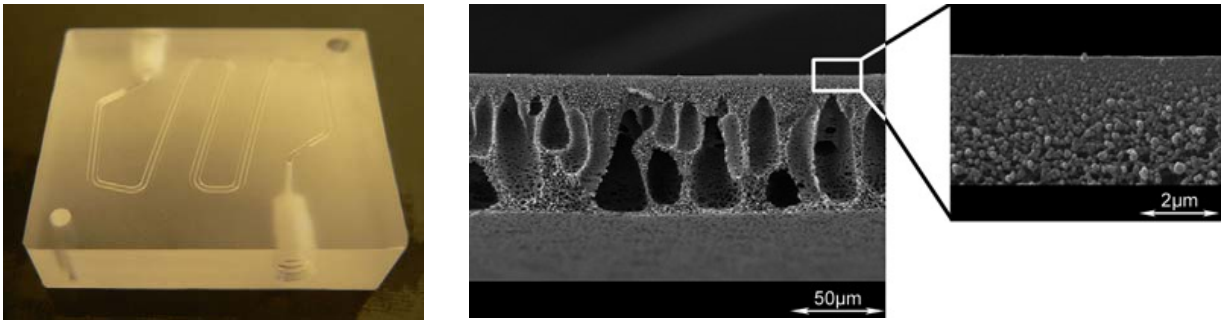


Figure 10a.) Microfluidic plates of PFTMD b.) Cross section of MD membrane: Magnification: 600 vs.20.000

2.3.2. Safety considerations

Safety considerations for the CMDS system are described in 2.2.3. Electrical safety is described in 2.2.3.2. The CMDS system is treated, similarly to the IABS system, as a Class I medical electrical systems with one applied component of class BF. The risk assessment revealed no need for an air bubble sensor since, in contrast to the IABS system, the CMDS system withdraws blood continuously and does not reinfuse blood. Accidental air infusion is therefore impossible. Pressure monitoring was furthermore not integrated into the CMDS system because a flow rate of 2ml/h instead of 10ml/min (IABS system) is used and injury to the patient's vein is thus very unlikely to occur. A low flow rate also enabled the use of heparin and perfusate containers large enough for the entire experiment, which rendered unnecessary the integration of a dripping sensor.
Biocompatibility of disposable tubing systems was guaranteed by choosing products (tubing, glue, connectors, etc.) certified according to EN ISO 10993 standard or USP Class VI Criteria, which were connected to the CE-marked tubing system of the DLC. Disposable tubing systems were also assembled in a laminar flow. The tubing systems and single use membranes were packed twice. Again the sterility of the disposable parts was guaranteed by EtO gas sterilisation at the local hospital according to GLP. As the PFTMD is a multi-use device, plates and connectors were steam-sterilised (FEDEGARI, AUTOCLAVI SPA, Albuzzano, Italy) at 121°C for 20min before each experiment.

2.3.3. Technical investigations - Optimization of flow cell recovery efficiency

Five PFTMDs were tested in vitro over 72h to find operating conditions maximising relative recovery whilst minimising transport delay times. Tests were performed using anticoagulated, glycolysis-inhibited and temperature controlled (37°C) bovine blood as test matrix (anticoagulation: potassium oxalate 500mg/l of blood; glycolysis inhibition: sodium fluoride 750mg/l of blood) and 5% Mannitol as perfusate. Bovine blood flow rate was fixed at 4ml/h, whereas perfusate flow rate was varied between 2, 3, 5 and 10µl/min. Reference blood samples (100µl) were taken directly from the blood pool at hourly intervals. Continuously withdrawn blood samples (100µl) were collected at the outflow of the PFTMD. All samples were centrifuged and the supernatant (plasma) collected for glucose analysis. Dialysate samples (25µl) were collected at the dialysate outflow of the PFTMD. All samples were frozen at −80°C for subsequent glucose analysis with a Roche Cobas Mira analyser using Cobas Gluco-quant and Glucose/Hexokinase (Roche Diagnostics GmbH, Mannheim, Germany).

2.3.4. Clinical investigations

The CMDS system was tested in parallel with the IABS system in the same study as described in 2.2.5.2. As well as the reference catheter, a DLC was inserted into the Vena mediana cubiti of the left arm of the subjects for

continuous blood sampling. Reference blood samples (100µl) were taken from the reference catheter and continuously withdrawn blood samples (100µl) were collected at the outflow of the PFTMD. Both samples were centrifuged and plasma was collected. Microdialysate samples were collected for 15min so as to obtain sufficient sample volumes for glucose analysis. Dialysate samples thus represent time-integrated glucose concentrations. Blood samples of the reference and the CMDS system were taken in the middle of each dialysate sampling period. All samples were immediately analysed for glucose concentration using a Beckman glucose analyser. In addition, three blood samples were taken from the reference catheter for the determination of the activated partial thromboplastin time (APTT) after 0, 5 and 12h, in order to verify the absence of any significant patient heparinisation.

2.3.5. Data analysis

Technical performance evaluation was carried out using reference samples and time delay corrected and prospectively one-point calibrated dialysate samples. Reference and dialysate data were therefore cross-correlated using a spline on a 1min-basis within in a ±20min time window to determine transport delay time, which was assumed to be most likely to occur at the maximum occurring coefficient of correlation. Calculated evaluation parameters include the R, MD, MAD, MRD, MARD, %PRESS and ISO-criteria. Clinical evaluation was performed by rating reference and delay time uncorrected dialysate data with the EGA and ITEGA. Data analysis was performed using Matlab (The Math- Works, Inc., Natick, MA, USA) and Microsoft Excel (Microsoft, Inc., Redmond, WA, USA).

2.3.6. Reference Methods

2.3.6.1. Beckman Glucose 2 analyser

See 2.2.7.2

2.3.6.2. Cobas Mira – Glucose analyser

The “Cobas Mira” from Roche, Basel, Switzerland is a bench-top biochemical analyzer designed to measure a range of metabolites, including glucose, which is measured using an enzymatic reaction. Up to 22 glucose measurements (double determinations) per hour can be performed using a total of 10µl of serum, plasma or ECF with a within-run CV of <10%. The device is calibrated manually with internal standards (low and high) at the beginning and end of each run.

2.4. STATISTICS

2.4.1. Technical evaluation of data

Accuracy assessment was performed using paired data in separate glucose ranges to calculate the Pearson’s coefficient of correlation (R) and also, according to the paper of Wentholt et al. [56], the mean difference (MD), mean absolute difference (MAD), the mean relative difference (MRD), also called the system error (SE), the absolute relative difference (ARD), the mean ARD (MARD), the Bland-Altman analysis [57] and the %Press [58], (Equation 3):

$$\%PRESS = \sqrt{\frac{\sum_{i=1}^{N}([Glucose_{NEW}] - [Glucose_{REF}])^2}{\sum_{i=1}^{N}[Glucose_{REF}]^2}}$$ Equation 3

The ISO criterion [59] is met if the system's glucose concentration is within ±15mg/dl (0.8mmol/l) or within 20% of the reference glucose concentration, for glucose concentrations <75mg/dl (4.2mmol/l) and ≥75mg/dl, respectively.

2.4.2. Clinical evaluation of data

Clinical evaluation was performed using the following methods:

2.4.2.1. Clark Error Grid analysis (EGA)

The EGA [60] is a clinically oriented approach for the validation of glucose data that describes the clinical significance of the degree of accuracy of a given glucose measurement system. The EGA takes into account the absolute values of the reference method (x-axis) and a newly introduced method (y-axis), the relative difference between these two values, and the clinical significance of this difference. The EGA categorizes individual reference glucose values into one of five zones, each of which represents a different degree of acceptability of a clinical treatment. These zones are A (accurate), B (acceptable), C, D, and E (unacceptable).

2.4.2.2. Insulin Titration Error Grid Analysis (ITEGA)

The [61] ITEGA is also a clinically oriented approach for the validation of glucose data that assesses the clinical significance of the accuracy of a given measurement system. Blood glucose concentrations determined by the reference method and by a new method are displayed respectively on the x- and y-axes, Glucose concentration ranges on the x- and y-axes are related to major treatment actions as suggested by the Leuven insulin titration guideline. Based on these treatment actions, different zones are defined as "appropriate treatment," "unacceptable violation," "major violation," and "life threatening treatment," according to the extent to which they violate the titration guideline.

3. RESULTS

3.1. Evaluation of SOTA manual blood sampling

A clinical study in 4 healthy humans revealed that a stepwise reduction of the withdrawn blood reservoir volume from 5ml to 0.5ml causes increased blood dilution for withdrawal volumes less than 1.5ml (Figure 11). This finding confirmed the use of a 5 ml syringe as reservoir for SOTA manual blood sampling as the associated system error caused by dilution is less than 0.16%.

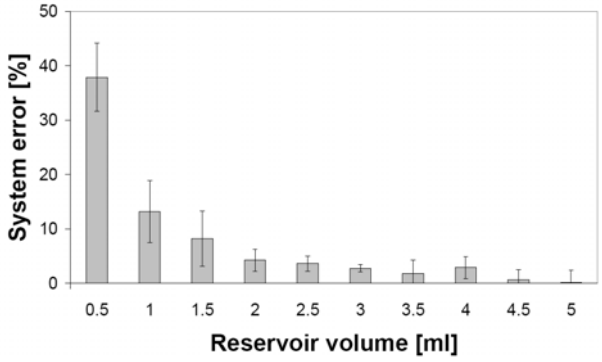


Figure 11: The size of the reservoir volume affects the degree of dilution of blood samples withdrawn by the SOTA manual blood sampling procedure. System errors are given as median ± standard deviations for each reservoir volume.

A reduction of the time interval between withdrawn blood samples from 10min to 1min did not significantly influence the dilution of these samples (Figure 12). Contrary to our expectations, the sampling interval between two withdrawn blood samples can be at least 1min since no trend was found for the median system error (range: -0.59 - 1.73%; for the 4 and 2min intervals).

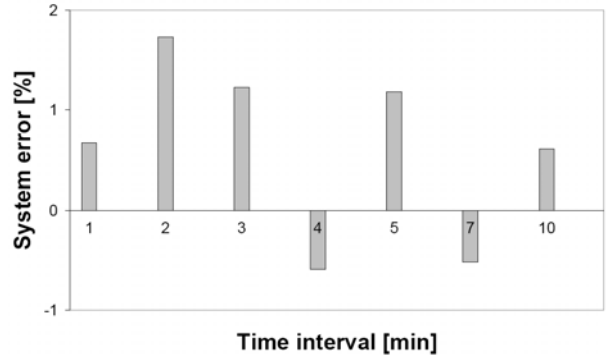


Figure 12: Influence of the time interval on the dilution of the blood samples using SOTA manual blood sampling in 4 healthy human subjects. System errors are given as median for each time interval.

3.2. Evaluation of the IABS and IAGM system

3.2.1. Evaluation of the SensLab biosensor

Evaluation of the biosensor using diluted bovine serum spiked with glucose to final concentrations of 0, 5, 10, 15 and 20mmol/l (Figure 13) revealed a sensor accuracy of 5.0%, a precision of 5.1% and a long term stability of −0.02mmol/l per hour.

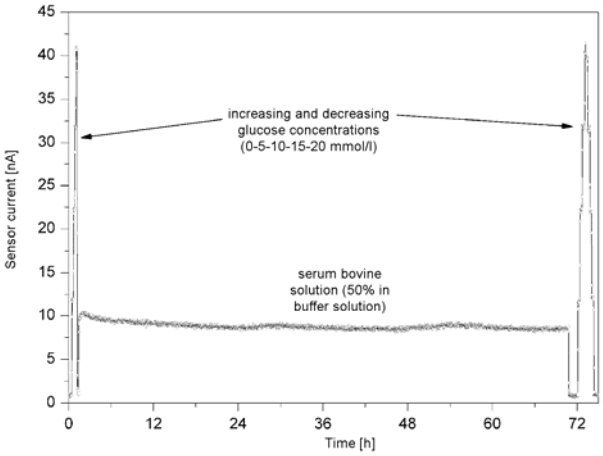


Figure 13: Sensor current for determination of long term stability, accuracy and precision, performed using diluted, glucose-spiked, bovine serum.

The online biosensors were furthermore calibrated using 5, 10, 15 and 20mmol/l standard aqueous glucose solutions prepared by spiking ELO-MEL (ELO-MEL, Fresenius Kabi, Graz, Austria) with glucose (D-glucose, Merck, Darmstadt, Germany) before the start of each experiment. Each concentration was measured 10 times in replicate. A representative sensor calibration curve is depicted in Figure 14. The corresponding derived linear equation was y=1.64x+6.33, R=0.998.

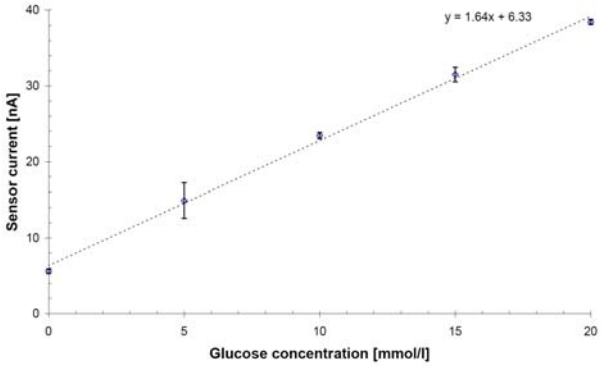


Figure 14: Sensor calibration curve. Sensor currents are given as means ± standard deviations for each glucose concentration.

3.2.2. 12h study in healthy subjects including OGTT

All eight volunteers successfully completed the study according to the study plan. The catheters were well tolerated throughout the study with no sign of local infection. Figure 15 shows an illustrative example of the comparison of glucose concentration profiles obtained using the IABS system (top) and the IAGM system (bottom) with the respective profiles obtained using SOTA manual blood sampling.

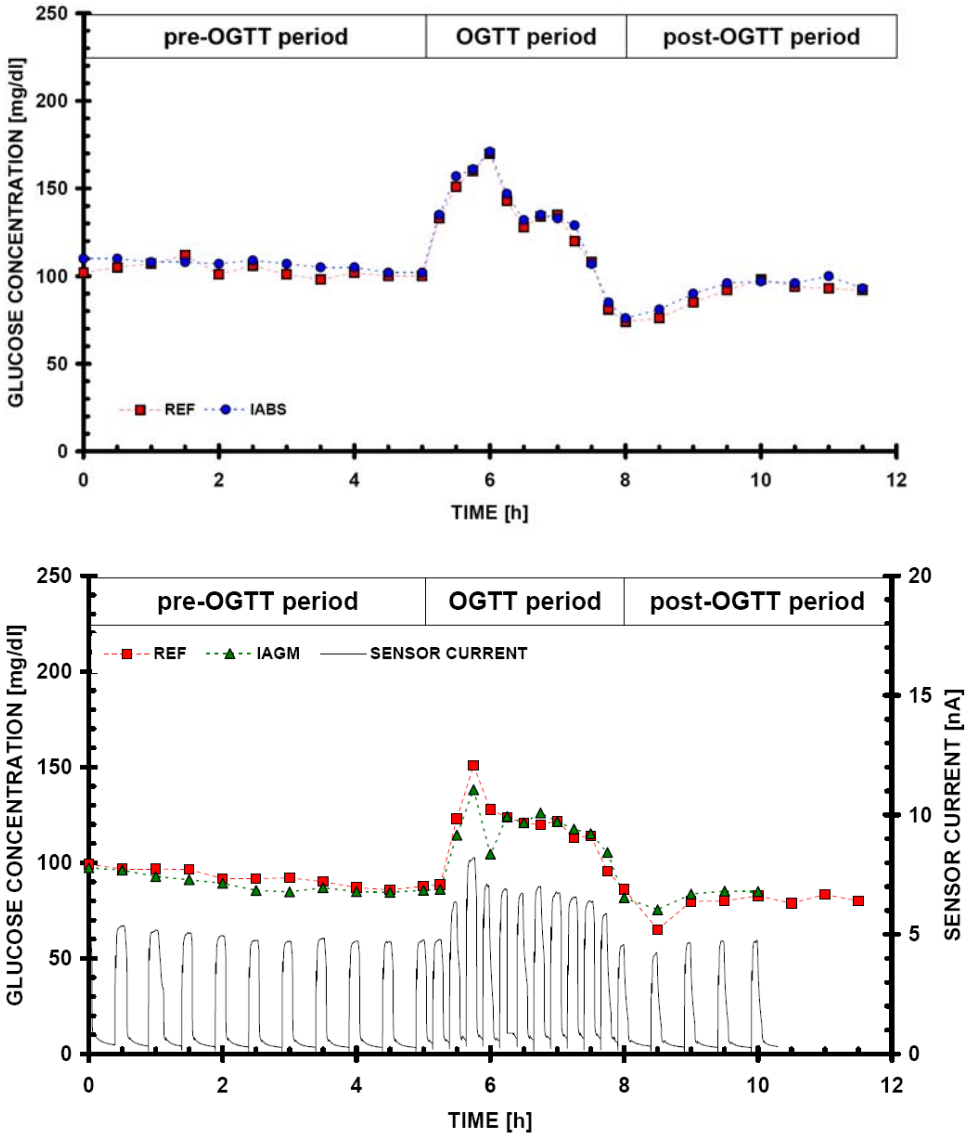


Figure 15: Illustrative 12h glucose profiles obtained with the IABS and the IAGM systema. The subjects underwent a 75g OGTT to enable observation of their pre- and postprandial glucose concentration profiles. Top: Comparison of glucose concentration profiles obtained for subject 3 using the IABS system (circles) and SOTA manual blood sampling (squares). Bottom: Comparison of glucose concentration profiles obtained for subject 7 using the IAGM system (triangles) and SOTA manual blood sampling (squares). Also shown are the corresponding amperometric measurements obtained with the biosensor. The sensor current in nA is displayed as a solid line on the 2nd y-axis. The response time t_{90} (time taken to reach 90% steady state signal) measured by step gradient from 0 to 100mg/dl glucose is about 2min. After the 10h time point, the biosensor showed leakage since it was initially designed to be operated with flow rates of around 1µl/min.

3.2.2.1. Technical evaluation

Of a total of 240 measurements, only 12 were invalid due to catheter blockage, coagulation, insufficient sample volume, haemolysis or leakage (7 for the IABS system and 5 for the IAGM system), resulting in a total of 228 valid data pairs. For two subjects, the catheters were renewed due to permanent occlusion. The measured glucose concentrations ranged from 61-236mg/dl (3.4 to 13.1mmol/l). Technical evaluation revealed an overall median frequency of successful sampling of 96.67±5.33% per subject [range: 86.67–100.00%]. The system error graphs for all subjects are shown in Figure 16. Circles and triangles represent respectively the system errors calculated for each data pair for the reference vs. the IABS system and for the reference vs. the IAGM system. The means (squares) ± standard deviations (bars) are, in addition, displayed for each subject. The calculated overall system error between the IABS system and reference measurements was −1.7±9.0% (subjects 1–6, circles). For the IAGM system the calculated system error was −1.0±6.6% (subjects 7 and 8, triangles). Individual system errors ranged from -18.2 to 56.0%. The system error of the IAGM system was lower than that of the IABS system because the former system was calibrated using the first valid reference measurement, whereas the values obtained with the IABS system were not calibrated. A notable feature of the initial results obtained for subject 2 was that the glucose concentrations in the manually drawn reference samples (=golden standard) were generally lower than those obtained with automatically sampled blood (Figure 16). This was found to be due to the location of the catheters for SOTA manual blood sampling and IABS system in the same forearm and thus in the same venous plexus. To begin with, samples were taken with the IABS system before blood was withdrawn by SOTA manual blood sampling. We reasoned that, because of this, the flushing fluid from the IABS system was afterwards withdrawn with the manual reference samples, leading to unwanted sample dilution. This was confirmed by the finding that, after synchronising the withdrawal and reinfusion procedure for both systems to avoid interaction between them, the system error improved for all remaining subjects (refer to Figure 16).

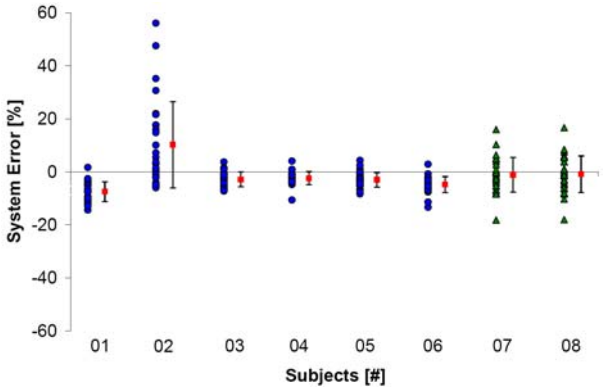


Figure 16: System error graph for each individual data pair for subjects 1–6 (IABS system, circles); subjects 7 and 8 (IAGM system, triangles). Squares and bars represent, respectively, the mean system error for each subject and the respective standard deviation.

Table 1 presents the calculated system errors for clinical parameters, taking into consideration the analyte values obtained using the IABS system and the respective values obtained using SOTA manual blood sampling. The investigated analytes are metabolites (glucose, lactate), ions (Ca^{++}, K^{+}, Na^{+}, Cl^{-}), haematocrit (Hct), blood gases (pH, pCO2, pO_2), haemoglobin (tHb, SO_2) and CO-oximetry (O_2Hb, COHb, HHb, MetHb). Values with a system error smaller than ±15% are marked in green [62], whereas values with a system error equal or larger than ±15% are marked in red.

		Subject [#]						
		1	2	3	4	5	6	ALL
System error of clinical parameters [%]	Gluc	-7.46	10.21	-2.88	-2.40	-3.02	-4.83	-1.66
	Lac	-11.80	1.70	-1.87	-0.33	-1.64	-5.83	-3.16
	Ca^{++}	-7.39	1.21	-4.10	-2.73	-2.65	-3.22	-3.15
	K^{+}	-10.33	4.55	-4.57	-3.66	-2.89	-6.71	-3.95
	Na^{+}	-0.36	-0.93	-0.29	-0.66	-0.46	-0.33	-0.50
	Cl^{-}	2.59	0.54	1.00	0.10	0.52	0.71	0.90
	Hct	-5.57	6.15	-0.64	-0.64	1.84	-1.35	-0.04
	pH	0.21	0.47	0.20	0.30	0.25	0.16	0.27
	pCO_2	-11.44	-11.68	-8.18	-9.35	-9.48	-8.22	-9.69
	pO_2	-4.75	87.62	16.78	43.21	18.20	8.07	28.94
	tHb	-5.02	3.58	-4.08	-1.80	-0.02	0.78	-0.98
	SO_2	-1.68	50.24	2.51	9.08	7.80	0.87	11.96
	O_2Hb	-1.67	50.64	2.56	9.19	7.90	0.89	12.08
	COHb	-2.69	-2.53	-0.74	-2.10	0.21	-0.28	-1.34
	HHb	35.17	-77.15	-35.33	-52.83	-41.21	-9.08	-31.58
	MetHb	3.23	-21.41	-0.87	-3.62	-9.55	-2.52	-6.06

Table 1: System errors for all subjects for all clinical parameters determined with the Roche Omni device: Glucose (Gluc), lactate (Lac), calcium (Ca^{++}), potassium (K^{+}), sodium(Na^{+}), chloride (Cl^{-}), haematocrit (Hct), pH value (pH), partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), total haemoglobin (tHb), oxygen saturation of haemoglobin (SO_2), Oxyhaemoglobin (O_2Hb), carboxyhaemoglobin (COHb), Deoxyhaemoglobin (HHb) and methaemoglobin (MetHb). Mean values for subjects 1 – 6 and for all subjects (ALL) are shown.

System errors lower than ±15% were achieved for glucose (Gluc) and lactate (Lac), calcium (Ca^{++}), potassium (K^{+}), sodium (Na^{+}) and chloride (Cl^{-}) ions and haematocrit (Hct). Blood gas measurement is complicated by exchange with the surrounding air. The pH value (pH) and the partial pressure of carbon dioxide (pCO_2) were nevertheless within the ±15% range. The partial pressure of oxygen (pO_2) was however 28.94%, most likely due to the fact that samples could not always be processed immediately due to calibration of the Roche Omni and thus underwent gaseous exchange with the surrounding air. Values of haemoglobin were within the range of ±15% for total haemoglobin (tHb) and oxygen saturation of haemoglobin (SO_2). For CO-oximetry, system errors smaller than ±15%, were achieved for oxyhaemoglobin (O_2Hb), carboxyhaemoglobin (COHb) and methaemoglobin (MetHb). Only deoxyhaemoglobin (HHb) showed a system error outside this range (-31.58%).

Table 2 summarises the technical data evaluation for all eight subjects. Mean coefficient of correlation (R) was found to be 0.982 (IABS) and 0.950 (IAGM). Mean difference (MD) was -1.8±10.3mg/dl (IABS) and -1.9±8.0mg/dl (IAGM). Mean absolute difference (MAD) was 6.5±8.1mg/dl (IABS) and 5.7±5.9mg/dl (IAGM). Mean relative difference (MRD) was -1.7±9.0% (IABS) and -1.0±6.6% (IAGM). Mean absolute relative difference (MARD) was 5.8±7.1% (IABS) and 5.1±4.4% (IAGM). %PRESS parameter was calculated as 7.4±5.8% (IABS) and 9.1±5.7% (IAGM). ISO criterion (ISO MET?) was met in 94.8% (IABS) and 100% (IAGM).

Subject [#]	R [-]	MD [mg/dl]	MAD [mg/dl]	MRD [%]	MARD [%]	%PRESS [%]	ISO MET? [Yes]
1	0.989	-9.1 ± 5.6	9.2 ± 5.3	-7.5 ± 3.8	7.6 ± 3.5	8.4	26 of 26
2	0.928	12.0 ± 17.7	14.4 ± 15.7	10.2 ± 16.3	12.7 ± 14.4	18.7	22 of 29
3	0.992	-3.1 ± 3.0	3.6 ± 2.3	-2.9 ± 2.8	3.4 ± 2.2	3.7	30 of 30
4	0.991	-2.6 ± 3.4	3.1 ± 3.0	-2.4 ± 2.5	2.8 ± 2.0	3.9	26 of 28
5	0.997	-3.4 ± 3.3	3.9 ± 2.7	-3.0 ± 2.7	3.5 ± 2.0	3.8	30 of 30
6	0.991	-5.2 ± 3.4	5.4 ± 3.1	-4.8 ± 3.0	5.0 ± 2.7	5.6	30 of 30
7	0.936	-1.7 ± 6.8	4.9 ± 5.0	-1.1 ± 6.6	4.9 ± 4.4	6.7	27 of 27
8	0.963	-2.1 ± 9.1	6.5 ± 6.6	-0.9 ± 6.9	5.2 ± 4.5	7.7	28 of 28
1 - 6	0.982	-1.8 ± 10.3	6.5 ± 8.1	-1.7 ± 9.0	5.8 ± 7.1	7.4 ± 5.8	164 of 173
7 - 8	0.950	-1.9 ± 8.0	5.7 ± 5.9	-1.0 ± 6.6	5.1 ± 4.4	9.1 ± 5.7	55 of 55

Table 2: Uncalibrated blood samples withdrawn with the IABS system (subjects 1-6) and one-point calibrated blood samples measured online with the IAGM system (subjects 7-8) are evaluated against respective samples withdrawn by SOTA manual blood sampling with respect to their glucose concentrations. Evaluation parameters include the Pearson's coefficient of correlation (R), mean difference (MD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria (ISO MET?). Evaluation parameters are expressed as means ± standard deviation

The actual blood consumption of the IABS and IAGM systems was 1.69±0.09ml per sample, of which 0.303±0.043ml was collected in vials for offline analysis or ~0.2ml was processed over the biosensor. The mean volume of saline solution used to flush the disposable tubing system to avoid clotting after each automatic blood withdrawal was 8.83±0.28ml. APTT levels were monitored to assess the level of unintended patient heparinisation. No statistically significant increase was however found in any of the subjects ($p>0.05$). Blood was retained within the tubing system for a maximum of 93 sec to minimise the risk of clotting. All blood samples were haemolysis free, judged by optical quantification following centrifugation. The setting-up of the IABS system, including the assembly of tubing, flushing, and connection to the patient, was always performed in less than 5 min, which, based on our experience in a routine hospital setting, is comparable to the time required to set-up and connect a standard infusion pump.

3.2.2.2. Clinical evaluation

The Clark Error Grid Analysis (EGA) is shown in Figure 17a. Of the 173 data pairs obtained using the IABS system (subjects 1–6; circles) and SOTA manual blood sampling, 166 (95.95%) were in zone A and only 7 (4.05%) in zone B. Furthermore, of the 55 data pairs obtained for the IAGM system (subjects 7 and 8; triangles) all were located in zone A. Thus, overall a total of 96.93% of the measurements were in zone A ("accurate") and only 3.07% in zone B ("acceptable") and none were found in zones C, D and E. Linear least squares regression analysis shows a linear correlation of $y=0.965x+2.004$.

The Insulin Titration Error Grid Analysis (ITEGA) shown in Figure 17b revealed that a total of 99.56% of the 228 valid data pairs (again IABS data represented by circles and IAGM data by triangles) generated were located in the acceptable treatment zone. The single data pair that lay outside of this zone was an erroneous measurement for subject 2 caused by dilution effects, already discussed in more detail in the preceding text.

These results are indicative of a high level of accuracy of the IABS system with respect to SOTA manual blood sampling.

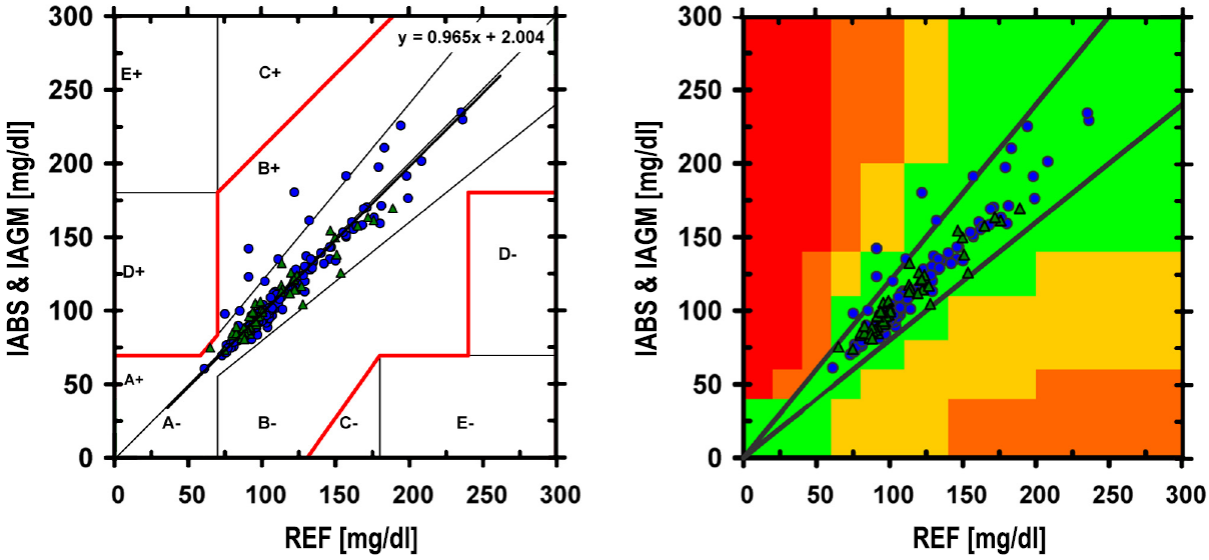


Figure 17a: Clark Error Grid analysis for IABS (circles) and IAGM system (triangles); 12h study

b: Insulin Titration Error Grid Analysis for IABS (circles) and IAGM system (triangles); 12h study

3.2.3. 30h study in healthy subjects including the ingestion of 4 meals

3.2.3.1. Technical evaluation

All six volunteers were successfully studied for the full planned study duration of 30h and the catheters were well tolerated without any sign of infection. Manual interventions were necessary for both the reference method and the IABS system to prevent catheter occlusion (e.g., additional flushing, movement, or replacement). For the first subject studied, a total of seven such manual interventions were necessary for the IABS system. These events generally occurred whilst drawing hourly samples during the night. For subjects 2–6, a keep vein open (KVO) saline infusion (2ml/h) which was stopped 10min prior blood sampling was therefore implemented into the IABS system. This highly effective method reduced the incidence of blood withdrawal failures from 9.86% for subject 1 (7 interventions for 71 samples) to an average failure rate of 0.56% for subjects 2–6 (2 interventions for 355 samples), a 17.5-fold reduction in the frequency of interventions. As in the 12h study, sample blood was allowed to remain in contact within the tubing system for a maximum of 93 sec to minimise the risk of clotting. A small and negligible level of clotting nevertheless was documented within the tubing system, though only within the most critical stagnation zones of the 3-way stop cock of the catheter (Figure 18a), and comparable to that observed in catheters used for SOTA manual blood sampling. The disposable pressure dome (Figure 18b) was in contrast fully clot-free.

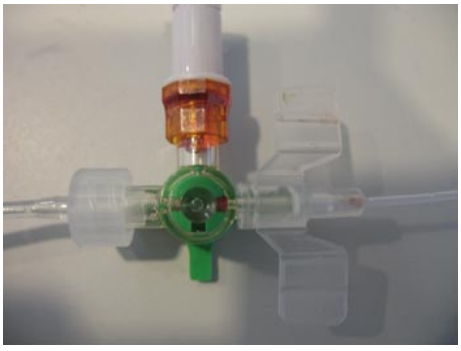
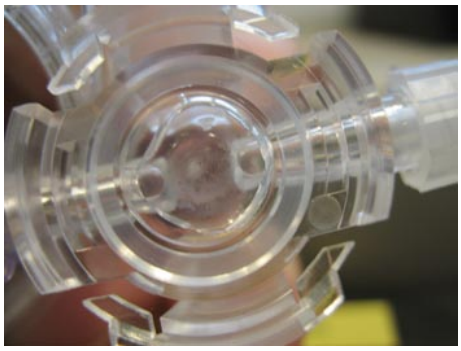


Figure 18a: Clot formation within the 3-way stop cock of the catheter



b: Clot-free disposable pressure dome

The median success rate of the system was 99.30% (range: 90.14–100.00%). Overall, 6 of 426 data pairs were incomplete or invalid, principally due to catheter blockage caused by coagulation, insufficient sample volume, or haemolytic samples, resulting in a total of 420 valid samples. The measured glucose concentrations for these samples ranged from 67.5 to 250mg/dl (3.75 to 13.88mmol/l). An illustrative glucose concentration profile comparing the results obtained with the IABS system (circles) to those obtained with the reference system (squares) is shown in Figure 19.

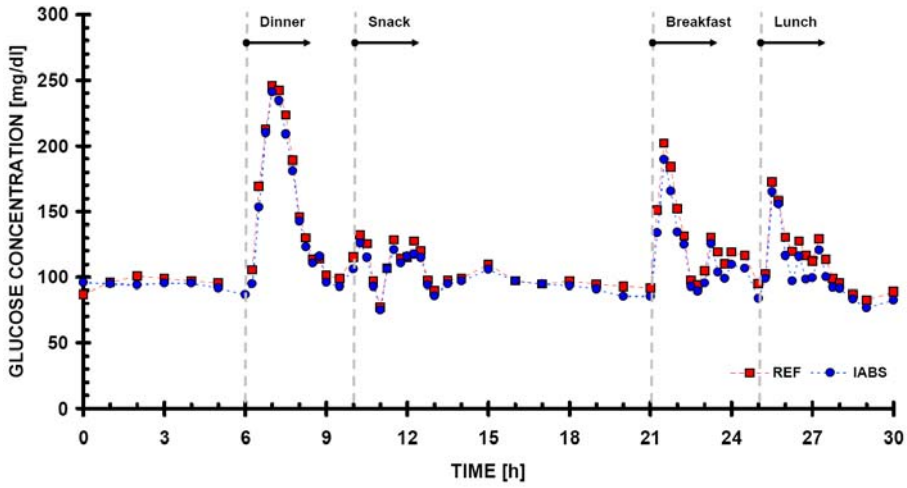


Figure 19: Illustrative glucose concentration profile for automatically (IABS system, circles) and manually (squares) withdrawn reference blood samples obtained for subject 3 during the 30h experiment. Subjects received meals after 6 (dinner), 10 (snack), 21 (breakfast) and 25 hours (lunch) to enable observation of their pre- and postprandial glucose concentration profiles.

Figure 20 shows the system error for each data pair from subjects 1 to 6 (circles). In addition, the means (squares) ± standard deviations (bars) are displayed for each subject. The calculated system error between the IABS system and reference measurements was -3.3 ± 5.5% [range: -6.0–0.50]. Individual system errors ranged from 24.1 to -29.0%.

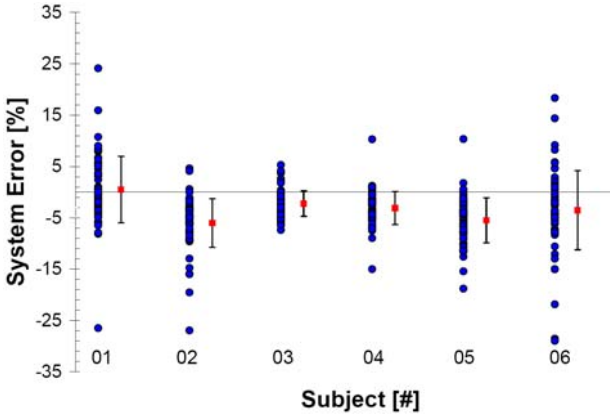


Figure 20: System error graph for each individual data pair from subjects 1–6 of the IABS system (circles). Squares represent the mean system error for each subject and bars, the respective standard deviation.

Figure 21 displays the individual system errors for each time point and subject. As a measure of system stability, the regression line was calculated (y =-0.1089x -1.5357). Starting with a system error of -1.54%, the system error increased until the end of the experiment to a final value of -4.80%.

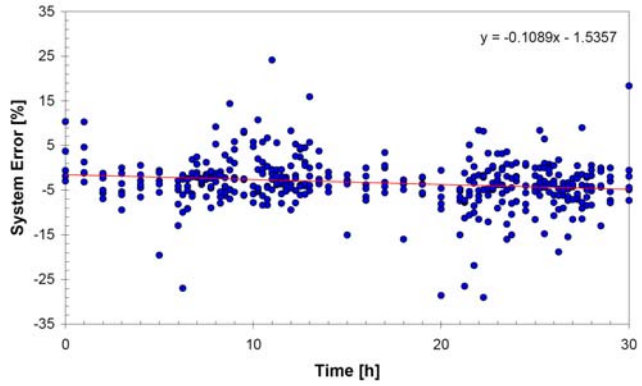


Figure 21: System error over time for subjects 1–6 plus the calculated regression line

Table 3 displays summarises the technical data evaluation for all six subjects. Mean coefficient of correlation (R) was found to be 0.976. Mean difference (MD) was -4.1±6.5mg/dl. Mean absolute difference (MAD) was 5.6±5.3mg/dl. Mean relative difference (MRD) was -3.3±5.5%. Mean absolute relative difference (MARD) was 4.8±1.5%. %PRESS parameter was calculated as 6.1±2.0%. ISO criterion (ISO MET?) was met in 98.3% of all cases.

Subject [#]	R [-]	MD [mg/dl]	MAD [mg/dl]	MRD [%]	MARD [%]	%PRESS [%]	ISO MET? [Yes]
1	0.953	0.2 ± 6.7	4.5 ± 4.9	0.5 ± 6.5	4.5 ± 4.7	6.3	68 of 70
2	0.971	-6.7 ± 5.4	6.9 ± 5.0	-6.0 ± 4.8	6.3 ± 4.4	7.6	70 of 71
3	0.991	-2.4 ± 2.6	3.0 ± 1.9	-2.2 ± 2.5	2.8 ± 1.8	3.1	71 of 71
4	0.996	-4.0 ± 4.2	4.4 ± 3.8	-3.1 ± 3.2	3.5 ± 2.7	4.3	69 of 70
5	0.989	-6.8 ± 5.5	7.2 ± 5.1	-5.5 ± 4.4	5.9 ± 3.8	6.9	70 of 70
6	0.954	-4.7 ± 9.8	7.3 ± 8.0	-3.5 ± 7.7	6.1 ± 5.9	8.4	65 of 68
ALL	0.976	-4.1 ± 6.5	5.6 ± 5.3	-3.3 ± 5.5	4.8 ± 1.5	6.1 ± 2.0	413 of 420

Table 3: Evaluation of uncalibrated blood samples withdrawn with the IABS system against corresponding samples withdrawn by the SOTA manual blood sampling procedure with respect to their glucose concentrations. Evaluation parameters include the Pearson's coefficient of correlation (R), mean difference (MD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria (ISO MET?). Evaluation parameters are given as means ± standard

3.2.3.2. Clinical evaluation

The Clark Error Grid Analysis (EGA) is shown in Figure 22a. Of the 420 data pairs obtained using the IABS system (subjects 1–6; circles) and SOTA manual blood sampling, 414 (98.6%) were in zone A ("accurate"), only 6 (1.4%) in zone B ("acceptable") and none were found in zones C, D and E. Linear least squares regression analysis shows a linear correlation of y=0.949x+1.7779.

The Insulin Titration Error Grid Analysis (ITEGA) shown in Figure 22b, revealed that, a total of 100% of the 420 valid data pairs generated (again IABS data depicted as circles) were located in the acceptable treatment zone. No single data point suggested an unacceptable or major violation or a life threatening treatment.

These results are indicative of a high level of accuracy of the IABS system with respect to SOTA manual blood sampling for the EGA as well as for the ITEGA.

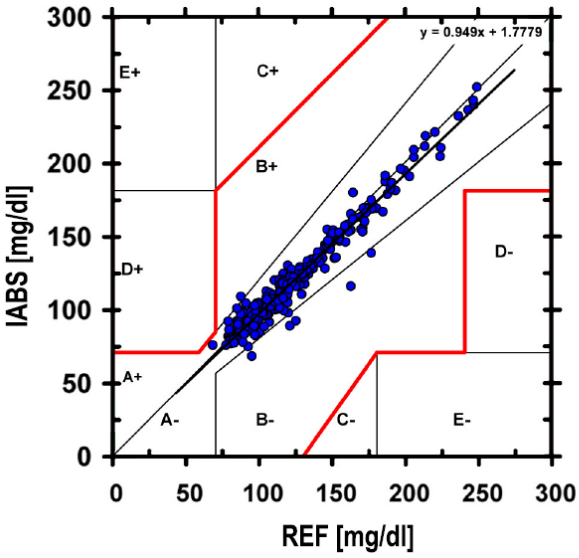
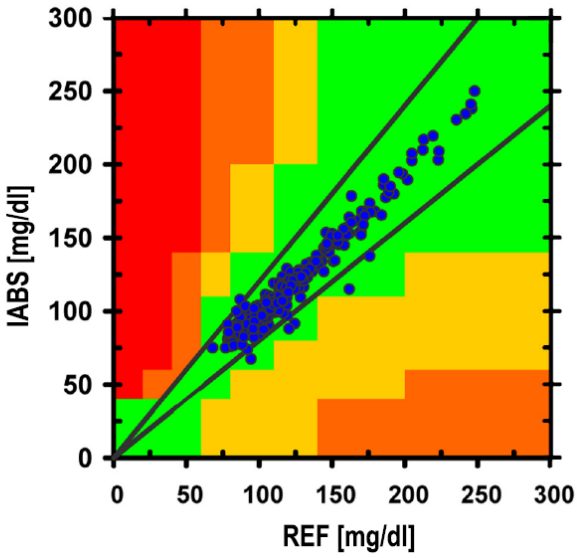


Figure 22a: Clark Error Grid analysis for IABS system (circles); 30h study



b: Insulin Titration Error Grid Analysis for IABS system (circles); 30h study

3.3. Evaluation of the CMDS system

3.3.1. Pre-clinical evaluation

In vitro investigation of the planar, flow through microdialysis device (PFTMD) over 72h in bovine blood revealed that the mean relative glucose recovery level remained stable at a level of around 100% for 72h at perfusate flow rates between 2–5µl/min and a blood flow rate of 4ml/min. A relative recovery of 100.4±4.2% was achieved at a perfusate flow rate of 5µl/min. This flow is regarded as the optimal perfusion flow rate combining complete relative recovery at a perfusion rate yielding an acceptably low system transport delay time. Thus, in the following clinical investigation 5µl/min was chosen as perfusion flow rate.

3.3.2. 12h study in healthy subjects including OGTT

3.3.2.1. Technical evaluation

All eight volunteers were successfully studied for the full planned duration of 12h. The DLC catheters were well tolerated throughout, with no sign of local infection and no trial-related adverse events occurred.
Blood-heparin withdrawal was monitored in all subjects and was found to be 66.9±5.6µl/min. As heparin was used to anti-coagulate blood for continuous withdrawal within a DLC, for safety reasons the subject's activated partial thromboplastin time (APTT) as a measure of systemic heparinisation was monitored. Of the three APTT measurements made per subject, none was significantly increased during the trial ($p>0.05$).
The system's mean delay time due to blood and dialysate transport was calculated as described above and found to be 10.5min. A total of 240 reference blood samples were taken (30 per subject) in a glucose range between 53.0 and 213.7mg/dl (<80mg/dl: 31 samples, 80–120mg/dl: 139 samples, >120mg/dl: 69 samples). 227 corresponding dialysate samples were successfully collected and analysed. In total, 13 dialysate samples (5.4%) were not taken due to system malfunction, mostly as a result of catheter occlusion. Figure 23 shows exemplary glucose concentration profiles derived from SOTA manual blood sampling (squares) and the microdialysis based CMDS system (circles). Microdialysis samples were prospectively one-point calibrated to the first reference blood glucose concentration and their glucose concentrations corrected by the calculated lag time.

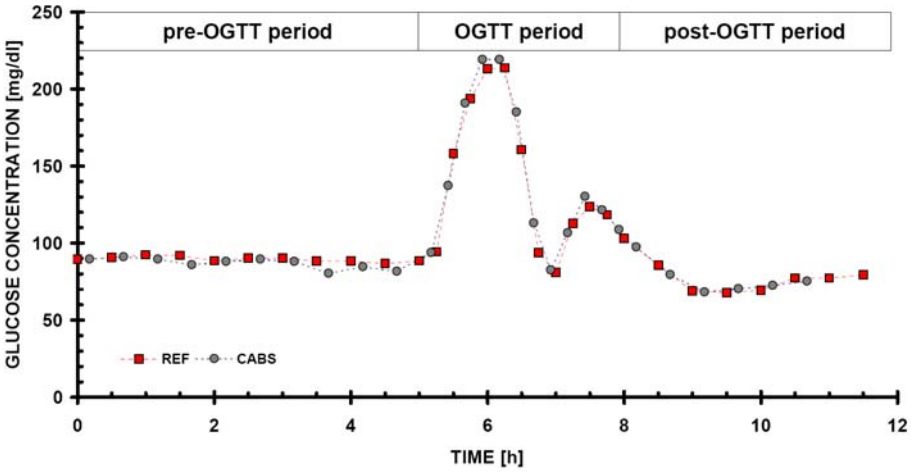


Figure 23: Illustrative glucose concentration profile showing reference (squares) and prospectively one point calibrated, transport-time-delay-corrected glucose concentration of the CMDS system (squares) for continuous extravascular monitoring of blood glucose concentration. The subjects underwent a 75g OGTT to enable observation of their pre- and postprandial glucose concentration profiles.

Table 4 summarises the technical data evaluation for all eight subjects. Mean coefficient of correlation (R) was found to be 0.960. Mean difference (MD) was 2.1±12.1mg/dl. Mean absolute difference (MAD) was 8.7±8.6mg/dl. Mean relative difference (MRD) was 1.9±11.2%. Mean absolute relative difference (MARD) was 8.4±7.7%. %PRESS parameter was calculated as 10.3±5.2%. ISO criterion (ISO MET?) was met in 91.6% of all cases.

Subject [#]	R [-]	MD [mg/dl]	MAD [mg/dl]	MRD [%]	MARD [%]	%PRESS [%]	ISO MET? [Yes]
1	0.876	6.6 ± 18.2	13.4 ± 13.8	6.7 ± 13.4	11.4 ± 9.5	15.3	21 of 25
2	0.935	5.2 ± 14.8	11.3 ± 10.6	4.0 ± 15.7	11.6 ± 11.0	14.3	21 of 26
3	0.989	-6.5 ± 3.4	6.5 ± 3.4	-6.4 ± 3.1	6.5 ± 3.1	7.1	30 of 30
4	0.936	15.9 ± 10.6	17.0 ± 8.7	16.3 ± 8.9	17.1 ± 7.3	17.8	20 of 27
5	0.995	0.9 ± 4.6	3.6 ± 2.9	0.6 ± 4.3	3.4 ± 2.6	3.9	28 of 28
6	0.984	-6.8 ± 4.6	7.1 ± 4.2	-6.9 ± 4.3	7.2 ± 3.7	8.2	30 of 30
7	0.971	7.5 ± 8.5	8.1 ± 7.9	6.8 ± 7.6	7.6 ± 6.9	11.2	27 of 30
8	0.994	-3.4 ± 3.8	4.0 ± 3.2	-3.6 ± 4.0	4.0 ± 3.5	4.3	30 of 30
ALL	0.960	2.1 ± 12.1	8.7 ± 8.6	1.9 ± 11.2	8.4 ± 7.7	10.3 ± 5.2	207 of 226

Table 4: Evaluation of prospectively, one-point calibrated, transport-time-delay-corrected microdialysate samples against reference blood samples with respect to their glucose concentrations. Evaluation parameters include the Pearson's coefficient of correlation (R), mean difference (MD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria (ISO MET?). Evaluation parameters are given as means ± standard deviation

3.3.2.2. Clinical evaluation

For this evaluation, the dialysate data were again prospectively one-point calibrated as described before but were not delay-time corrected, as they would also not be in a clinical setting.

The Clark Error Grid Analysis (EGA) is shown in Figure 24a. Of the 227 data pairs obtained using the CMDS system (subjects 1–6; circles) and SOTA manual blood sampling, 85.9% (195) were in zone A ("accurate"), 13.7%

(31) in zone B (“acceptable”) and only one glucose sample (0.4%) was found to be in zone D “not acceptable”. Linear least squares regression analysis shows a linear correlation of y=0.983x+3.931.

The Insulin Titration Error Grid Analysis (ITEGA) shown in Figure 24b revealed that a total of 99.1% (225) of all 227 valid data pairs (again CMDS data depicted as circles) would have led to appropriate treatment, whereas 0.9% (2) would have led to an unacceptable violation in insulin therapy. No single data pair suggested a major violation or a life threatening treatment.

These results of both EGA and ITEGA are thus indicative of a high level of accuracy of the CMDS system with respect to SOTA manual blood sampling.

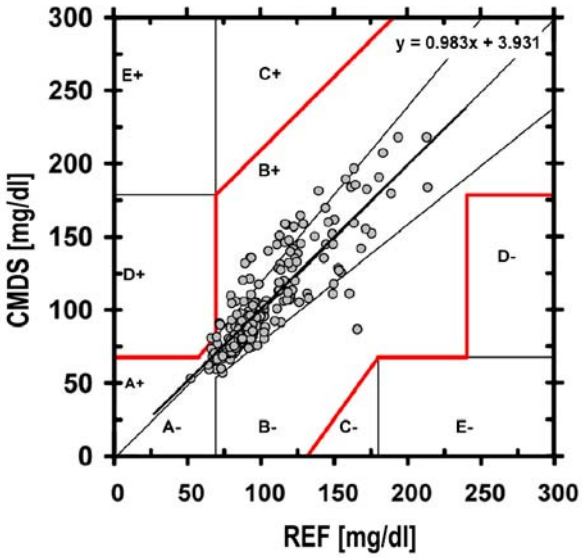
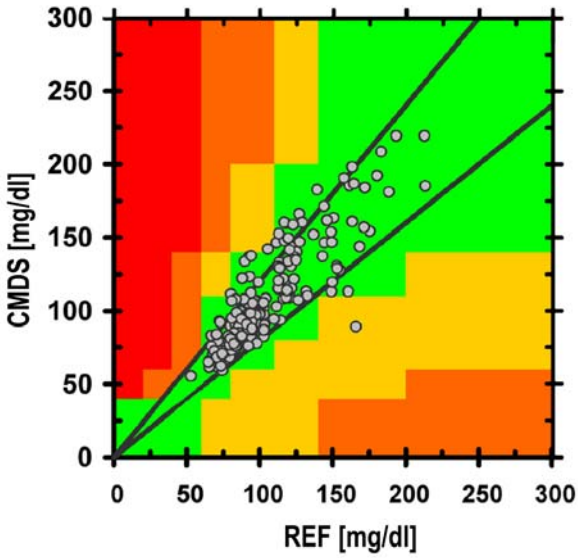


Figure 24a: Clark Error Grid analysis for CMDS system (circles); 12h study



b: Insulin Titration Error Grid Analysis for CMDS system (circles); 12h study

4. DISCUSSION

Glyceamic management has been shown be beneficial for critically ill patients that develop hyperglycaemia and insulin resistance. Frequent blood sampling for glucose monitoring can however conflict with other aspects of patient care.

In order to contribute to improved management of stress diabetes, the main objectives of this thesis were to develop and evaluate two robust, reliable and simple bedside devices for glucose monitoring in ICU patients. We present here a controlled clinical evaluation of two prototypes: firstly, an intermittent automated blood glucose monitoring system (the "IAGM-system") comprising an intermittent automated blood sampling system ("IABS system") coupled to a glucose biosensor and secondly, a continuous blood microdialysis system ("CMDS system"). Our goal was to examine the performance and clinical suitability of these novel systems.

IABS/ IAGM system

Clinical suitability of the intermittent system was evaluated in a total of fourteen volunteers. The performance of the IABS system was studied with six volunteers and the performance of the complete IAGM system with two volunteers over 12h. In addition, long term coagulation behaviour within the disposable tubing system of the IABS system was studied in a further six volunteers over 30h.

A key feature of the IAGM system is that sampling from a peripheral vein is carried out using a single lumen catheter that is available in every hospital. Unlike the CMDS system, which uses a double lumen catheter (DLC), the IAGM system can therefore be used without the need for additional expenditure in this regard. In addition to this, medical personnel are well acquainted with such catheters whereas a DLC needs special handling. The performance of the IABS system's catheter was improved 17.5-fold by implementing a Keep Vein Open (KVO) saline infusion at a rate of 2ml/h between successive blood withdrawals. Also, the volume of saline solution used to flush the disposable tubing system to avoid clotting after each automatic blood withdrawal was low at 8.83±0.28ml. Despite this however, the peripheral venous catheter might still represent a weak link in the system during long-term use, due to coagulation. The use of a central venous or arterial catheter could avoid this issue and so improve the long-term stability of the system. In addition, it would also be feasible in intensive care patients with whom central catheters are commonly used. In the 30h study a small amount of clotting was observed within the tubing system, though only within the stagnation zones of the 3-way stop cock of the catheter. This was, however, negligible and comparable to that observed in catheters used for SOTA manual blood sampling.

An excellent degree of correlation was observed between glucose concentrations obtained with manually drawn reference samples and those taken with the IABS system: Pearson's correlation coefficient R=0.982 (range 0.928–0.997), system error 1.7±9.0% for 12h study and R=0.976 (range: 0.953–0.996); system error -3.3±5.5% (range: -6.0–0.5) for the 30h study. Glucose values obtained fully automatically with the IAGM system also exhibited a very high degree of correlation with those obtained by conventional analysis and SOTA manual blood sampling R=0.950 (range 0.936–0.963), system error 1.0±6.6% (range: -1.1 to -0.9).

The system errors for clinical parameters determined additionally with the Roche Omni revealed values < ±15% for glucose and lactate, Ca^{++}, K^{+}, Na^{+} and Cl^{-} ions, Hct, pH, pCO_2, tHb, SO_2, O_2Hb , COHb and MetHb. Only the values for pO_2 and HHb were elevated. These results indicate that the IABS system can also be used to "feed" a standard blood gas analyser with whole blood and thus might enable bedside analysis of metabolites, ions, blood gases and CO-oximetry. As bedside analysers are classified as in vitro diagnostic devices, coupling to an IABS system is only allowed if a sterile barrier is guaranteed. IntelliDx [63] solved this problem by interconnecting an IABS system to the sensor via a sterile "blood transfer disk". Moreover, on the basis of these correlation data the

IAGM system could in principle be used to drive an algorithm for insulin infusion and enable tight, fully automated glycaemic control.

The amount of blood consumed by the IABS/ IAGM system was 1.69±0.09 ml per withdrawal procedure. On the basis of a subsequent survey performed at the Medical University of Graz, the maximum amount of blood that can be withdrawn from patients in the intensive care unit (ICU) is 50 ml per day. This would allow, on average, hourly glucose measurements of ICU patients to be made. More frequent glucose determinations would be enabled by reducing the diameters and lengths of the system's disposable tubing system to reduce the amount of blood consumed per measurement.

The clinically oriented Clark Error Grid analysis (EGA) is the most commonly used method to compare glucose values and evaluate the clinical significance of a particular system's accuracy. The EGA revealed that 96.93% (221) of the data points obtained in the study were located in zone A (accurate treatment) and 3.07% (7) in zone B (acceptable treatment) for the 12h study and 98.6% (414) in zone A and 1.4% (6) in zone B for the 30h study The data were also analysed using Insulin Titration Error Grid analysis which confirmed the conclusion drawn from EGA: 99.56% (227) of the data pairs suggested an acceptable treatment whereas 0.44% (1) suggested an unacceptable violation for the 12h study and 100% (420) suggested an acceptable treatment for the 30h study. Taken together, our results demonstrate that the IAGM system would be suitable for use in a variety of clinical applications, in particular in ICUs to enable frequent blood sampling, necessary to maintain tight glycaemic control (TGC) in critically ill hyperglycaemic patients, with significantly less staff effort than is currently required. To underline this, the IAGM system could always be assembled in less than 5min, which is comparable to the time required to set-up a standard infusion pump. In addition, the system incorporates acoustic and optical alarms to alert staff when interventions are required. The time saved by the system is, therefore not offset by a demanding and extended set-up time and a high level of maintenance. The constant APTT levels that were observed throughout the study confirm that the use of the IAGM system did not cause unintended systemic heparinisation and thus support its use in ICUs. Finally, we believe that our system can be an alternative to systems which have already been introduced and subsequently removed from the market [64], investigational tools [65,66,67] where clinical performance has to be shown in human studies, or tools for animal studies [68,69] which are not certified for human use.

CMDS system

Clinical suitability of the continuous system was evaluated in a total of eight volunteers over 12h. A key feature of the CMDS system is that this novel microdialysis-based device for continuous extravascular monitoring of blood glucose, incorporates a planar flow-through microdialyser and a newly developed membrane. In contrast to the IABS system, it delivers a protein free matrix which might increase the sensor's life time. In contrast to reported recovery degradation, the glucose recovery levels in our investigations remained stable using the newly designed membrane. This membrane is superior to those described in the literature with respect to recovery stability when exposed to heparinised blood. The CMDS system, in contrast to the IAGM system, uses a double lumen catheter (DLC) and thus creates additional expenditure and the need to teach medical staff the special handling required, particularly if the inner lumen is inserted or removed (e.g. in case of coagulation).

An excellent degree of correlation was observed between glucose concentrations obtained with manually drawn reference samples and dialysate samples taken with the CMDS system: Pearson's correlation coefficient R=0.960 (range: 0.876–0.995), system error 1.9±11.2% (range: -6.9-16.3) for 12h study. Finally, the constant APTT levels that were observed throughout the study confirm that the use of the CMDS system did not cause unintended systemic heparinisation and support its use in ICUs.

The current limitations of the system include the blood loss of 2ml/h, the time delay of 10.5min and the lack of an integrated online glucose sensor. However, it can be speculated that blood loss might be reduced by further downscaling system dimensions, by re-infusing the analysed blood or by integrating the analytics for other

relevant blood gases and metabolites. Due to the inherent time delay of the CMDS system, a therapy decision would be based on 'old' glucose data. Nevertheless, even with the existing time delay, the EGA revealed that 85.9% (195) of the data points obtained in the 12h study were located in zone A (accurate treatment) and 13.7% (31) in zone B (acceptable treatment). The data were also analysed using ITEGA, which confirmed the conclusion drawn from EGA: 99.1% (225) of the data pairs suggested an appropriate treatment whereas 0.9% (2) suggested an unacceptable violation. It might be worth considering here that the performed clinical and technical evaluation is based on 15min time-integrated glucose concentrations of dialysate samples which were compared to spot measurements of reference blood glucose concentration. Dialysate samples thus represent a 15min glucose average, which, especially in periods with pronounced glucose changes (OGTT period), can result in relatively high discrepancies between glucose concentrations obtained from reference and dialysate samples. We therefore believe that the precision of the system could be further improved, e.g. by using online glucose sensors together with an online recovery monitor [[70]], and that the system would then be suitable to enable continuous glucose monitoring to maintain TGC in critically ill hyperglycaemic patients with less staff effort than is currently required.

5. CONCLUSION AND OUTLOOK

In conclusion, the studies herein described demonstrate the feasibility of intermittent, automated real time ex vivo glucose determinations with the IAGM system. Following appropriate calibration, the amperometric biosensor used to quantify glucose in blood, delivered glucose values closely matching those determined by standard analysis methods. Also, the analysis of clinical parameters determined additionally with the Roche Omni revealed a good correlation between automatically and manually drawn samples. The amount of blood consumed by the system per sampling currently imposes a lower limit of, on average, hourly measurements in ICU patients. It might be speculated that the reduction in the dimension of the disposable tubing system and optimisation of the sample transport will decrease the amount of blood per withdrawal and so allow more frequent measurements.
Additionally, within these studies it could be demonstrated that the CDMS system facilitates continuous extraction of whole blood via a DLC to deliver protein-free dialysate samples using a newly developed dialysis cell and membrane for the purpose of continuous glucose monitoring. The membrane showed superior long-term stability against clotting, which was confirmed by a stable recovery rate. The next step towards an automated continuous glucose monitoring system is to integrate online glucose sensors into the devices presented here and to investigate aspects of long-term stability in future in-vivo studies.
We view peripheral catheters as the weakest link in both the IAGM and CMDS systems, even if other manufacturers follow this approach. Our future work will examine the performance of both systems together with a central venous or arterial catheter, as these are commonly used in ICU patients. Additional biosensors should be integrated in both systems to enable primary continuous glucose monitoring to maintain TGC in critically ill hyperglycaemic patients and, secondarily, also blood gases and electrolytes. Such a system would have the potential to support clinical decisions and to change, at a fundamental level, the ability of clinicians to improve patient care. Finally, to close the loop for complete glyceamic management, both systems should be tested together with algorithms and insulin infusion pumps under controlled conditions during human studies.

ACKNOWLEDGEMENTS

This thesis is based on experimental as well as theoretical work carried out during my work from 2004 – 2010 at Joanneum Research Forschungsgesellschaft mbH at the Institute of Medical Technologies and Health Management and the Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine at the Medical University of Graz. I would like to thank everyone involved in the completion of this thesis and to express my particular appreciation to the following people:

Zlatko Trajanoski, for his support during my PHD and especially for teaching me effectiveness
Thomas Pieber, for his expertise in the field of diabetes and open-minded style to coordinate our interdisciplinary team
Franz Feichtner, for his invaluable collaboration and alliance
Werner Regittnig, for his endless discussions and expertise in the field of Type 1 diabetes
Martin Ellmerer, for coordination of the Clinicip project, fruitful discussions, creative solutions and support
Andrea Wutte, for her maternal care concerning the Clinical Research Centre, study organisation and GCP issues and her belief in us.
Lukas Schaupp, for his indescribable, manifold input

I thank those who have actively contributed to this work in alphabetical order:

Andrea Groselj-Strele, Andreas Fercher, Barbara Semlitsch, Christopher Wrighton, Cornelia Missbrenner, Gerd Köhler, Gerlies Bock, Hannes Fiechtner, Hans Köhler, Heiko Wedig, Johannes Plank, Julia Mader, Karin Pickl, Manfred Bodenlenz, Maria Ratzer, Reinhard Hainisch, Robert Hellmich and Stefan Korsatko.

Special thanks go to my family, especially to my mother Monika, my father Josef and to my love Michèle.

This work was founded by grants from the European Commission: EC-Project Clinicip (www.clinicip.org) FP6 IST 506965, Federal Ministry of Economics and Labour of the Republic of Austria, Medingo Ltd. and Science Park Graz

REFERENCES

[1] Berger and Michael (Hrsg.), Diabetes mellitus, 2. Auflage. 2. 2000. Urban & Schwarzenberger

[2] http://www.who.int/mediacentre/factsheets/fs312/en/index.html; accessed 3rd of March 2010

[3] Wild S., Roglic G., Green A., Sicree R., King H., Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030; Diabetes Care; 27(5):1047-1053; 2004

[4] Williams L.S., Rotich J., Qi R., Fineberg N., Espay A., Bruno A., Fineberg S.E., Tierney W.R. Effects of admission hyperglycemia on mortality and costs in acute ischemic stroke; Neurology 59:67–71; 2002

[5] Umpierrez G.E., Isaacs S.D., Bazargan N., You X., Thaler L.M., Kitabchi A.E., Hyperglycemia: an independent marker of in-hospital mortality in patients with undiagnosed diabetes; J Clin Endocrinol Metab 87:978–982; 2002

[6] Finney S.J., Zekveld C., Elia A., Evans T.W., Glucose Control and Mortality in Critically Ill Patients; JAMA 290(15): 2041–2047; 2003

[7] Mizock B.A., Alterations in carbohydrate metabolism during stress: a review of the literature; Am J Med 98:75–107; 1995

[8] Inzucchi S.E., Goldberg P.A., Dziura J.D., Siegel M., Lee M., Halickman J., Sherwin R., Diabetes 52(1):A96; 2003

[9] Van den Berghe G., Beyond diabetes: saving lives with insulin in the ICU; Int J Obes Relat Metab Disord. 26 (3): S3–8; 2002

[10] Malmberg K., Norhammar A., Wedel H., Ryden L., Glycometabolic State at Admission: Important Risk Marker of Mortality in Conventionally Treated Patients With Diabetes Mellitus and Acute Myocardial Infarction; Circulation 99:2626–2632; 1999

[11] Nakamura H, Tanaka M, Shinohara S, Gotoh M, Karube I., Development of a self-sterilizing lancet coated with a titanium dioxide photocatalytic nano-layer for self-monitoring of blood glucose; Biosens Bioelectron; 22:1920–1925; 2007

[12] American Diabetes Association; Standards of Medical Care in Diabetes; Diabetes Care 30(1):S4–S41; 2007

[13] Harris M., Frequency of Blood Glucose Monitoring in Relation to Glycemic Control in Patients with Type 2 Diabetes; Diabetes Care 24(6):979–982; 2001

[14] Sternberg F., Meyerhoff C., Mennel F.J., Mayer H., Bischof F., Pfeiffer E.F., Does fall in tissue glucose precede fall in blood glucose?; Diabetologia 39:609–612; 1996

[15] Bantle J.P., Thomas W., Glucose measurement in patients with diabetes mellitus with dermal interstitial fluid; J Lab Clin Med 130:436–441; 1997

[16] Lindpointner S., Korsatko S., Köhler G., Köhler H., Schaller R., Kaidar R., Yodfat O., Schaupp L.A., Ellmerer M., Pieber T.R., Regittnig W., Use of the Site of Subcutaneous Insulin Administration for the Measurement of Glucose in Patients with Type 1 Diabetes; Diabetes Care 33 (3); March 2010

[17] Kadish A.H., Physiologic monitoring of blood glucose; Calif Med 98(6):325-327; 1963

[18] Hovorka R., Kremen J., Blaha J., Matias M., Anderlova K., Bosanska L., Roubicek T., Wilinska M.E., Chassin L.J., Svacina S., Haluzik M., Blood glucose control by a model predictive control algorithm with variable sampling rate vs. a routine glucose management protocol in cardiac surgery patients: a randomized controlled trial; J Clin Endocrinol Metab; 92(8):2960-2964; 2007

[19] Van den Berghe G., Wouters P., Weekers F., Verwaest C., Bruyninckx F., Schetz M., Vlasselaers D., Ferdinande P., Lauwers P., Bouillon R., Intensive insulin therapy in the critically ill patients; N Engl J Med 345(19):1359-1367; 2001

[20] Van den Berghe G., Wouters P.J., Kesteloot K., Hilleman D.E., Analysis of healthcare resource utilization with intensive insulin therapy in critically ill patients; Crit Care Med 34(3):612-616; 2006

[21] NICE-SUGAR Study Investigators, Intensive versus conventional glucose control in critically ill patients; N Engl J Med 360:1283-97; 2009

[22] Griesdale D.E.G., De Souza R.J., Van Dam R.M, Heyland D.K., Cook D.J., Malhotra A., Dhaliwal R., Henderson W.R., Chittock D.R., Finfer S., Talmor D., Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data; CMAJ 180(8): 821-827; 2009

[23] Van den Berghe G., Mesotten D., Vanhorebeek I. Intensive insulin therapy in the intensive care unit, CMAJ 180(8): 799-800; 2009

[24] Cryer P.E., Binder C., Bolli G.B., Cherrington A.D., Gale E.A., Gerich J.E., Sherwin R.S. Hypoglyceamia in IDDM; Diabetes 38(9):1193–1199;1989

[25] Perriello G., De Feo P., Torlone E., Calcinaro F., Ventura M.M., Basta G., Santeusanio F., Brunetti P., Gerich J.E., Bolli G.B., The effect of asymptomatic nocturnal hypoglyceamia on glyceamic control in diabetes mellitus; N Engl J Med 319:1233–1239, 1988

[26] Bremer T., Gough D.A. Is Blood Glucose Predictable From Previous Values?; Diabetes 48: 445-451; 1999

[27] Briggs A.L., Cornell S. Self-monitoring blood glucose (SMBG): now and the future; J Pharm Pract 17(1): 29-38; 2004

[28] Lourido J., Ederoth P., Sundvall N., Ungerstedt U., Nordstrom C.H., Correlation between blood glucose concentration and glucose concentration in subcutaneous adipose tissue evaluated with microdialysis during intensive care; Scand J Clin Lab Invest. 62(4):285–292; 2002

[29] Vlasselaers D., Schaupp L., Van den Heuvel I., Mader J., Bodenlenz M., Suppan M., Wouters P., Ellmerer M., Van den Berghe G., Monitoring blood glucose with microdialysis of interstitial fluid in critically ill children; Clin Chem 53, 536–537; 2007

[30] Kulcu E., Tamada J.A., Reach G., Potts R.O., Lesho M.J., Physiological differences between interstitial glucose and blood glucose measured in human subjects; Diabetes Care 26(8):2405-2409; 2003

[31] Regittnig W., Ellmerer M., Fauler G., Sendlhofer G., Trajanoski Z., Leis H.J., Schaupp L., Wach P., Pieber T.R., Assessment of transcapillary glucose exchange in human skeletal muscle and adipose tissue; Am J Physiol Endocrinol Metab 285, E241-E251; 2003

[32] Klonoff D.C., Continuous Glucose Monitoring - Roadmap for 21st century diabetes therapy; Diabetes Care 28(5):1231–1239; 2005

[33] Yates K., Hasnat Milton A., Dear K., Ambler G., Continuous Glucose Monitoring–Guided Insulin Adjustment in Children and Adolescents on Near-Physiological Insulin Regimens; Diabetes Care 29(7):1512–1517, 2006

[34] Basmadjian D., Sefton M.V., Baldwin S.A.; Coagulation on biomaterials in flowing blood: some theoretical considerations; Biomaterials Vol 18(23):1511-1522, 1997

[35] Weller C., Linder M., Macaulay A., Ferrari A., Kessler G., Continuous in vivo determination of blood glucose in human subjects; Ann N Y Acad Sci 87:658-668, 1960

[36] Ganesh A., Hipszer B., Loomba N., Simon B., Torjman M.C., Joseph J., Evaluation of the VIA blood chemistry monitor for glucose in healthy and diabetic volunteers; J Diabetes Sci Technol 2(2):182–193, 2008

[37] Stjernstrom H., Karlsson T., Ungerstedt U., Hillered L., Chemical monitoring of intensive care patients using intravenous microdialysis; Intensive Care Med 19:423-428; 1993

[38] Ungerstedt U., Microdialysis – principles and applications for studies in animals and humans; J Intern Med 230:365-373; 1991

[39] De Lange E.C., De Boer A.G., Breimer D.D., Methodological issues in microdialysis sampling for pharmacokinetic studies; Adv Drug Deliv Rev 45:125-148, 2000

[40] Stjernstrom H., Karlsson T., Ungerstedt U., Hillered L., Chemical monitoring of intensive care patients using intravenous microdialysis; Intensive Care Med 19:423–428; 1993

[41] O'Connell M.T., Tison F., Quinn N.P., Patsalos P.N., Clinical drug monitoring by microdialysis: application to levodopa therapy in Parkinson's disease; Br J Clin Pharmacol 42:765–769, 1996

[42] Patsalos P.N., O'Connell M.T., Doheny H.C., Sander J.W., Shorvon S.D., Antiepileptic drug pharmacokinetics in patients with epilepsy using a new microdialysis probe: preliminary observations; Acta Neurochir 67:59–62, 1996

[43] Paez X., Hernandez L., Blood microdialysis in humans: a new method for monitoring plasma compounds; Life Sci 61:847–856, 1997

[44] Castejon A.M., Paez X., Hernandez L., Cubeddu L.X., Use of intravenous microdialysis to monitor changes in serotonin release and metabolism induced by cisplatin in cancer patients: comparative effects of granisetron and ondansetron; J Pharmacol Exp Ther 291(3):960–966, 1999

[45] Costa F., Sulur P., Angel M., Cavalcante J., Haile V., Christman B., Biaggioni I., Intravascular source of adenosine during forearm ischemia in humans: implications for reactive hyperemia; Hypertension 33:1453–1457, 1999

[46] Dizdar N., Granerus A.K., Hannestad U., Kullman A., Ljungdahl A., Olsson J.E., Kagedal B., L-dopa pharmacokinetics studied with microdialysis in patients with Parkinson's disease and a history of malignant melanoma; Acta Neurol Scand 100:231–237, 1999

[47] Elshoff J.P., Laer S., Development of an intravenous microdialysis method for pharmacokinetic investigations in humans; J Pharmacol Toxicol Methods 52:251–259, 2005

[48] Yokel R.A., Allen D.D., Burgio D.E., McNamara P.J., Antipyrine as a dialyzable reference to correct differences in efficiency among and within sampling devices during in vivo microdialysis; J Pharmacol Toxicol Methods 27: 135–142, 1992

[49] Chen Z., Steger R.W., Plasma microdialysis, A technique for continuous plasma sampling in freely moving rats; J Pharmacol Toxicol Methods 29:111–118, 1993

[50] Verbeeck R.K., Blood microdialysis in pharmacokinetic and drug metabolism studies; Adv Drug Deliv Rev. 45: 217–228, 2000

[51] De Lange E.C., De Boer A.G., Breimer D.D., Methodological issues in microdialysis sampling for pharmacokinetic studies; Adv Drug Deliv Rev 45:125-148, 2000

[52] Commission Directive 2004/9/EC; http://www.snas.sk/files/pdf/GLP_Directive_2004_9-EC_en.pdf; accessed 3rd of March 2010

[53] World medical association - Declaration of Helsinki, http://www.wma.net/en/30publications/10policies/b3/index.html; accessed 3rd of March 2010

[54] ICH Topic E 6 (R1) Guideline for Good Clinical Practice; www.emea.europa.eu/pdfs/human/ich/013595en.pdf; accessed 3rd of March 2010

[55] Commission Directive 2005/28/EC; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:091:0013:0019:en:PDF; accessed 3rd of March 2010

[56] Wenthold I.M., Hart A.A., Hoekstra J.B., Devries J.H.; How to assess and compare the accuracy of continuous glucose monitors?; Diabetes Technol Ther 10(2):57–68, 2008

[57] Bland J.M., Altman D.G., Statistical methods for assessing agreement between two methods of clinical measurement; Lancet 1(8476):307–310; 1986

[58] Lodwig V., Heinemann L., Glucose Monitoring Study Group, Continuous glucose monitoring with glucose sensors: calibration and assessment criteria; Diabetes Technol Ther 5(4):572–86; 2003

[59] In vitro diagnostic test systems - Requirements for blood-glucose monitoring systems for self-testing in managing diabetes mellitus (ISO 15197:2003)

[60] Clarke W.L., Cox D., Gonder-Frederick L.A., Carter W., Pohl S.L., Evaluating clinical accuracy of systems for self-monitoring of blood glucose; Diabetes Care 10(5):622–8; 1987

[61] Ellmerer M., Haluzik M., Blaha J., Kremen J., Svacina S., Toller W., Mader J., Schaupp L., Plank J., Pieber T.R., Clinical evaluation of alternative-site glucose measurements in patients after major cardiac surgery; Diabetes Care 29(6):1275–81, 2006

[62] Guidance for Industry - Bioanalytical Method Validation; FDA; 2001

[63] http://intellidx.com; accessed 3rd of March 2010

[64] http://www.viamedical.com/; accessed 3rd of March 2010

[65] http://www.cascademetrix.com; accessed 3rd of March 2010

[66] http://www.spheremedical.com/; accessed 3rd of March 2010

[67] http://www.mtb-zier.de; accessed 3rd of March 2010

[68] http://www.dilab.se/; accessed 3rd of March 2010

[69] http://www.instechlabs.com/Infusion/bloodsampling/; accessed 3rd of March 2010

[70] Schaupp L., Ellmerer M. Brunner G.A, Wutte A., Sendlhofer G., Trajanoski Z., Skrabal F., Pieber T.R., Wach P. Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion; Am J Physiol Endocrinol Metab 276:E401-E408; 1999

Publication I

“A novel automated discontinuous venous blood monitoring system for ex vivo glucose determination in humans”

Schaller, R., Feichtner F., Köhler H., Bodenlenz M., Plank J., Wutte A., Mader J.K., Ellmerer M., Hellmich R., Wedig H., Hainisch R., Pieber T.R., Schaupp L.A.

.

Biosens. Bioelectron. Vol. 24 (7), 15th of March 2009

Biosensors and Bioelectronics 24 (2009) 2239–2245

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A novel automated discontinuous venous blood monitoring system for ex vivo glucose determination in humans

R. Schaller[a,*], F. Feichtner[a], H. Köhler[a], M. Bodenlenz[a], J. Plank[b], A. Wutte[b], J.K. Mader[b], M. Ellmerer[b], R. Hellmich[d], H. Wedig[d], R. Hainisch[c], T.R. Pieber[a,b], L. Schaupp[a,b]

[a] Joanneum Research Forschungsgesellschaft mbH, Institute of Medical Technologies and Health Management, Elisabethstrasse 11a, 8010 Graz, Austria
[b] Medical University of Graz, Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Austria
[c] FH OOE Forschungs & Entwicklungs GmbH, Department of Social/Health, Austria
[d] SensLab Gesellschaft zur Entwicklung und Herstellung bioelektrochemischer Sensoren mbH, Germany

ARTICLE INFO

Article history:
Received 14 August 2008
Received in revised form
27 November 2008
Accepted 28 November 2008
Available online 7 December 2008

Keywords:
Glucose
Sensors
Monitoring
Blood
Automated
Ex vivo

ABSTRACT

Intensive insulin therapy reduces mortality and morbidity in critically ill patients but imposes great demands on medical staff who must take frequent blood samples for the determination of glucose levels. A solution to this resourcing problem would be provided by an automated blood monitoring system. The aim of the present clinical study was to evaluate such a system comprising an automatic blood sampling unit linked to a glucose biosensor. Our approach was to determine the correlation and system error of the sampling unit alone and of the combined system with respect to reference levels over 12 h in humans. Two venous cannulae were inserted to connect the automatic and reference systems to the subjects. Blood samples were taken at 15 and 30 min intervals. The median Pearson coefficient of correlation between manually and automatically withdrawn blood samples was 0.982 for the sampling unit alone and 0.950 for the complete system. The biosensor had a linear range up to 20 mmol l^{-1} and a 95% response time of <2 min. Clark Error Grid analysis showed that 96.93% of the data (228 data pairs) was in zone A and 3.07% in zone B. Insulin Titration Error Grid analysis suggested an acceptable treatment in 99.56% of cases. Implementation of a "Keep Vein Open" saline infusion into the automated blood sampling system reduced blood withdrawal failures through occluded catheters fourfold. In summary, automated blood sampling from a peripheral vein coupled with automatic glucose determination is a promising alternative to frequent manual blood sampling.

1. Introduction

Critically ill patients undergoing intensive medical treatment may develop hyperglycaemia and insulin resistance despite the lack of a previous history of diabetes (McCowen et al., 2001). The use of intensive insulin therapy is very helpful for such patients as illustrated by a 12-month study involving 1548 critically ill surgical patients (Berghe et al., 2001). In this study, maintenance of blood glucose levels within a range of 4.4–6.1 mmol l^{-1} was shown to reduce the rate of mortality from 8.0% (conventional treatment) to 4.6%, an apparent risk reduction of around 42%. The risk of complications and the consumption of intensive care resources were moreover also reduced.

To maintain euglycaemic glucose levels, skilled medical staff must take frequent blood samples. In practice this is often not feasible however, due to the high workload associated with the care of critically ill patients. Reproducible manual blood sampling is moreover technically challenging. The quality of the sample obtained from the fingertip with a lancet is affected by factors such as the location and depth of puncture and dilution by ethanol used for surface sterilisation. On the other hand, sampling from a peripheral vein can be problematic owing to a lack of suitable veins. These technical challenges are rendered more acute by the general lack of available time and the need to minimize patient discomfort. Finally, the complicated logistics of sample processing can potentially lead to delayed results or even sample mix-ups.

Fully automated blood sampling and real time glucose analysis would elegantly avoid these problems and moreover provide valuable trend information (direction and rate of change). Armed with this, and freed from the need to perform routine blood sampling and await the analysis results, an intensive care nurse could more effectively maintain euglycaemia. A prerequisite for the clinical acceptance of such a system would be the avoidance of systemic heparinisation, especially of patients following major surgery.

Systems using double lumen catheters (Weller et al., 1960; Gfrerer et al., 1998) such as the Biostator and Glucostator or ex vivo

* Corresponding author.
E-mail address: roland.schaller@joanneum.at (R. Schaller).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.11.029

bedside devices employing mid-infrared spectroscopy (Heise et al., 2007) have been successfully used to sample peripheral venous blood. These systems have only gained acceptance for clinical studies however and are in addition more expensive to use than those employing conventional catheters. Via Medical introduced a blood glucose monitoring device using standard venous access but did not supply the European market (Ganesh et al., 2008).

Glucose levels can also be determined in subcutaneous adipose tissue by microdialysis (Jungheim et al., 2001) or by open-flow microperfusion (Trajanoski et al., 1997). Systems using microdialysis and a needle-type sensor include the Abbott Freestyle Navigator (Weinstein et al., 2007), the Dexcom STS sensor (Garg et al., 2006) and the MiniMed CGMS Gold portable system (Mastrototaro, 1999). Non-invasive, continuous measurement systems are also commercially available. These include iontophoresis-based systems such as the Cygnus GlucoWatch (Tierney et al., 2001) or impedance spectroscopy-based devices (Caduff et al., 2003). As well as the above, several glucose sensors have been validated for future use with automatic blood sampling units (Ricci et al., 2007).

The aim of the EU-funded project closed loop insulin infusion for critically ill patients (CLINICIP) was to develop a low-risk, invasive decision support system comprising biosensors and an adaptive control algorithm for the glycaemic control of intensive care patients (Hovorka et al., 2004). Our goal within this project was to develop an automated glucose monitoring system ("AGM-system") comprising an automated blood sampling system ("ABS-system") in combination with an online glucose biosensor ("biosensor"). In the present study, the ability of the resultant system to deliver clinically reliable glucose measurements was determined over a 12-h period in two healthy volunteers using good clinical practice in a purpose built clinical study unit. The glucose values obtained with the AGM-system were compared with those obtained from manually withdrawn blood samples using correlation, system error and error grid analyses. Prior to this, the ABS-system was validated alone in six subjects since this was the most technically complex part of the AGM-system and thus, we reasoned, the most error-prone part (the biosensor was already known to function well over an extended period). The study safety endpoints were the determination of the activated partial thromboplastin time (APTT) as a measure of systemic heparinisation caused by the system and the monitoring of adverse events.

2. Experimental

2.1. Blood sampling

Physicians and nurses conventionally obtain peripheral venous blood samples using a manual procedure employing two stopcocks and two syringes. This procedure was used as the study reference. The overall configuration and a description of the AGM-system are provided in Fig. 1. The following materials were used: single lumen catheters (18 gauge × 45 mm, BBraun, Melsungen, Germany) sterile 0.9% saline solution (Fresenius, Fesenius Kabi, Graz, Austria), peristaltic pumps (Minipuls MP3, Gilson, Cedex, France), heparin (Novo Nordisk 5000 IE ml^{-1}, Bagsvaerd, Denmark), tubing (TYGON® S-50-HL and TYGON® 3350, Saint-Gobain Performance Plastics, Beaverton, France), drop chambers (Infusiomatleitung, BBraun, Melsungen, Germany), dripping sensors (Type: 3450578 A, BBraun, Melsungen, Germany), pinch valves (PM-0815W, Takasago Electric Inc., Japan), check valves (Part No. 11582, Qosina, Edgewood, USA), reusable pressure transducers and disposable pressure domes (SP854, Memscap, Norway) and air bubble sensors (customized sensor, Zevex, Salt Lake City, Utah, USA). The system housing was designed by Solid Works (Solid works, Concord, MA, USA) and

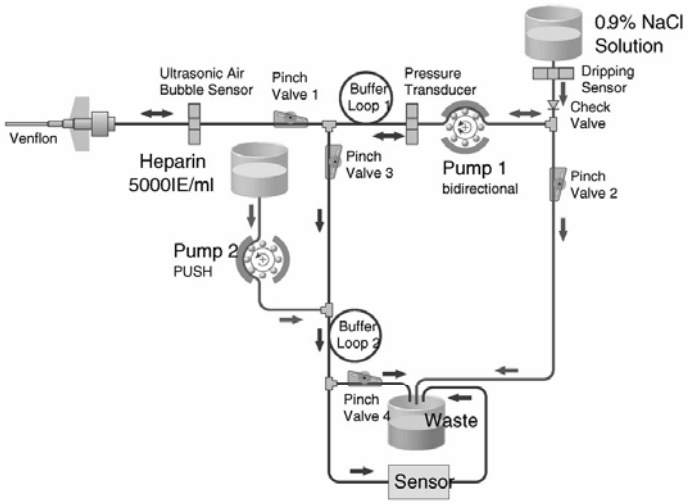


Fig. 1. AGM-system. *Construction*: The in-house produced tubing system was sterilised with ethylene oxide and afterwards completely flushed using saline solution attached to the system using a standard fluid administration set containing a drop chamber and a dripping sensor to monitor the fluid level. Flow was controlled using pinch valves compatible with the tubing used. A check valve was used to prevent reflux and to maintain the sterility of the saline solution. For safety reasons, pressure changes within the accessed vessel were monitored using a reusable pressure transducer and a disposable pressure dome. The maximum allowed pressure was set at 150 kPa which is comparable to the maximum pressure applied by infusion pumps. As well as this, an air bubble sensor was used to avoid accidental air infusion. Alarms were displayed both optically and acoustically. *Operation*: Blood (4.0 ml) was withdrawn at 10 ml min^{-1} using peristaltic pump 1 and stored in buffer loop 1. From this, 1.69 ± 0.09 ml were branched off, heparinised (final concentration 50 IE ml^{-1}) and afterwards transferred to loop 2 for storage and analysis using pump 2. The remaining non-heparinised blood was immediately re-infused into the subject to minimize blood consumption. The line was then flushed to avoid clotting. Closure of a pinch valve prevented the reflux of heparinised blood into the subject. The blood in buffer loop 2 was then uni-directionally pumped through the biosensor for 10 min at 20 μl min^{-1} and immediately discarded. The biosensor current was recorded every 10 s.

rapidly prototyped (FH OOE, Linz, Austria). All materials used (e.g. tubing) are approved for use in humans.

2.2. Glucose biosensor

In fundamental terms, the Senslab glucose biosensor consists of a micro-structured planar flow-through cell and a screen-printed amperometric thick film sensor coated with glucose oxidase (GOD). The sample flows along the elliptic flow channels and is drawn by capillary forces into the gap between the indicating window of the biosensor and the upper embossed area of the flow-through cell. Microair bubbles present in the analyte solution collect in the elliptic flow channels and are thus excluded from the sensitive area of the indicating window. Glucose is detected by a primary indicating reaction mediated by immobilised GOD, which catalyses the oxidation of glucose to glucono delta-lactone and hydrogen peroxide according to Eq. (1):

$$\text{D-(+)-}\beta\text{glucose} + O_2 + H_2O \xrightarrow{\text{glucose Oxidase(EC1.1.3.4)}} \text{D-glucono-1, 5-lactone} + H_2O_2 \quad (1)$$

The hydrogen peroxide generated is then quantified by a secondary electrochemical reaction at the amperometric sensor. Oxidation of hydrogen peroxide according to Eq. (2) at the surface of the working electrode caused by the application of a polarisation voltage of +400 mV with respect to the internal silver/silver chloride reference electrode generates an anodic electron flow that is directly proportional to the glucose content of the sample, which is measured by an ampere meter as part of the potentiostat.

$$2H_2O_2 \xrightarrow{\text{working electrode(anode)}} O_2 + 2H^+ + 2H_2O + 2e^- \quad (2)$$

A diffusion-controlled reaction rate was obtained by the application of a diffusion barrier layer.

The biosensor comprises a plastic support (Polycarbonate, ThyssenKrupp Schulte, Duesseldorf, Germany) with outer dimensions of 10 mm × 10 mm × 2 mm ($L \times W \times H$) on which three different polymeric carbon paste conducting paths are printed. In the area of the indicating window, formed later by the dielectric layer, a platinised carbon paste is overprinted on two of these three conducting paths to create working and counter electrodes, respectively. The third electrode is covered by a silver/silver chloride paste to create the reference electrode. In a final screen printing step, an isolating (dielectric) paste is overprinted to define the indicating window of the sensor. The total inner volume of the measuring chamber is 0.5 μl. The three electrode system consists of the working, counter and reference electrodes according to the potentiostatic principle. Approximately 31 units (0.5 μg) of GOD (Serva, Heidelberg, Germany) are immobilised on the surface of the working electrode using polycarbamoyl sulfonate (PCS; Prepolymer of Polycarbomoyl sulfonate, SensLab, Leipzig, Germany) as an entrapping matrix, after which siloprene (Fluka, Schnelldorf), Germany is deposited at the GOD-PCS matrix layer to form a diffusion barrier and protective layer (Fig. 2).

Prior to the present study we evaluated the precision, accuracy and long-term stability of the biosensor using diluted bovine serum containing 10 mmol l^{-1} glucose. Typical values obtained during these pre-investigations (unpublished data) were: accuracy: 5.0%, precision: 5.1% and long-term stability −0.02 mmol l^{-1} per hour (Fig. 1, Supplementary material).

2.3. Data acquisition

Data were acquired with a notebook computer using a DAQ-Card 6036-E 16-bit analogue/digital data acquisition PCMCIA card, an SC-2345 configurable connector and a data acquisition programme based on Lab VIEW® 7.0 (all from National Instruments Inc., Austin, TX, USA). Data were stored in an Excel worksheet (Microsoft Inc., Redmond, WA, USA). Biosensor data (1–100 nA) were acquired at 10 s intervals and stored after signal processing using a custom-made data logger containing a 12-bit analogue/digital converter (Disetronic, Burgdorf, Switzerland). Data were transmitted to a personal computer via an infrared interface, acquired using PC software (Disetronic, Burgdorf, Switzerland) and stored in an ASCII format.

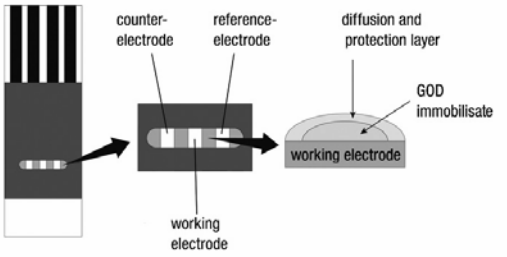


Fig. 2. Assembly of the biosensor showing immobilised GOD and diffusion/protective layer.

2.4. Subjects and protocols

The study, a 12-h open mono-centre trial at the Medical University of Graz, was approved by the local ethics committee and written informed consent was obtained from all participants. Eight suitable healthy volunteers (7 males and 1 female aged 28.88 ± 3.52 years with a body mass index of 25.11 ± 1.49 kg m^{-2}) were recruited and attended the trial centre at 8:00 a.m. in a fasting state. For reference blood sampling a catheter was inserted into a peripheral vein in the left forearm with controlled saline infusion to prevent occlusion. A second catheter was then placed in the same arm and connected to the ABS-system. The first blood sample was then taken manually for determination of the baseline activated partial thromboplastin time (APTT).

The study comprised of two parts. The objective of the first part (subjects 1–6) was to validate the ABS-system through a comparison with the manual sampling technique. In the second part (subjects 7 and 8), the complete AGM-system (ABS-system and online biosensor) was validated. Blood samples were generally withdrawn at 30 min intervals. Following oral administration of 75 g of glucose at 1:00 p.m. to modify the subjects' glucose concentration profiles, the rate of sampling was increased to four samples/hour for 3 h. The theoretical total blood consumption was 79.5 ml per volunteer.

2.5. Laboratory analysis, calibration of the biosensor and system, data analysis and statistics

Manually and automatically drawn blood samples were analysed using a laboratory glucose analyser (Omni S6, Roche, Basel, Switzerland). The ABS-system is designed to be operated without calibration. The online biosensor was calibrated before the start of each experiment using 5, 10, 15 and 20 mmol l^{-1} standard aqueous glucose solutions prepared by spiking ELO-MEL (ELO-MEL, Fresenius Kabi, Graz, Austria) with glucose (D(+)-Glucose, 108337, Merck KGaA, Darmstadt, Germany) according to good laboratory practice (GLP). The online biosensor current signal (95% of the peak value) was recalibrated 1 h after the start of the online measuring procedure using the reference blood glucose values. The agreement between the glucose concentrations obtained by automatic blood

withdrawal (with and without the biosensor) and those obtained with manually withdrawn blood was assessed by calculating the Pearson coefficient of correlation (Bland and Altman, 1986) and the system error (Lodwig and Heinemann, 2003). A tool for assessing the degree of clinical agreement between two methods (Clarke et al., 1987) was applied to the glucose values obtained with automatically sampled blood and those obtained from the corresponding manually drawn samples. The data were also compared using the recently published Insulin Titration Error Grid analysis Fig. 2, Supplementary material (Ellmerer et al., 2006).

3. Results

3.1. General

All eight volunteers successfully completed the study according to the study plan. The catheters were well tolerated throughout with no sign of local infection. For two subjects, the catheters were renewed due to permanent occlusion. From a total of 240 measurements made, only 12 were invalid due to catheter blockage, coagulation, insufficient sample volume or haemolysis (7 for the ABS-system and 5 for the complete AGM-system). The overall median frequency of successful sampling was 96.67 ± 5.33% per subject [range: 86.67–100.00%].

3.2. Calibration

The biosensor responded linearly to increasing glucose concentrations up to 20 mmol l^{-1} during in vitro calibration (Fig. 3). Base current and sensitivity of the sensor were typically 5 nA and 1.8 nA $mmol^{-1}$ glucose, respectively.

3.3. Correlation

The median Pearson coefficient of correlation between glucose levels determined with manually and automatically withdrawn blood samples was 0.982 [range: 0.928–0.997, subjects 1–6]. The corresponding value for manually withdrawn blood samples and glucose levels obtained fully automatically was 0.950 [range: 0.936–0.963, subjects 7 and 8]. Illustrative examples of the comparison of glucose concentration profiles obtained using (i) the ABS-system alone and (ii) the AGM-system with the respective profiles obtained using manually withdrawn blood samples are shown in Fig. 4.

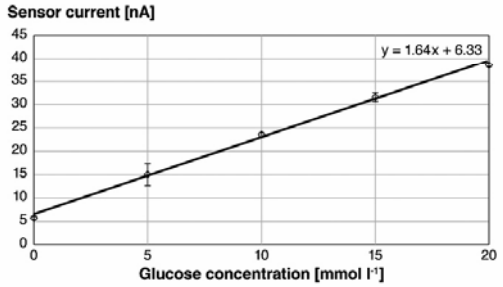


Fig. 3. Biosensor calibration curve. Standard glucose solutions (0, 5, 10, 15 and 20 mmol l^{-1}) were used for the construction of the biosensor calibration curve. The linear equation used was $y = 1.64x + 6.3$, $R = 0.9984$. The 95% response time was less than 2 min.

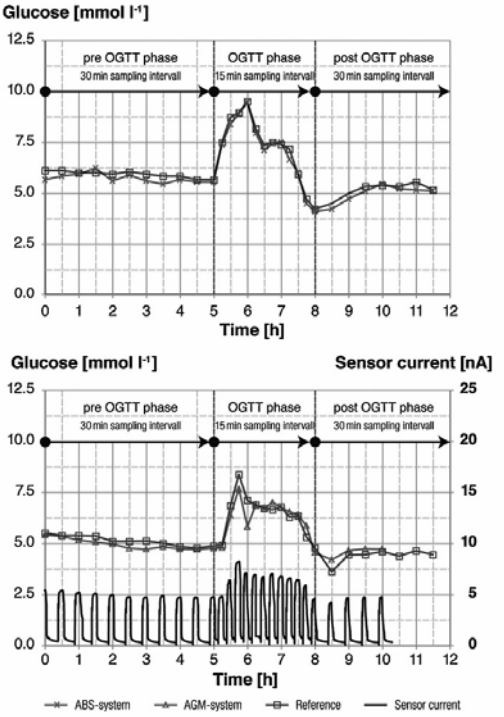


Fig. 4. Top: Comparison of glucose concentration profiles obtained for subject 03 with automatically (crosses) and manually (squares) withdrawn blood. Bottom: Comparison of glucose concentration profiles obtained for subject 7 using the AGM-system (triangles) with manually withdrawn reference blood samples (squares). Also shown are the corresponding chronoamperometric measurements obtained with the biosensor. The sensor current in nA (2nd y-axis) for each reading is displayed as a solid line.

3.4. System error

The system error is defined according to the following equation:

$$\text{system error} = \frac{\text{estimated value} - \text{reference value}}{\text{reference value}} \times 100 \qquad (3)$$

Fig. 5 shows the system error for subjects 1–8. The individual and mean system error + the standard deviations are indicated for each subject. The calculated system error between the ABS-system and reference measurements was −1.66 ± 9.01% (subjects 1–6). For the complete AGM-system this was −1.00 ± 6.61% (subjects 7 and 8). The system error for subjects 7 and 8 was around zero due to the calibration of the ABS-system together with the sensor in the complete AGM-system.

In order to minimize unwanted coagulation, especially when sampling at 30 min intervals, a "Keep Vein Open" saline infusion (2 ml h^{-1}) was implemented into the ABS-system and found to reduce the frequency of blood withdrawal failures due to occluded catheters from 13.33% to 3.33%. The mean volume of saline used to flush the disposable tubing system of the ABS-system after each automatic blood withdrawal was 8.83 ± 0.28 ml. The total amount of flushing fluid used for the complete study was less than 270 ml.

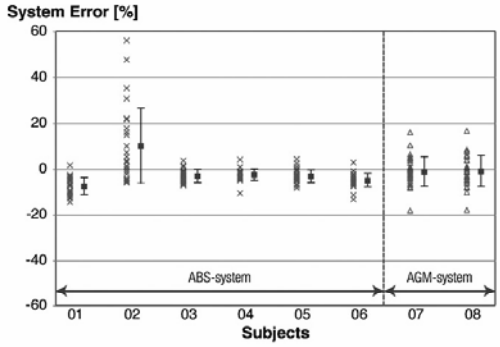


Fig. 5. Error graph for all subjects: subjects 1–6 (ABS); subjects 7 and 8 (ABS + biosensor). Crosses and triangles indicate data pairs of reference vs. ABS-system or reference vs. the complete AGM-system. Squares represent the mean values for each subject and the bars the respective standard deviations.

3.5. Clark Error Grid analysis

Clark Error Grid analysis (EGA) is a clinically oriented graphical method for the comparison of blood glucose data obtained with a novel method and with an established reference system. EGA is conducted by plotting sample data on a graph (estimated blood glucose value on the ordinate and the reference glucose value on the abscissa) divided into five zones, each representing a different degree of clinical acceptability as follows: A (accurate), B (acceptable), C, D and E (not acceptable). EGA thus categorizes individual glucose values obtained with the new method with respect to the corresponding reference values according to the possible clinical consequences of the inaccuracy of the investigated method.

EGA analysis of the study results is shown in Fig. 6. Of the 173 valid data pairs obtained by conventional glucose analysis of blood samples drawn with the ABS-system and with the reference method, 166 (95.95%) were in zone A and only 7 (4.05%) in zone B. Furthermore, of the 55 data pairs obtained for the complete AGM-system (i.e. ABS-system plus biosensor) and corresponding reference measurements, all were located in zone A. Thus, overall a total of 96.93% of the measurements were in zone A ("accurate") and only 3.07% in zone B ("acceptable"). This result is indicative of a high level of accuracy of both the ABS-system alone and in combination with the biosensor with respect to the manual state of the art procedure.

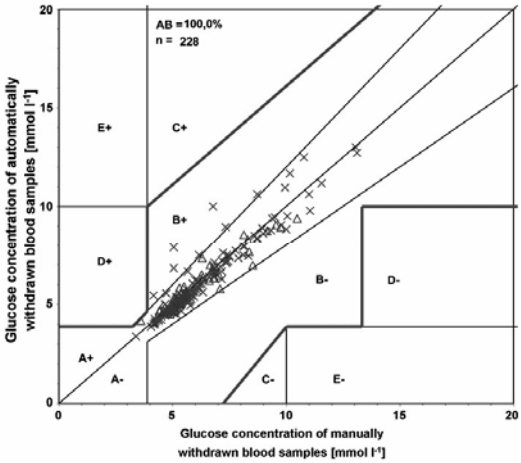


Fig. 6. Clark Error Grid analysis of glucose concentration data obtained using the ABS-system alone (subjects 1–6; crosses) and with the online biosensor (subjects 7 and 8; triangles); ABS: 173 data pairs, zone AB (100%); combined system: 55 data pairs, zone AB (100%).

3.6. Insulin Titration Error Grid analysis

Glucose measurements can also be clinically evaluated by Insulin Titration Error Grid analysis, which also involves the plotting of blood glucose concentrations for the reference method and the method under evaluation on the x- and y-axis, respectively. Glucose ranges on the x- and y-axis are related to major treatment interventions as suggested by the Leuven insulin titration guideline (Berghe, 2002). Different zones have been defined on the basis of these treatment actions, according to the severity of violation of the titration guidelines as: "appropriate treatment", "unacceptable violation", "major violation" and "life threatening treatment". A total of 99.56% of the 227 valid data pairs generated by the study were located in the acceptable treatment zone. The single data pair that lay outside of this zone was an erroneous measurement for subject 2 caused by dilution effects (and considered in more detail in Section 4).

3.7. Safety endpoints and adverse events

None of the subjects exhibited a statistically significant increase in APTT levels ($p = 0.05$), from which we conclude that unintended heparinisation of the subjects did not take place and that the use of heparin to avoid coagulation within the AGM-system is both effective and harmless. No adverse trial-related events occurred during the study.

4. Discussion

Intensive insulin therapy has been shown to benefit critically ill patients that develop hyperglycaemia and insulin resistance. The frequent blood sampling that is required can however conflict with other aspects of patient care. We present here a controlled clinical evaluation of a prototype automatic blood glucose monitoring system (the "AGM-system") comprising an automated blood sampling system (the "ABS system") coupled to a glucose biosensor (the "biosensor"). Our goal was to examine the clinical suitability and accuracy of this novel system. Eight volunteers participated in the evaluation. The performance of the ABS-system was studied with six of these and the performance of the complete AGM-system with two subjects.

A key feature of the AGM-system is that sampling from a peripheral vein is carried out using a single lumen catheter that is available in every hospital. Unlike other products which use double lumen catheters (DLC) the AGM-system can thus be used without the need for additional expenditure in this regard. Medical personnel are moreover well acquainted with such catheters whereas a DLC needs special handling, particularly if the inner lumen is inserted or removed. The performance of the ABS-system's catheter was improved fourfold by implementing a Keep Vein Open (KVO) saline infusion at a rate of $2\,\mathrm{ml\,h^{-1}}$. Despite this however, the peripheral venous catheter might represent a weak link in the system during long-term use due to coagulation. The use of a central venous or arterial catheter could avoid this and so improve the long-term stability of the system and would also be feasible in

intensive care patients with whom central catheters are commonly used.

An excellent degree of correlation was observed between glucose concentrations obtained with manually drawn reference samples and those taken with the ABS-system (median Pearson correlation coefficient 0.982, range 0.928–0.997, system error 1.66 ± 9.01%). Glucose values obtained fully automatically with the AGM-system also exhibited a very high degree of correlation with those obtained by conventional analysis of manually drawn blood samples (median Pearson correlation coefficient 0.950, range 0.936–0.963, system error 1.00 ± 6.61%). On the basis of these correlation data the AGM-system could in principle be used to drive an algorithm for insulin infusion and enable tight, fully automated glycaemic control.

The amount of blood consumed by the ABS-system was consistently low (1.69 ± 0.09 ml per withdrawing procedure). On the basis of a survey performed at the Medical University of Graz that calculated that the maximum amount of blood that can be withdrawn from patients in the intensive care unit (ICU) is 50 ml per day, this would allow on average hourly glucose measurements of ICU patients to be made. More frequent glucose determinations would be enabled by reducing the diameters and lengths of the system's disposable tubing system to reduce the amount of blood consumed per measurement.

A notable feature of the initial results obtained for subject 2 was that the glucose concentrations in the manually drawn reference samples were generally lower than those obtained with automatically sampled blood (see Fig. 5). This was found to be due to the location of the catheters for both manual and automatic blood withdrawal in the same forearm to allow patient's comfort and thus in the same venous plexus. At the beginning samples were taken with the ABS-system before manual withdrawal. We reasoned that because of this, the flushing fluid from the sampling unit was afterwards withdrawn with the manual reference samples, leading to sample dilution. This was confirmed by the finding that after synchronising the withdrawing and reinfusion procedure for both systems from time point 9 h onwards to avoid interaction between them, both system error and correlation improved (see Fig. 5). This modified procedure was used for all remaining subjects.

The clinically oriented and highly visual Clark Error Grid analysis is the most commonly used method for the comparison of glucose values. This revealed that 96.93% (221) of the data points obtained in the study were located in zone A (accurate treatment) and 3.07% (7) in zone B (acceptable treatment). The data were also analysed using Insulin Titration Error Grid analysis which confirmed the conclusion drawn from EGA: 99.56% (227) of the data pairs suggested an acceptable treatment whereas 0.44% (1) suggested an unacceptable violation. The latter data pair was one of those measured in subject 2 where the ABS-system biased the reference measurements by causing dilution of the corresponding manually withdrawn samples.

Taken together, our results thus demonstrate that the AGM-system would be suitable for use in a variety of clinical applications, in particular in ICUs to enable the frequent blood sampling necessary to maintain tight glycaemic control in critically ill hyperglycaemic patients, with significantly less staff effort than is currently required. Although many systems have already been introduced to the market, these have been found to be unsuitable for use in a clinical setting due to their long set-up time. The AGM-system, in contrast, could always be assembled in less than 5 min, which is comparable to the time required to set-up a standard infusion pump. The system moreover incorporates acoustic and optical alarms to alert staff to required interventions. Thus the time saved by the system is not offset by a demanding and extended set-up time and a high level of maintenance.

Finally, the constant APTT levels that were observed throughout the study confirm that the use of the AGM-system did not cause unintended systemic heparinisation and support its use in ICUs.

5. Conclusions

In conclusion, this study demonstrated the feasibility of automated real time ex vivo glucose determination. Following appropriate calibration the amperometric biosensor used to quantify glucose in blood, delivered glucose values closely matching those determined by standard analysis methods. Permanent flushing of the catheter reduced the number of occlusions fourfold. We view the peripheral catheter as the weakest link in the current system however and future work will examine its performance together with a central venous or arterial catheter. Biosensors from other manufacturers should also be examined. The amount of blood consumed by the system per sampling currently imposes a ceiling of on average hourly measurements in ICU patients. A reduction in the dimension of the disposable tubing system will decrease the amount of blood per withdrawal and so allow more frequent measurements.

The AGM-system presented here could be a valuable device in situations in which tight glycaemic control is required, especially in ICUs and/or after major surgery. It could also be a valuable research tool for clinical investigations requiring real time glucose monitoring.

Acknowledgements

Financial support from the European Commission under the CLINICIP project (contract no. 506965 within the 6th Framework program) is gratefully acknowledged.

Thanks to my love Michèle Ammann for supporting me.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.11.029.

References

Berghe, G., Wouters, P., Weekers, F., Verwaest, C., Bruyninckx, F., Schetz, M., Vlasselaers, D., Gerdinande, P., Lauwers, P., Bouillon, R., 2001. N. Engl. J. Med. 345, 1359–1367.
Berghe, G., 2002. Int. J. Obes. 26 (Suppl. 3), 3–8.
Bland, J.M., Altman, D.G., 1986. Lancet 327 (8476), 307–310.
Caduff, A., Hirt, E., Feldman, Y., Ali, Z., Heinemann, L., 2003. Biosens. Bioelectron. 19 (3), 209–217.
Clarke, W.L., Cox, D., Gonder-Frederick, L.A., Carter, W., Pohl, S.L., 1987. Diabetes Care 10 (5), 622–628.
Ellmerer, M., Haluzik, M., Blaha, J., Kremen, J., Svacina, S., Toller, W., Mader, J., Schaupp, L., Plank, J., Pieber, T.R., 2006. Diabetes Care 29 (6), 1275–1281.
Ganesh, A., Hipszer, B., Loomba, N., Simon, B., Torjman, M.C., Joseph, J., 2008. J. Diabetes Sci. Technol. 2 (2), 182–193.
Garg, S., Zisser, H., Schwartz, S., Bailey, T., Kaplan, R., Ellis, S., Jovanovic, L., 2006. Diabetes Care 29 (1), 44–50.
Gfrerer, R.J., Brunner, G.A., Trajanoski, Z., Schaupp, L., Sendlhofer, G., Skrabal, F., Jobst, G., Moser, I., Urban, G., Pieber, T.R., Wach, P., 1998. Biosens. Bioelectron. 13 (12), 1271–1278.
Heise, H.M., Damm, U., Bodenlenz, M., Kondepati, V.R., Köhler, G., Ellmerer, M., 2007. J. Biomed. Opt. 12 (2), 024004.
Hovorka, R., Canonico, V., Chassin, L.J., Haueter, U., Massi-Benedetti, M., Orsini-Federici, M., Pieber, T.R., Schaller, H., Schaupp, L., Vering, T., Wilinska, M.E., 2004. Physiol. Meas. 25 (4), 905–920.
Jungheim, K., Wientjes, K.J., Heinemann, L., Lodwig, V., Koschinsky, T., Schoonen, A.J., 2001. Diabetes Care 24 (12), 2030–2034.
Lodwig, V., Heinemann, L., 2003. Diabetes Technol. Ther. 5 (4), 573–587.
Mastrototaro, J., 1999. J. Pediatr. Endocrinol. Metab. 12 (Suppl. 3), 751–758.
McCowen, K.C., Malhotra, A., Bistrian, B.R., 2001. Crit. Care Clin. 17 (1), 107–124.
Ricci, F., Caprio, F., Poscia, A., Valgimigli, F., Messeri, D., Lepori, E., Dall'Oglio, G., Palleschia, G., Moscone, D., 2007. Biosens. Bioelectron. 22 (9–10), 2032–2039.

Tierney, M.J., Tamada, J.A., Potts, R.O., Jovanovic, L., Garg, S., 2001. Biosens. Bioelectron. 16 (9–12), 621–629.
Trajanoski, Z., Brunner, G.A., Schaupp, L., Ellmerer, M., Wach, P., Pieber, T.R., Kotanko, P., Skrabal, F., 1997. Diabetes Care 20 (7), 1114–1121.
Weinstein, R.L., Schwartz, S.L., Brazg, R.L., Bugler, J.R., Peyser, T.A., McGarraugh, G.V., 2007. Diabetes Care 30 (5), 1125–1130.
Weller, C., Linder, M., Macaulay, A., Ferrari, A., Kessler, G., 1960. Ann. NY Acad. Sci. 87, 658–668.

Publication II

“An Automated discontinuous venous blood sampling system for ex vivo glucose determination in humans”

Schaller R., Feichtner F., Köhler H., Bodenlenz M., Plank J., Wutte A., Mader J., Ellmerer M., Hainisch R., Pieber T.R., Schaupp L.A.

Journal of Diabetes Science and Technology, Vol. 3 (1), January 2009

Journal of Diabetes Science and Technology
Volume 3, Issue 1, January 2009

ORIGINAL ARTICLES

An Automated Discontinuous Venous Blood Sampling System for *Ex Vivo* Glucose Determination in Humans

Roland Schaller, M.Sc.,[1] Franz Feichtner, M.Sc.,[1] Hans Köhler, M.Sc.,[1] Manfred Bodenlenz, M.Sc.,[1] Johannes Plank, M.D.,[2] Andrea Wutte, M.Sc.,[2] Julia K. Mader, M.D.,[2] Martin Ellmerer, Ph.D.,[2] Reinhard Hainisch, M.Sc.,[3] Thomas R. Pieber, M.D.,[1,2] and Lukas Schaupp, Ph.D.[1,2]

Abstract

Background:

Intensive insulin therapy reduces mortality and morbidity in critically ill patients but places great demands on medical staff who must take frequent blood samples for the determination of glucose levels. A cost-effective solution to this resourcing problem could be provided by an effective and reliable automated blood sampling (ABS) system suitable for *ex vivo* glucose determination.

Method:

The primary study aim was to compare the performance of a prototype ABS system with a manual reference system over a 30 h sampling period under controlled conditions in humans. Two venous cannulae were inserted to connect the ABS system and the reference system. Blood samples were taken with both systems at 15, 30, and 60 min intervals and analyzed using a Beckman glucose analyzer. During the study, blood glucose levels were altered through four meal ingestions.

Results:

The median Pearson coefficient of correlation between manually and automatically withdrawn blood samples was 0.976 (0.953–0.996). The system error was -3.327 ± 5.546% (-6.03–0.49). Through Clark error grid analysis, 420 data pairs were analyzed, showing that 98.6% of the data were in zone A and 1.4% were in zone B. Insulin titration error grid analysis revealed an acceptable treatment in 100% of cases. A 17.5-fold reduction in the occurrence of blood-withdrawal failures through occluded catheters was moreover achieved by the added implementation in the ABS system of a "keep vein open" saline infusion.

Conclusions:

Our study showed that the ABS system described provides a user-friendly, reliable automated means for reproducible and accurate blood sampling from a peripheral vein for blood glucose determination and thus represents a promising alternative to frequent manual blood sampling.

J Diabetes Sci Technol 2009;3(1):110-116

Author Affiliations: [1]Institute of Medical Technologies and Health Management, Joanneum Research Forschungsgesellschaft mbH, Graz, Austria; [2]Department of Internal Medicine Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Graz, Austria; [3]Department of Social/Health, FH OOE Forschungs and Entwicklungs GmbH, Linz, Austria

Abbreviations: (ABS) automated blood sampling, (APTT) activated partial thromboplastin time, (CEGA) Clark error grid analysis, (CLINICIP) Closed Loop INsulin Infusion for Critically Ill Patients, (ICU) intensive care unit, (ITEGA) insulin titration error grid analysis, (KVO) keep vein open

Keywords: automated, blood, glucose, monitoring, sampling, *ex vivo*

Corresponding Author: Roland Schaller, M.Sc., Institute of Medical Technologies and Health Management, Joanneum Research Forschungsgesellschaft mbH, Elisabethstraße 11a, 8010 Graz, Austria; email address roland.schaller@joanneum.at

Introduction

Critically ill patients undergoing intensive medical treatment may develop insulin resistance associated with hyperglycemia even if they have no previous history of diabetes.[1] Intensive insulin therapy is very helpful for such patients as shown in a 12 month study involving 1548 critically ill surgical patients.[2] In this study, mortality was reduced by 42% by maintaining glucose levels between 4.4 and 6.1 mmol/liter. Morbidity, the consumption of intensive care resources, and the risk of complications were moreover also reduced.

To maintain euglycemic glucose levels, skilled medical staff must take frequent blood samples for glucose determination. In practice, this is often not feasible, however, due to the high workload associated with the care of critically ill patients and the high cost of monitoring. Manual sampling moreover introduces the possibility of handling errors and delay when using laboratory services. These factors may be associated with therapy errors or delays.

Automated real-time glucose monitoring would address these problems and, at the same time, provide valuable information concerning the direction and rate of change in blood glucose concentrations. This trend information could be used by a clinician or by an algorithm to titrate the delivery of insulin and glucose to maintain euglycemia with greater precision.

A number of systems using a double-lumen catheter to access peripheral veins with continuous flow have been developed.[3,4] This technology has been used to create glucose monitoring systems based on midinfrared spectroscopy.[5,6] These have, however, only found acceptance as tools for clinical studies. A blood glucose monitoring system for intensive care units (ICUs) using standard venous access has been introduced by Via Medical[7] but is not available in Europe.

As an alternative to blood analysis, glucose profiles can be determined in subcutaneous adipose tissue with needle-type sensors, such as Medtronic/MiniMed's CGMS Gold,[8] Abbott's Freestyle Navigator,[9] and Dexcom's STS sensor,[10] or with systems based on microdialysis[11] and open-flow microperfusion.[12]

Noninvasive, continuous iontophoresis-based systems, such as the Cygnus GlucoWatch[13] and impedance spectroscopy-based systems[14] have been launched but have achieved little market success.

An automated blood sampling (ABS) system coupled to one of the several validated glucose sensors[15] could provide a workable alternative to the mentioned systems as a means of avoiding frequent manual blood sampling, especially when ongoing control is required as in ICUs. A system of this type is, however, currently unavailable.

A low-risk decision supporting system comprising biosensors and an adaptive control algorithm for the maintenance of glycemic control of ICU patients was developed within the European Union-funded project, Closed Loop INsulin Infusion for Critically Ill Patients (CLINICIP, www.clinicip.org).[16] Our aim within this overall project was to test a prototype ABS system over a 30 h period in human volunteers to determine its technical and clinical suitability for incorporation into the CLINICIP setup. Blood glucose concentrations obtained with automatically sampled blood were compared to those obtained with blood taken in parallel using an established manual sampling method. In the course of this study, setup times, success rate, flushing volume, and blood consumption were also determined.

Methods

Reference Method and Automated Blood Sampling System

The reference method used in the study to obtain manually drawn peripheral venous blood samples used a conventional single-lumen catheter (18 gauge x 45 mm, B. Braun, Melsungen, Germany), two stopcocks, and two syringes. Clotting was avoided at the catheter tip by flushing with physiological saline at a very low flow rate. To ensure that saline-free, undiluted blood was sampled, flushing was stopped 10 min prior to each sampling. In each case, a total blood volume of 0.5 ml was drawn, and approximately 300 µl was transferred to a vial for analysis (PCR Softtubes, Biozym, Oldendorf, Germany).

The ABS system mimics this manual state-of-the-art technique (**Figure 1**). Before connecting it to the subjects via a second catheter, it was flushed with sterile 0.9% saline solution (Fresenius, Fresenius Kabi, Graz, Austria) to remove air. For each sampling, a blood volume of 4 ml was withdrawn by a peristaltic pump (Minipuls MP3, Gilson, Cedex, France) at a flow rate of 10 ml/min and collected in a buffer loop. Then 1.69 ± 0.09 ml of this drawn blood was immediately branched off, and approximately 1 ml was pumped into the waste container

before collecting samples (0.303 ± 0.043 ml) in a vial of the same type used for manually drawn blood for analysis. The remaining blood in the buffer loop (approximately 2.3 ml) was then immediately reinfused into the subject so that the actual blood consumption of the ABS system was 1.69 ± 0.09 ml per sample. We previously found this procedure to ensure that undiluted blood, i.e., not containing saline, is collected (unpublished data), which can thus be directly compared to blood drawn manually as described earlier. Following sampling with the ABS system, 8.83 ± 0.28 ml of flushing fluid was pumped through the tubing to clean it.

The in-house assembled tubing system (TYGON® S-50-HL, Saint-Gobain Performance Plastics, Beaverton, France) was sterilized using ethylene oxide and afterward was completely flushed using sterile 0.9% saline solution to prevent clotting. The sterile saline solution was attached to the system using a standard fluid administration set (Infusiomatleitung, B. Braun), and its fluid level was monitored via a dripping sensor (Type: 3450578 A, B. Braun). Flow (see arrows in **Figure 1**) was controlled using pinch valves (PM-0815W, Takasago Electric, Inc., Japan) compatible with the tubing used (TYGON). A check valve (Part No. 11582, Qosina, Edgewood, NY) was used to prevent reflux and to maintain the sterility of the saline solution. The dead space of the system from the IV catheter to the sampling site was 0.75 ml. For safety reasons, pressure changes within the accessed vessel were monitored using a reusable pressure transducer and a disposable pressure dome (SP854, Memscap, Norway). The maximum allowed pressure was set at 150 kPa. In addition to this, an air-bubble sensor (customized sensor, Zevex, Salt Lake City, UT) was used to avoid accidental air infusion. The system's housing was designed in Solid Works (Solid Works, Concord, MA) and rapidly prototyped (FH OOE, Linz, Austria). All materials used (e.g., tubing) had been previously approved for *in vivo* use in humans.

Data Acquisition and System Control

The data acquisition system comprised a notebook computer, a 16-bit PCMCIA card (DAQ-Card 6036-E, National Instruments, Inc., Austin, TX), a configurable connector (SC-2345, National Instruments, Inc.), and Lab VIEW® 7.0 (National Instruments, Inc.). Data were stored in Microsoft Excel worksheet files (Microsoft, Inc., Redmond, WA).

Subjects and Protocols

The study, a 30 h open single-center trial at the Medical University of Graz, was approved by the local ethics committee (18-011 ex 06/07), and written consent (signed and dated) was obtained from all participants before commencement of the study. Six healthy male volunteers (age: 28.17 ± 2.04 years; body mass index: 23.00 ± 2.41 kg/m) that passed the inclusion and exclusion criteria were recruited. Eligible subjects were between 19 and 60 years in age. Exclusion criteria were the presence of severe acute and chronic diseases, mental illness, lack of cooperation or language barriers precluding adequate understanding or cooperation, prescription of vasoactive substances or medication for anticoagulation or medication that interferes with coagulation, blood donation within the previous 4 weeks, skin diseases that could interfere with catheter placement, pregnancy, and breastfeeding.

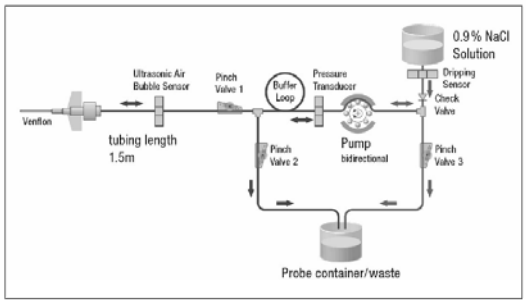


Figure 1. Schematic of the ABS system. A peristaltic pump withdraws and reinfuses peripheral venous blood. Physiological saline solution flushes the system. A Beckman glucose analyzer was used for glucose determination.

The study subjects attended the trial center at 11:00 AM on Day 1, having fasted from at least midnight onward (i.e., for at least 11 h). The sampling systems were connected by a physician by inserting the catheters into pronounced veins in the left forearm. Both catheters were inserted into the left arm to allow the patient to move freely throughout the study period.

The subjects received four meals at 5:00 PM (dinner), 9:00 PM (snack), 8:00 AM (breakfast), and 12:00 PM (lunch) to enable the observation of their pre- and postprandial glucose concentration profiles. Blood samples were generally taken at hourly intervals. After each meal, however, the frequency of sampling (manual and automatic) was increased to four samples per hour for 3 h. Thereafter, two samples were taken at 30 min intervals before resumption of hourly monitoring. A further seven blood samples were also taken throughout the study for the determination of activated partial thromboplastin times (APTTs). The expected blood consumption was 187.7 ml per volunteer.

Laboratory Analysis and Statistics

Manually and automatically drawn blood samples were analyzed using a laboratory glucose analyzer (Glucose Analyzers 2, Beckman Coulter, Brea, CA). In order to be able to perform a valid comparison of the results obtained with manually and automatically drawn blood, preanalytical variables were controlled to as great an extent as possible. Thus, undiluted samples (see *Reference Method and Automated Blood Sampling System* in the *Methods Section* of this article) obtained with the ABS system and the reference method were collected in parallel into the same type of vials, in both cases, without the use of additives (e.g., anticoagulants or clot activators), and immediately centrifuged to prepare plasma prior to analysis using the Beckmann glucose determination device. This process was completed for each sample pair within 5 min. Sample processing was moreover carried out directly in the subject room and exclusively by qualified study assistants trained according to good laboratory practice.

The relationship between the glucose concentrations determined for the automatically and manually withdrawn blood samples was assessed by calculating the Pearson coefficient of correlation,[17] the system error,[18] and the mean absolute relative difference[19] for each sample pair. The degree of clinical correlation was assessed using Clark error grid analysis (CEGA)[20] and insulin titration error grid analysis (ITEGA).[21] All calculations were made on a personal computer using Microsoft Excel worksheet files.

Results

General

All six volunteers were successfully studied for the full planned duration of the study (30 h) and the catheters were well tolerated. Manual interventions were necessary for both the reference method and the ABS system to prevent catheter occlusion (e.g., additional flushing, movement, or replacement). For the first subject studied, a total of seven such manual interventions were necessary for the ABS system. These events generally occurred whilst drawing hourly samples during the night. For Subjects 2 to 6, we therefore implemented a keep-vein-open (KVO) saline infusion with the ABS system at a rate of 2 ml/h. This highly effectively reduced the incidence of blood withdrawal failures from 9.86% for Subject 1 (7 interventions for 71 samples) to an average failure rate of 0.56% for Subjects 2–6 (2 interventions for 355 samples), i.e., a 17.5-fold reduction in the frequency of interventions. The median success rate of the system was 99.30% (range: 90.14–100.00%). Overall, 6 of 426 data pairs were incomplete or invalid, principally due to catheter blockage caused by coagulation, insufficient sample volume, or hemolytic samples, resulting in a total of 420 valid samples. The measured glucose concentrations for these samples ranged from 3.75 to 13.88 mmol/liter.

Correlation

The median Pearson coefficient of correlation between manually and automatically withdrawn blood samples was 0.976 (range: 0.953–0.996). An illustrative glucose concentration profile comparing the results obtained with the ABS system (crosses) to those with the reference system (squares) is depicted in **Figure 2**.

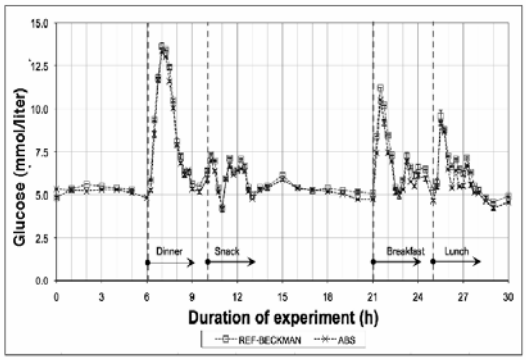


Figure 2. Illustrative glucose concentration profile for automatically (crosses) and manually (squares) withdrawn reference blood samples obtained during one experiment over 30 h. The subjects received four regular meal ingestions after 6 (dinner), 10 (snack), 21 (breakfast), and 25 hours (lunch) to enable observation of their pre- and postprandial glucose concentration profiles.

System Error and Mean Absolute Relative Difference

Figure 3 shows the system error for each data pair from Subjects 1 to 6 (crosses). In addition, the mean (squares) ± standard deviations (bars) are displayed for each subject. The calculated system error between the ABS system and the manual reference measurements was -3.327 ± 5.546% [range: -6.03–0.49]. Individual system errors ranged from 24.14 to -29.01%. Mean absolute relative difference was 4.84 ± 1.46% (range: 2.80–6.29%).

System Error over Time

Figure 4 displays the individual system errors for each time point and for all subjects. As a measure of system stability, the regression line was calculated (y = -0.1089 x -1.5357). Starting with a slight dilution of -1.54%, the dilution increased until the end of the experiment to a final system error of -4.80%.

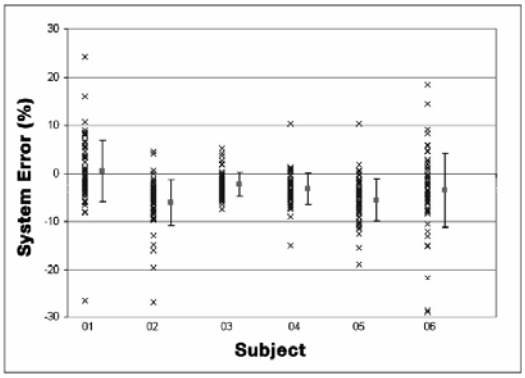


Figure 3. Error graph for Subjects 1 to 6. Crosses indicate data pairs between the reference method and the ABS system. Bars indicate the standard deviations, and squares display the mean values.

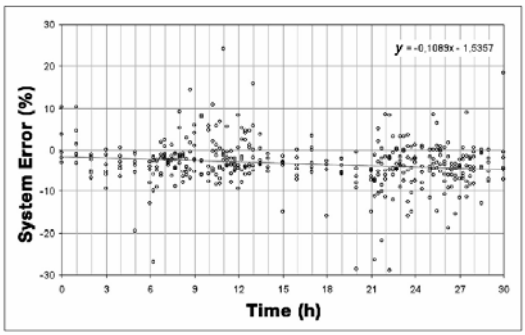


Figure 4. System error over time for Subjects 1 to 6 plus the equation of the regression line as a measure of system stability.

Clark Error Grid Analysis

Figure 5 depicts the CEGA, a clinically oriented approach to evaluating glucose data and thus the clinical significance of the degree of accuracy of the ABS system. Clark error grid analysis takes into account the absolute values of automatically generated (ordinate) and reference (abscissa) glucose measurements, the relative difference between these two values, and the clinical significance of this difference. Clark error grid analysis categorizes individual reference glucose values into one of five zones, each of which represents a different degree of clinical treatment appropriateness. These zones are A (accurate), B (acceptable), C, D, and E (not acceptable). The analysis of the 420 valid data pairs via CEGA showed that 98.6% (414) of the data were in zone A and only 1.4% (6) were in zone B (crosses).

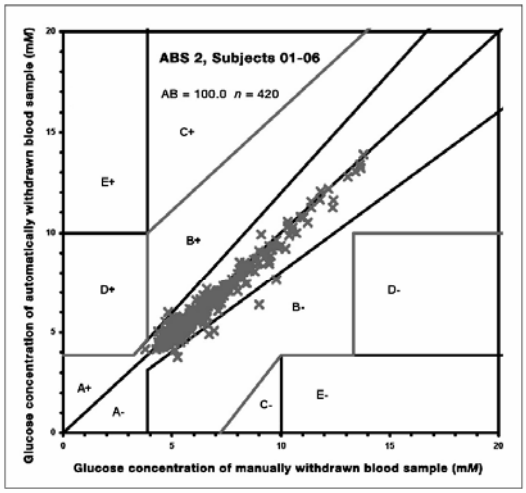


Figure 5. Clark error grid analysis comparing the data obtained using the ABS system to that of the reference method (Subjects 1–6: crosses), 420 data pairs, Zone A (98.6%, 414), and Zone B (1.4%, 6).

Insulin Titration Error Grid Analysis

The clinical significance of glucose measurement differences can also be assessed by ITEGA. Blood glucose concentrations of reference samples and automatically withdrawn samples are displayed on the x and y axis, respectively. Glucose ranges on x and y axis are related to major treatment actions as suggested by the Leuven insulin titration guideline.[22] Based on these treatment actions, different zones have been defined as "appropriate treatment," "unacceptable violation," "major violation," and "life threatening treatment," according to the extent to which they violate the titration guidelines. In our study, 100% of the 420 valid data pairs were located in the acceptable treatment zone.

Coagulation Disturbance and Adverse Events

No statistically significant increase in APTT levels was found in any of the subjects ($p = 0.05$) compared to the initial levels, providing good evidence that the coagulation status of the subjects was not deleteriously affected by the saline infusion used to clean the disposable tubing system and to maintain catheter patency. Furthermore, no adverse trial-related events occurred during the study.

Discussion and Conclusions

Intensive insulin therapy has been shown to benefit critically ill patients but requires frequent blood

sampling, which can conflict with other aspects of patient care. We present a controlled clinical evaluation of a prototype ABS device. The results of our study allow the following conclusions to be drawn concerning the reliability of the device (degree of intervention-free operation) and the degree of correlation between blood glucose concentrations determined with automatically and manually withdrawn blood.

The ABS system was found to be extremely reliable. The median success rate for the entire study was 99.30% (range: 90.14–100.00%). This overall value, however, disguises the fact that a higher rate of intervention was initially observed with the first subject (9.86%). After implementation of a KVO saline infusion of 2 ml/h^{-1} for the remaining 5 subjects, the success rate was close to 100% (99.44%), a 17.5-fold improvement. This is clearly very high in absolute terms. We are unaware of a similar system elsewhere and are thus unable, at this point, to comment on the relative reliability of the ABS system.

The amount of blood consumed by the automated sampling unit was consistently low (1.69 ± 0.09 ml per withdrawing procedure). According to a survey performed at the Medical University of Graz, the maximum amount of blood that can be safely withdrawn from ICU patients per day is 50 ml. On this basis, on average, hourly glucose measurements of ICU patients could be made using the current ABS system. There is, moreover, scope to enable more frequent sampling to reduce the amount of blood consumed by the system by reducing the diameters and lengths of the system's disposable tubing.

An excellent degree of correlation was observed between glucose concentrations obtained with manually drawn reference samples and those taken with the automatic sampling device (median Pearson correlation coefficient 0.976 [range: 0.953–0.996]). The average system error of the ABS system was relatively low, with values of -3.327 ± 5.546% (range: -6.03–0.49) compared to the reference measurements. The system error was moreover relatively stable over the whole trial period (y = -0.1089 x -1.5357). Mean absolute relative difference was 4.84 ± 1.46% with a range of 2.8–6.29.

The clinically oriented, highly visual CEGA method revealed that 98.6% (414) of the data points obtained in the study were located in zone A (accurate treatment) and just 1.4% (6) were in zone B (acceptable treatment). Analysis of the data using ITEGA confirmed the conclusion drawn from CEGA: 100% (420) of the data pairs suggested an acceptable treatment.

A common feature of other systems for semi or fully automated glucose determination is a lack of suitability for use in a clinical setting, due either to a long setup time or unexpected maintenance steps (or both). The setting-up of the ABS system, including assembly of tubing, flushing, and connection to the patient, was, in marked contrast, always performed in less than 5 min, which, based on our experience in a routine hospital setting, is comparable to that required to setup and connect a standard infusion pump. This and the operational reliability shown by our results provide strong support for significant time savings for medical staff in a clinical setting that could be used to focus on patient care.

In conclusion, we have demonstrated that the ABS system has the potential to replace manual blood sampling for glucose determination. This would be particularly valuable in ICUs in which frequent blood glucose determination to maintain tight glycemic control has been shown to benefit critically ill patients that develop hyperglycemia and insulin resistance, but which is hard to perform using the limited available staff. Further clinical studies under ICU conditions and with diabetes patients would be necessary to confirm this. Fully automated blood glucose determination could, in principle, be achieved by online coupling of a glucose sensor or other glucose analyzer. Looking further ahead, such a system could potentially be used to drive an algorithm for insulin infusion and enable fully automated real-time glycemic control.

Funding:

We received financial support from the European Commission under the CLINICIP project (contract 506965 within the 6th Framework program).

Acknowledgments:

This study was conducted in the Clinical Research Centre of the Medical University of Graz. Special thanks go to our collaborators and physicians who were on duty during the study: S. Korsatko, H. Weinhandl, C. Pachler, D. Ikeoka, R. Kulnik, and G. Bock. Graphics were done by B. Trübswasser..

References:

1. McCowen KC, Malhotra A, Bistrian BR. Stress-induced hyperglycemia. Crit Care Clin. 2001;17(1):107–24.
2. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in critically ill patients. N Engl J Med. 2001;345(19):1359–67.
3. Weller C, Linder M, Macaulay A, Ferrari A, Kessler G. Continuous *in vivo* determination of blood glucose in human subjects. Ann N Y Acad Sci. 1960;87:658–68.

4. Gfrerer RJ, Brunner GA, Trajanoski Z, Schaupp L, Sendlhofer G, Skrabal F, Jobst G, Moser I, Urban G, Pieber TR, Wach P. Novel system for real-time *ex vivo* lactate monitoring in human whole blood. Biosens Bioelectron. 1998;13(12):1271–8.
5. Heise HM, Damm U, Bodenlenz M, Kondepati VR, Köhler G, Ellmerer M. Bedside monitoring of subcutaneous interstitial glucose in healthy individuals using microdialysis and infrared spectrometry. J Biomed Opt. 2007;12(2):024004.
6. Heise HM, Kondepati VR, Damm U, Licht M, Feichtner F, Mader JM, Ellmerer M. Microdialysis based monitoring of subcutaneous interstitial and venous blood glucose in type 1 diabetic subjects by mid-infrared spectrometry for intensive insulin therapy. Optical Diagnostics Sensing VIII. 2008;6863:686308.
7. Ganesh A, Hipszer B, Loomba N, Simon B, Torjman MC, Joseph J. Evaluation of the VIA blood chemistry monitor for glucose in healthy and diabetic volunteers. J Diabetes Sci Technol. 2008;2(2):182–193.
8. Boyne MS, Silver DM, Kaplan J, Saudek CD. Timing of changes in interstitial and venous blood glucose measured with a continuous subcutaneous glucose sensor. Diabetes. 2003;52(11):2790–4.
9. Weinstein RL, Schwartz SL, Brazg RL, Bugler JR, Peyser TA, McGarraugh GV. Accuracy of the 5-day FreeStyle Navigator continuous glucose monitoring system: comparison with frequent laboratory reference measurements. Diabetes Care. 2007;30(5):1125–30.
10. Garg S, Zisser H, Schwartz S, Bailey T, Kaplan R, Ellis S, Jovanovic L. Improvement in glycemic excursions with a transcutaneous, real-time continuous glucose sensor: a randomized controlled trial. Diabetes Care. 2006;29(1):44–50.
11. Dempsey E, Diamond D, Smyth MR, Malone MA, Rabenstein K, McShane A, McKenna M, Keaveny TV, Freaney R. *In vitro* optimisation of a microdialysis system with potential for on-line monitoring of lactate and glucose in biological samples. Analyst. 1997;122(2):185–9.
12. Trajanoski Z, Brunner GA, Schaupp L, Ellmerer M, Wach P, Pieber TR, Kotanko P, Skrabal F. Open-flow microperfusion of subcutaneous adipose tissue for on-line continuous *ex vivo* measurement of glucose concentration. Diabetes Care. 1997;20(7):1114–21.
13. Tierney MJ, Tamada JA, Potts RO, Jovanovic L, Garg S, Cygnus Research Team. Clinical evaluation of the GlucoWatch biographer: a continual, non-invasive glucose monitor for patients with diabetes. Biosens Bioelectron. 2001;16(9-12):621–9.
14. Caduff A, Hirt E, Feldman Y, Ali Z, Heinemann L. First human experiments with a novel non-invasive, non-optical continuous glucose monitoring system. Biosens Bioelectron. 2003;19(3):209–17.
15. Ricci F, Caprio F, Poscia A, Valgimigli F, Messeri D, Lepori E, Dall'Oglio G, Palleschia G, Moscone D. Toward continuous glucose monitoring with planar modified biosensors and microdialysis. Study of temperature, oxygen dependence and *in vivo* experiment. Biosens Bioelectron. 2007;22(9-10):2032–9.
16. Plank J, Blaha J, Cordingley J, Wilinska ME, Chassin LJ, Morgan C, Squire S, Haluzik M, Kremen J, Svacina S, Toller W, Plasnik A, Ellmerer M, Hovorka R, Pieber TR. Multicentric, randomized, controlled trial to evaluate blood glucose control by the model predictive control algorithm versus routine glucose management protocols in intensive care unit patients. Diabetes Care. 2006;29(2):271–6.
17. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307–10.
18. Lodwig V, Heinemann L, Glucose Monitoring Study Group. Continuous glucose monitoring with glucose sensors: calibration and assessment criteria. Diabetes Technol Ther. 2003;5(4):572–86.
19. Wenthold IM, Hart AA, Hoekstra JB, Devries JH. How to assess and compare the accuracy of continuous glucose monitors? Diabetes Technol Ther. 2008;10(2):57–68.
20. Clarke WL, Cox D, Gonder-Frederick LA, Carter W, Pohl SL. Evaluating clinical accuracy of systems for self-monitoring of blood glucose. Diabetes Care. 1987;10(5):622–8.
21. Ellmerer M, Haluzik M, Blaha J, Kremen J, Svacina S, Toller W, Mader J, Schaupp L, Plank J, Pieber T. Clinical evaluation of alternative-site glucose measurements in patients after major cardiac surgery. Diabetes Care. 2006;29(6):1275–81.
22. Van den Berghe G. Beyond diabetes: saving lives with insulin in the ICU. Int J Obes Relat Metab Disord. 2002;26 Suppl 3:S3–8.

Publication III

“Microdialysis based device for continuous extravascular monitoring of blood glucose“

Feichtner F., Schaller R., Fercher A., Ratzer M., Ellmerer M., Plank J., Krause B., Pieber T.R., Schaupp L.A.
.

Biomedical Microdevices; published online 26. January 2010

Biomed Microdevices
DOI 10.1007/s10544-010-9396-3

Microdialysis based device for continuous extravascular monitoring of blood glucose

Franz Feichtner · Roland Schaller · Andreas Fercher · Maria Ratzer · Martin Ellmerer · Johannes Plank · Bernd Krause · Thomas Pieber · Lukas Schaupp

Abstract Glycemic control of intensive care patients can be beneficial for this patient group but the continuous determination of their glucose concentration is challenging. Current continuous glucose monitoring systems based on the measurement of interstitial fluid glucose concentration struggle with sensitivity losses, resulting from biofouling or inflammation reactions. Their use as decision support systems for the therapeutic treatment is moreover hampered by physiological time delays as well as gradients in glucose concentration between plasma and interstitial fluid. To overcome these drawbacks, we developed and clinically evaluated a system based on microdialysis of whole blood. Venous blood is heparinised at the tip of a double lumen catheter and pumped through a membrane based microfluidic device where protein-free microdialysate samples are extracted. Glucose recovery as an indicator of long term stability was studied *in vitro* with heparinised bovine blood and remained highly stable for 72 h. Clinical performance was tested in a clinical trial in eight healthy volunteers undergoing an oral glucose tolerance test. Glucose concentrations of the new system and the reference method correlated at a level of 0.96 and their mean relative difference was 1.9±11.2%. Clinical evaluation using Clark's Error Grid analysis revealed that the obtained glucose concentrations were accurate and clinically acceptable in 99.6% of all cases. In conclusion, results of the technical and clinical evaluation suggest that the presented device delivers microdialysate samples suitable for accurate and long term stable continuous glucose monitoring in blood.

Keywords Microdialysis · Sampling · Glucose monitoring · Extravascular · *In vitro* study · Clinical trial

F. Feichtner (✉) · R. Schaller · A. Fercher · M. Ratzer · T. Pieber · L. Schaupp
Institute of Medical Technologies and Health Management, Joanneum Research GmbH,
Elisabethstrasse 11a,
8010 Graz, Austria
e-mail: franz.feichtner@joanneum.at

M. Ellmerer · J. Plank · T. Pieber · L. Schaupp
Division of Endocrinology and Nuclear Medicine, Department of Internal Medicine, Medical University of Graz,
Auenbruggerplatz 15, 8036, Graz, Austria

B. Krause
Gambro Dialysatoren GmbH,
Holger-Crafoord-Straße 26,
72379 Hechingen, Germany

Published online: 26 January 2010

1 Introduction

Tight glycemic control of critically ill patients was shown to substantially reduce mortality and morbidity in this patient group (van den Berghe et al. 2001) and is associated with substantial reduction in medical care cost (van den Berghe et al. 2006). Thus, the EU funded project CLINICIP (Closed Loop Insulin Infusion for Critically Ill Patients) aimed to develop a *continuous* low-risk monitoring and control device which allows maintaining metabolic control in intensive care patients. Our task in this project was to develop the very first part of such a glycemic control loop, the continuous glucose monitoring system and to evaluate it technically and clinically.

Most current glucose monitoring systems for diabetic (Skyler 2009) and intensive care patients (Kondepati and Heise 2007) are based on sensing in the extra cellular fluid (ECF) of the subcutaneous adipose tissue. However, none of these systems may be used as a decision support system for therapeutic treatment. Additionally, various studies

calculated or measured a physiological time lag between the systemic blood glucose concentration and the glucose concentration of ECF (Regittnig et al. 2003: ~28 min; Roe and Smoller 1998: 0–45 min, average lag 8–10 min; Kulcu et al. 2003: 5 min in average in diabetic subjects). Furthermore, a gradient between interstitial and plasma glucose concentrations was reported, that varied between 20% (Sternberg et al. 1996) and 110% (Bantle and Thomas 1997). Bad correlation between blood and ECF glucose concentration was also found in critically ill children (Vlasselaers et al. 2007) and critically ill patients with severe traumatic brain injuries (Lourido et al. 2002). From a therapeutic point of view it is therefore obvious not to access ECF but blood for continuous glucose monitoring in intensive care patients, moreover, because blood access is available in these patients anyway.

Years before the van den Berghe study (van den Berghe et al. 2001) initialised the debate about tight glycemic control of intensive care patients, Stjernstrom and co-workers measured different metabolites, including glucose, in intensive care patients accessing the patients intravenously. They first used a technique of intravenous microdialysis (Stjernstrom et al. 1993), but only a few publications arose since then repeating this technique in humans (O'Connell et al. 1996; Patsalos et al. 1996; Paez and Hernandez 1997; Castejon et al. 1999; Costa et al. 1999; Dizdar et al. 1999a, b; Elshoff and Laer 2005). A reason for this small number of studies might be the risks associated with implanting a fragile membrane into the vascular system as such probes were hand-made and non-commercially available. What's more, decreasing microdialysis efficiencies of membranes were repeatedly reported to occur *in vitro* and *in vivo* shortly after probe implantation (Yokel et al. 1992; Chen and Steger 1993; Yang et al. 1997; Verbeeck 2000) even in heparinised blood (Sauernheimer et al. 1994), which might be due to clot formation around intravenously implanted probes (De Lange et al. 2000). However, in the meantime CMA Microdialysis AB has launched a product for intravenous microdialysis, the IView, but there are no published data available yet.

As solution for the limitations mentioned above we decided to develop a device that *continuously* withdraws heparinised blood from a peripheral vein to an *ex vivo* microdialyser. Thereby we are able to combine various advantages of current state-of-the-art techniques. Direct access to blood enables to *continuously* monitor the systemic glucose concentration and performing microdialysis outside the patient is safer than placing a fragile membrane into the vascular system. To overcome the described drawback of decreasing efficiencies of microdialysis membranes that are exposed to blood, we developed also a new membrane with a very "smooth" blood contacting surface, onto which blood clot formations are very unlikely to adhere. The membrane is thus very long-term stable with respect to diffusive transport properties.

In this paper, we report on the design, the technical and the clinical validation of this microdialysis based device for continuous extravascular monitoring of blood glucose.

2 Materials and methods

2.1 Continuous blood sampling

A double lumen catheter (DLC; mtb GmbH, Lonsee, Germany) is used to continuously withdraw blood from a peripheral vein using a Gilson Minipuls MP3 peristaltic pump (Gilson, Cedex, France). To prevent coagulation a NaCl-Heparin solution (50 IU/ml) is delivered to the tip of the catheter via the outer lumen of the DLC using another Gilson Minipuls 3 peristaltic pump at a flow rate of 2 ml/h. Anti-coagulated blood is withdrawn simultaneously through the inner lumen of the DLC at a flow rate of 4 ml/h. This well-established technique is described in detail elsewhere (Weller et al. 1960) and has been used in blood sampling and blood glucose analysers such as the Biostator and the Glucostator, respectively. Anti-coagulated blood is pumped through the planar flow-through microdialysis (PFTMD) device and is collected in 30 µl samples for glucose analysis. Pumps are PC-controlled using Lab VIEW® 7.0 software on a notebook and a NI 9263 4-Channel 16-Bit Analog Voltage Output Module (all from National Instruments, Inc., Austin, TX, USA). Backflux of potentially contaminated blood from the waste compartment is prevented by using peristaltic pumps unidirectionally.

2.2 Microdialysis

Microdialysis (MD) is a technique used to extract components of the ECF via a semi-permeable membrane, which goes back to the pioneering work of Ungerstedt (Ungerstedt 1991). Molecules below the membrane's molecular weight cut-off diffuse through the membrane to an analyte-free aqueous solution (perfusate), which is pumped through the microdialysis device. The analyte concentration reached in the aqueous solution (dialysate) is related to the concentration in the extra-cellular fluid and depends on parameters including flow rate and temperature of both liquids, the analyte's molecular weight, its charge and the membrane's surface area. Important to know is that analyte concentrations in microdialysis samples do not fully equilibrate with the surrounding tissue unless inapplicably low perfusion flow rates (~1 µl/min) are chosen (Rosdahl et al. 1998; Ekberg et al. 2005). Thus MD data always require calibration procedures.

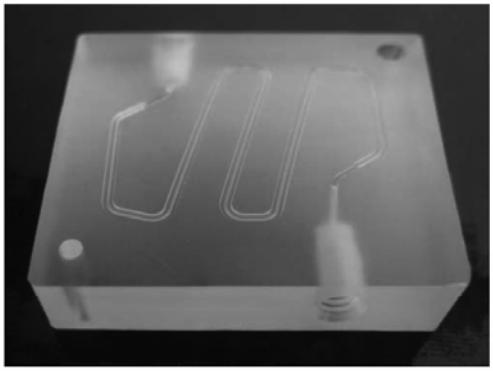


Fig. 1 One of two microfluidic plates with engraved microfluidic channels (8 µl): Perfusate or blood is connected to the plate via HPLC screw connectors. A semipermeable membrane is sandwiched between two of these plates. Analytes below the membrane's molecular weight cut off diffuse from one side of the plate to the other. Analyte enriched perfusate (=dialysate) is collected for glucose analysis

2.3 The planar flow-through microdialysis device (PFTMD)

The PFTMD consists of two polycarbonate plates (37×33×8 mm, Makrolon Rx-1805 from Bayer AG, Leverkusen, Germany) that sandwich a semi-permeable membrane in-between (Fig. 1). On the plate's surfaces meander-like microfluidic channels are engraved with a volume of 8 µl each. The first plate is connected to the DLC with TYGON® tubing (S-50-HL, ID=0.25 mm, OD=0.75 mm, l=1,500 mm, Saint-Gobain Performance Plastics, Beaverton, France) using re-usable custom made HPLC screw-connectors and perfused with heparinised blood. The other plate is connected to a 5% Mannitol solution (Fresenius Kabi, Graz, Austria) with TYGON® tubing (R-3606, ID=0.19 mm, OD=2.01 mm) and is perfused countercurrently.

The PFTMD is a multi-use device. Its polycarbonate plates meet biocompatibility criteria according to ISO 10993-1 standard. Plates and connectors were steam-sterilised at 121°C for 20 min before each experiment. Tubing and membrane are single-use only and were EtO sterilised before use. A schematic illustration of the complete system including the continuous blood sampling system is shown in Fig. 2.

2.4 Membrane

Custom made high-porous hydrophilic PAES flat sheet membranes (Gambro, Hechingen, Germany, molecular weight cut-off=10 kDa, thickness=60 µm, liquid permeability=2–3×10 $^{-4}$ cm³/(cm² sec bar)) were prepared by phase separation. A polymer solution was formed by dissolving polyethersulfone (Ultrason 6020, BASF, Ludwigshafen, Germany) and polyvinylpyrrolidone (K30 and K90, BASF) in N-Methylpyrrolidone (NMP). The viscosity of the resulting polymer solution was 5,000 mPa·s. The final degassed homogeneous polymer solution was casted onto a smooth glass plate using an Erichsen Coatmaster 5097 MC-I and a coating knife (gap height 100 µm) at a casting speed of 12.5 mm/s at a temperature of 50°C. The glass plates were immediately immersed into a coagulation bath containing a mixture of NMP and water. The membranes formed were washed with water and dried at 60°C.

The custom made membrane combines high diffusive and controlled convective transport characteristics. Its selective layer is on the blood contacting side with selective pore diameters between 2–10 nm, building an extremely "smooth" blood contacting surface (compare Fig. 3), which reduces the probability of cell and protein adhesion to the membrane surface and thus prevents a loss in dialysis efficiency.

2.5 Pre-clinical experiments

Five PFTMDs were tested *in vitro* in 72 h experiments to find optimum operating conditions with respect to achieve high relative recovery while having short transport delay times. Tests were performed using anticoagulated, glycolysis-inhibited and temperature controlled (37°C) bovine blood as test matrix (anticoagulation: 500 mg potassium oxalate per 1,000 ml blood, glycolysis-inhibition: 750 mg sodium fluoride per 1,000 ml blood) and 5% Mannitol as perfusate. Bovine blood flow rate was fixed at 4 ml/hour, whereas perfusate flow rate was varied between 2, 3, 5 and 10 µl/min.

Reference blood samples (100 µl) were taken directly from the blood pool in hourly intervals. Continuously withdrawn blood samples were collected at the outflow of the PFTMD in 100 µl fractions. Both were centrifuged and supernatant plasma was collected for glucose analysis. Dialysate samples were collected at the dialysate outflow of the PFTMD in 25 µl fractions. All samples were frozen at −80°C for subsequent glucose analysis with a Roche Cobas Mira analyser using Cobas Gluco-quant and Glucose/Hexokinase (Roche Diagnostics GmbH, Mannheim, Germany).

2.6 Clinical investigations

After optimal operating conditions were found in the pre-clinical experiments a technical and clinical evaluation of the PFTMD was performed in a 12 h open mono-centre clinical feasibility trial in eight healthy, non-diabetic volunteers (7 males, 1 female; age: 28.9±3.5 years; BMI: 25.1±1.5 kg/m²). The study was performed according to

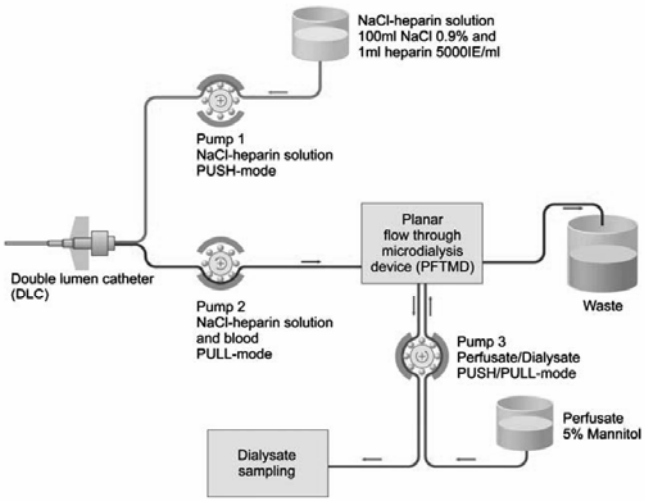


Fig. 2 Schematic illustration of the microdialysis based device for continuous extravascular monitoring of blood glucose. A NaCl heparin solution is pumped to the tip of a double lumen catheter and anti-coagulated blood is continuously withdrawn and pumped to a planar flow through microdialysis device that sandwiches a membrane between two microfluidic plates in a clamp-device. 5% Mannitol as perfusate is delivered to the second microfluidic plate to obtain protein-free dialysate for analysis

Good Clinical Practice (GCP) guidelines at the Clinical Research Centre located at the Medical University of Graz. Ethical approval was obtained from the local ethical committee. Signed informed consent was obtained from each subject before any trial related activities.

2.6.1 Study protocol

Subjects arrived in the morning of the trial in a fasting condition. A peripheral 20 Gauge venous catheter (CODAN pvb Medical GmbH, Lensahn, Germany) and the DLC were applied at the Vena mediana cubiti of the right and the left arm of the subjects for reference and continuous blood sampling, respectively. The DLC was connected to the PFTMD and continuous blood sampling was performed as described above. After this setup procedure the study protocol started (sampling interval: 30 min).

Five hours after the trial start an Oral Glucose Tolerance Test (OGTT) was performed. 75 g of glucose were dissolved in 250 ml of water and were given orally to the subjects. After

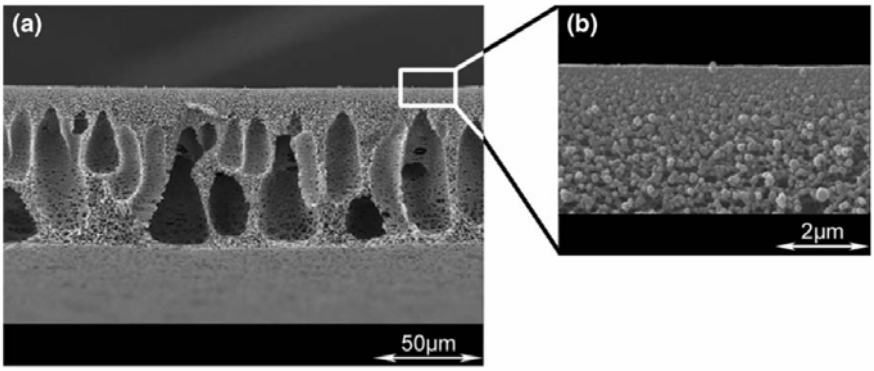


Fig. 3 Scanning Electron Micrographs of the cross section of a hydrophilic microdialysis membrane: (**a**) Whole cross section of the flat sheet membrane (Magnification: 600). (**b**) Active separation layer (Magnification: 20.000). The cross section of the flat sheet membrane in (**a**) clearly allows to identify the novel 3 layer structure: the selective layer on top shows a narrow pore size distribution. It is responsible for the separation of different molecules based on size exclusion (thickness 0.1–0.5 µm, blood contacting side). The support layer on the bottom has a more open membrane structure, with stabilising but no sieving function (perfusate contacting side). In between, the finger-type layer has a very open structure, which gives additional mechanical stability. Due to the high void fraction of this layer the diffusive resistance in this part is nearly identical to the one in water

this glucose bolus 3 h of highly dynamic glucose levels were expected (OGTT period, sampling interval: 15 min). Then a 4-h post-OGTT period followed (sampling interval: 30 min). The trial ended after 12 h.

2.6.2 Sampling

Reference blood samples (REF, 100 µl) were taken from the reference catheter and continuously withdrawn blood samples (CON, 100 µl) were collected at the outflow of the PFTMD. Both samples were centrifuged and supernatant plasma was collected. Microdialysate samples (DIA) were collected for 15 min to get enough sample volume for glucose analysis and were vortexed afterwards. Thus, DIA samples reflect a time integrated glucose concentration. REF and CON samples were taken in the middle of each DIA sampling period. All samples were immediately analysed for glucose concentration using a Beckman glucose analyser.

Additionally, three blood samples were taken from the reference catheter for determining the activated partial thromboplastin time (APTT) at 0 h, 5 h and 12 h in order to verify the absence of any significant patient heparinisation.

2.7 Data analysis

Technical evaluation and data analysis was performed according to Wentholt et al. (2008). The evaluation criteria include relative recovery levels, Bland and Altman analysis, Pearson's coefficient of correlation (R), absolute (AD) and relative differences (RD), mean absolute relative difference (MARD) and mean relative difference (MRD), which is also called the system error (SE) and %PRESS (Lodwig and Heinemann 2003). The latter is very sensitive on outliers and can be calculated according to (1). ISO criterion is met if the system's glucose concentration is within ±15 mg/dl or within 20% of the reference glucose concentration, for glucose concentrations <75 mg/dl and ≥75 mg/dl, respectively. Evaluation was performed on REF and CON glucose data to obtain information about heparin-blood-mixing ratio. REF and DIA data were splined on minute basis and cross-correlated in a ±20 min time window to determine transport delay time which was assumed to be found most likely at the maximum occurring coefficient of correlation. DIA data were prospectively one-point calibrated using the first valid DIA and corresponding REF sample.

Clinical evaluation included Clark Error Grid analysis (Clarke et al. 1987) and Insulin Titration Error Grid Analysis (Ellmerer et al. 2006). Technical performance evaluation was performed using time-delay corrected and prospectively one-point calibrated DIA samples. Clinical performance evaluation was performed using not delay-time corrected DIA data, as they would also not be time corrected in a clinical setting.

Data analysis was performed using Matlab (The MathWorks, Inc., Natick, MA, USA) and Microsoft Excel (Microsoft, Inc., Redmond, WA, USA).

$$\%PRESS = \sqrt{\frac{\sum_{i=1}^{N}\left([Glucose_{DIA}] - [Glucose_{REF}]\right)^2}{\sum_{i=1}^{N}[Glucose_{REF}]^2}} \quad (1)$$

3 Results

3.1 Pre-clinical evaluation

In vitro investigation in bovine blood revealed that the mean relative glucose recovery level (calculated according to (2)) remained stable at a level of around 100% for 72 h at perfusate flows between 2–5 µl/min and a blood flow of 4 ml/min. Detailed results are given in Table 1.

$$recovery = \frac{[Glucose_{DIA}]}{[Glucose_{REF}]} \quad (2)$$

Relative recovery of 100.4±4.2% was achieved at a perfusate flow of 5 µl/min. This flow is regarded as the optimal perfusion flow rate combining complete relative recovery at a perfusion rate yielding in acceptable low system transport delay time. Thus, in the following clinical investigation 5 µl/min was chosen as perfusion flow rate.

3.2 Clinical study—Technical performance evaluation

Blood-heparin withdrawal was monitored in all eight subjects and was found to be 66.9±5.6 µl/min. The system's mean delay time due to blood and dialysate transport was calculated as described above and found to be 10.5 min. In total 240 reference blood samples were taken

Table 1 Relative recovery levels at different perfusate flow rates in 72 h *in vitro* MD experiments using five PFTMD devices, custom made MD membranes and heparinised bovine blood at a flow of 4 ml/h

Perfusate flow [µl/min]	Relative recovery [%]
2	103.6±4.3
3	102.6±2.1
5	100.4±4.2
10	82.7±6.9

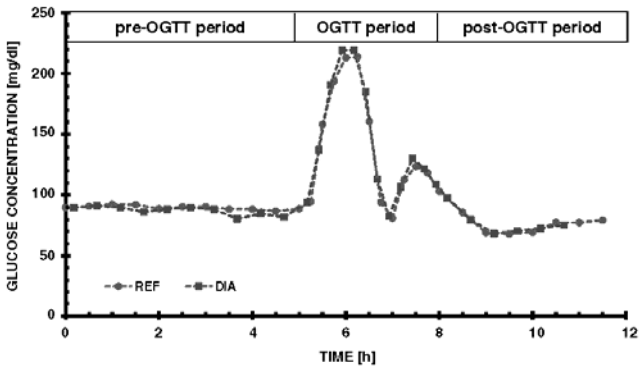


Fig. 4 Exemplary glucose time-profile showing reference (REF, circles) and prospectively one-point calibrated, transport-time-delay-corrected glucose concentration of the microdialysis based device (DIA, squares) for continuous extravascular monitoring of blood glucose concentration during a 12 h feasibility trial in a healthy volunteer undergoing an oral glucose tolerance test (OGTT)

(30 per subject) in a glucose range between 53.0 and 213.7 mg/dl (<80 mg/dl: 31 samples, 80–120 mg/dl: 139 samples, >120 mg/dl: 69 samples). 227 corresponding DIA samples were successfully collected and analysed. In total, 13 DIA samples (5.4%) were not taken due to malfunction of the system, mostly as a result of catheter occlusion.

Figure 4 shows exemplary glucose concentration profiles derived from the reference (REF, circles) and the microdialysis based system (DIA, squares). Microdialysis samples were prospectively one-point calibrated to the first reference blood glucose concentration and their glucose concentrations are corrected by the calculated lag time.

Technical data evaluation was performed according to Wentholt et al. (2008) for all eight subjects accordingly (Table 2). Mean coefficient of correlation (CORR) was found to be 0.96±0.042. Mean difference (MD) was 2.1± 12.1 mg/dl. Mean absolute difference (MAD) was 8.7± 8.6 mg/dl. Mean relative difference (MRD) was 1.9± 11.2%. Mean absolute relative difference (MARD) was 8.4±7.7%. %PRESS parameter was calculated as 10.3± 5.2%. ISO criterion (ISO MET?) was met in 91.6% of all cases. Four out of eight trials were performed with 100% success rate (ISO criterion met).

A Bland and Altman analysis on transport-time-delay-corrected DIA and REF data is provided in Fig. 5.

3.3 Clinical study—Clinical performance evaluation

The Clark Error Grid analysis (EGA, Clarke et al. 1987) is a standard clinical evaluation method classifying data pairs of a new glucose sensing method and a reference method into five zones with different levels of clinical acceptability (A: accurate, B: acceptable, C, D and E: not acceptable).

Table 2 Summary of the technical performance evaluation of the 12-h clinical trials performed in eight healthy volunteers undergoing an oral glucose tolerance test

Subject [#]	CORR [−]	MD [mg/dl]	MAD [mg/dl]	MRD [%]	MARD [%]	%PRESS [%]	ISO MET? [Yes]
1	0.876	6.6±18.2	13.4±13.8	6.7±13.4	11.4±9.5	15.3	21 of 25
2	0.935	5.2±14.8	11.3±10.6	4.0±15.7	11.6±11.0	14.3	21 of 26
3	0.989	−6.5±3.4	6.5±3.4	−6.4±3.1	6.5±3.1	7.1	30 of 30
4	0.936	15.9±10.6	17.0±8.7	16.3±8.9	17.1±7.3	17.8	20 of 27
5	0.995	0.9±4.6	3.6±2.9	0.6±4.3	3.4±2.6	3.9	28 of 28
6	0.984	−6.8±4.6	7.1±4.2	−6.9±4.3	7.2±3.7	8.2	30 of 30
7	0.971	7.5±8.5	8.1±7.9	6.8±7.6	7.6±6.9	11.2	27 of 30
8	0.994	−3.4±3.8	4.0±3.2	−3.6±4.0	4.0±3.5	4.3	30 of 30
ALL	0.960±0.042	2.1±12.1	8.7±8.6	1.9±11.2	8.4±7.7	10.3±5.2	207 of 226

Prospectively one-point calibrated, transport-time-delay-corrected microdialysate samples are evaluated against reference blood samples with respect to their glucose concentrations. Evaluation parameters include the Pearson's coefficient of correlation (CORR), mean difference (MD), mean absolute difference (MAD), mean relative difference (MRD), mean absolute relative difference (MARD), %PRESS and ISO-criteria (ISO MET?). Evaluation parameters are given as means ± standard deviation for each subject and for all subjects in summary

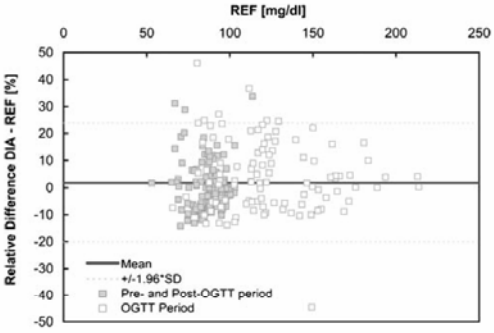


Fig. 5 Bland and Altman plot showing relative differences (y-axis) of transport-time-delay-corrected DIA and REF data plotted against REF data (x-axis). Data pairs are shown as full squares during periods of relatively stable glucose concentrations (pre- and post-OGTT period: 0–5 h and 8–12 h, respectively) and as open squares during periods with pronounced glucose excursions (OGTT-period, 5–8 h)

For this evaluation the DIA data were again prospectively one-point calibrated as described before but were not delay-time corrected, as they would also not be in a clinical setting. The EGA analysis revealed that 85.9% (n=195) and 13.7% (n=31) were in zones A and B, respectively. One glucose sample (0.4%) was found to be in zone D. The EGA plot is depicted in Fig. 6, left.

Another clinical evaluation method for glucose monitoring systems is the insulin titration error grid analysis (ITEGA). It was developed for intensive care patients undergoing intensive insulin therapy (Ellmerer et al. 2006). Four zones in a scatter-plot represent different degrees of accurate therapeutic treatment assuming the therapy decision was based on the actual glucose measurement (appropriate treatment (a), unacceptable violation (b), major violation (c), life threatening violation (d)). 99.1% (=225) of all 227 microdialysate (DIA) glucose concentrations would have led to appropriate treatment, whereas 0.9% (n=2) would have led to an unacceptable violation in insulin therapy (compare Fig. 6, right).

3.4 Safety endpoints

All eight subjects successfully finished the trial at the foreseen trial end. No trial related adverse event occurred. As heparin was used to anti-coagulate blood for continuous withdrawal within a double lumen catheter, we—for safety reasons—monitored the subject's activated partial thromboplastin time (APTT) as a measure of systemic heparinisation. Three APTT levels were measured per subject and none of them significantly increased during the trial (p>0.05).

4 Discussion and conclusion

This study presents a novel microdialysis based device for continuous extravascular monitoring of blood glucose. A planar flow-through microdialyser was designed and technically evaluated in combination with a continuous blood sampling system in 72 h *in vitro* investigations. In contrast to reported recovery degradation following probe implantation into a blood vessel (Yokel et al. 1992; Chen and Steger 1993; Yang et al. 1997; Verbeeck 2000) we can report that the glucose recovery levels remained stable in our investigations using a newly designed membrane, letting us conclude that the membrane is superior to those

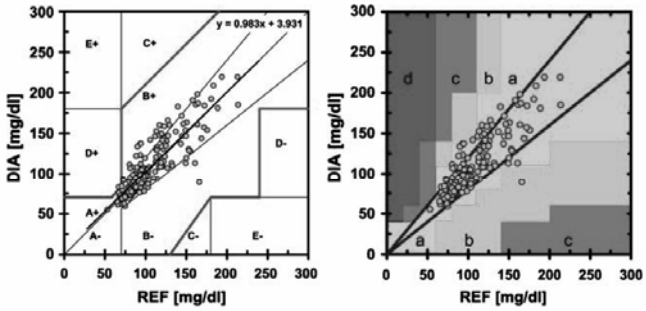


Fig. 6 Left: Clark Error Grid Analysis (EGA) comparing prospectively one-point calibrated microdialysate (DIA) with reference blood (REF) sample's glucose concentrations. 99.6% of all 227 data pairs are found in accurate and clinically acceptable zones A and B. One data pair is found in clinically unacceptable zone D. Right: Insulin Titration Error Grid Analysis (ITEGA) using the same data. 99.1% of all 227 data pairs are found in the (a)-zone, representing the "appropriate treatment" zone. Two are found in the (b)-zone "unacceptable violation"

presented in the literature with respect to recovery stability when exposed to heparinised blood.

After optimising the operating conditions, a clinical study was performed in eight healthy subjects in order to evaluate the system technically and clinically. The study was finished successfully in all subjects. A clinical performance evaluation showed that the device is able to deliver glucose information that in 99.6% of all cases was found to be accurate and clinically acceptable.

Also a technical performance evaluation was done considering Bland and Altman analysis and state of the art estimate parameters including absolute and relative differences, mean absolute relative difference, the system error %PRESS and ISO criterion. The latter was met in four out of eight experiments and we identified that 78% of all ISO deviations occurred during the OGTT period, thus during times of pronounced glucose changes. We could not find any correlation between met or unmet ISO criterion and the length or the amplitude of glucose concentration during the OGTT period, but it might be worth considering here that the performed clinical and technical evaluation is based on 15 min-time-integrated glucose concentrations of dialysate samples that were compared to spot measurements of reference blood glucose concentration. Dialysate samples thus represent a 15 min glucose average, which especially in periods with pronounced glucose changes (OGTT period) can result in relatively high discrepancies between glucose concentrations of reference and dialysate blood samples. All other evaluated clinical and technical performance parameters, let us conclude that the developed system stably delivers a continuous dialysate-matrix upon which the estimation of blood glucose concentrations can be performed precisely and highly accurate. However, we believe that the precision of the system could even be further improved, e.g. by using online glucose sensors instead of offline glucose analysis of time-integrated dialysate samples and by incorporating an online recovery monitor such as suggested by Schaupp et al. (1999) or Yokel et al. (1992).

The current limitations of the system include blood loss, time delay and the lack of an integrated online glucose sensor. The former is inherent and cannot be diminished in our approach, but the blood withdrawal rate of 2 ml/h was chosen according to specifications of the DLC-manufacturer and approved to be safe by intensive care professionals. However, it can be speculated that blood waste might be reduced by further downscaling system dimensions, by re-infusing the analysed blood or by integrating the analytics for other relevant blood gases and metabolites.

The time delay of our system currently is 10.5 min, therefore therapy decision would be based on 'old' glucose data. However, it was shown by the EGA and ITEGA analysis that this time delay would not result in unappropriate treatment. Moreover, the delay time could be improved by increasing perfusate flow or by introducing low-volume online sensors.

The system has currently not been combined with online glucose sensors and thus allows only a limited number of samples to be analysed when used with offline laboratory glucose meters such as the herein used Beckman glucose analyser, which requires at least 10 µl per sample. Integration of low volume online sensors (e.g. 0.5 µl) such as presented by Schaller et al. (Schaller et al. 2009) is feasible, obtaining highly resolved glucose signals using a 5 µl/min perfusate flow and will be investigated in future studies where aspects of longterm *in vivo* stability will also be addressed.

In summary, we have successfully developed and tested a device that is able to continuously deliver dialysate that is extracted from whole blood outside the human body for the purpose of continuous glucose monitoring. The next step towards an *automated* continuous glucose monitoring system is to integrate online glucose sensors into the herein presented microdevice.

Acknowledgements The authors gratefully acknowledge financial support from the European Commission under the CLINICIP project (contract no. 506965 within the 6th Framework program). Thanks to my love Iris Yukon.

Competing interests statement The authors declare to have no competing interests.

References

J.P. Bantle, W. Thomas, Glucose measurement in patients with diabetes mellitus with dermal interstitial fluid. J. Lab. Clin. Med. **130**, 436–441 (1997)

A.M. Castejon, X. Paez, L. Hernandez, L.X. Cubeddu, Use of intravenous microdialysis to monitor changes in serotonin release and metabolism induced by cisplatin in cancer patients: comparative effects of granisetron and ondansetron. J. Pharmacol. Exp. Ther. **291**, 960–966 (1999)

Z. Chen, R.W. Steger, Plasma microdialysis. A technique for continuous plasma sampling in freely moving rats. J. Pharmacol. Toxicol. Methods **29**, 111–118 (1993)

W.L. Clarke, D. Cox, L.A. Gonder-Frederick, W. Carter, S.L. Pohl, Evaluating clinical accuracy of systems for self-monitoring of blood glucose. Diabetes Care **10**, 622–628 (1987)

F. Costa, P. Sulur, M. Angel, J. Cavalcante, V. Haile, B. Christman, I. Biaggioni, Intravascular source of adenosine during forearm ischemia in humans: implications for reactive hyperemia. Hypertension **33**, 1453–1457 (1999)

E.C. De Lange, A.G. De Boer, D.D. Breimer, Methodological issues in microdialysis sampling for pharmacokinetic studies. Adv Drug Deliv Rev **45**, 125–148 (2000)

N. Dizdar, A.K. Granerus, U. Hannestad, A. Kullman, A. Ljungdahl, J.E. Olsson, B. Kagedal, L-dopa pharmacokinetics studied with microdialysis in patients with Parkinson's disease and a history of malignant melanoma. Acta Neurol. Scand. **100**, 231–237 (1999a)

N. Dizdar, A. Kullman, B. Norlander, J.E. Olsson, B. Kagedal, Human pharmacokinetics of L-3, 4-dihydroxyphenylalanine studied with microdialysis. Clin. Chem. **45**, 1813–1820 (1999b)

N.R. Ekberg, N. Wisniewski, K. Brismar, U. Ungerstedt, Measurement of glucose and metabolites in subcutaneous adipose tissue during hyperglycemia with microdialysis at various perfusion flow rates. Clin. Chim. Acta **359**, 53–64 (2005)

M. Ellmerer, M. Haluzik, J. Blaha, J. Kremen, S. Svacina, W. Toller, J. Mader, L. Schaupp, J. Plank, T.R. Pieber, Clinical evaluation of alternative site glucose measurement in patients after major cardiac surgery. Diabetes Care **29**, 1275–1281 (2006)

J.P. Elshoff, S. Laer, Development of an intravenous microdialysis method for pharmacokinetic investigations in humans. J. Pharmacol. Toxicol. Methods **52**, 251–259 (2005)

V.R. Kondepati, H.M. Heise, Recent progress in analytical instrumentation for glycaemic control in diabetic and critically ill patients. Anal. Bioanal. Chem. **388**, 545–563 (2007)

E. Kulcu, J.A. Tamada, G. Reach, R.O. Potts, M.J. Lesho, Physiological differences between interstitial glucose and blood glucose measured in human subjects. Diabetes Care **26**, 2405–2409 (2003)

V. Lodwig, L. Heinemann, Glucose Monitoring Study Group. Continuous glucose monitoring with glucose sensors: calibration and assessment criteria. Diabetes Technol. Ther. **5**, 573–587 (2003)

J. Lourido, P. Ederoth, N. Sundvall, U. Ungerstedt, C.H. Nordstrom, Correlation between blood glucose concentration and glucose concentration in subcutaneous adipose tissue evaluated with microdialysis during intensive care. Scand. J. Clin. Lab. Invest. **62**, 285–292 (2002)

M.T. O'Connell, F. Tison, N.P. Quinn, P.N. Patsalos, Clinical drug monitoring by microdialysis: application to levodopa therapy in Parkinson's disease. Br. J. Clin. Pharmacol. **42**, 765–769 (1996)

X. Paez, L. Hernandez, Blood microdialysis in humans: a new method for monitoring plasma compounds. Life Sci. **61**, 847–856 (1997)

P.N. Patsalos, M.T. O'Connell, H.C. Doheny, J.W. Sander, S.D. Shorvon, Antiepileptic drug pharmacokinetics in patients with epilepsy using a new microdialysis probe: preliminary observations. Acta Neurochir. Suppl. **67**, 59–62 (1996)

W. Regittnig, M. Ellmerer, G. Fauler, G. Sendlhofer, Z. Trajanoski, H. J. Leis, L. Schaupp, P. Wach, T.R. Pieber, Assessment of transcapillary glucose exchange in human skeletal muscle and adipose tissue. Am. J. Physiol. Endocrinol. Metab. **285**, E241–E251 (2003)

J.N. Roe, B.R. Smoller, Bloodless glucose measurements. Crit. Rev. Ther. Drug Carrier Syst. **15**, 199–241 (1998)

H. Rosdahl, K. Hamrin, U. Ungerstedt, J. Henriksson, Metabolite levels in human skeletal muscle and adipose tissue studied with microdialysis at low perfusion flow. Am. J. Physiol. **274**, E936–E945 (1998)

C. Sauernheimer, K.M. Williams, K. Brune, G. Geisslinger, Application of microdialysis to the pharmacokinetics of analgesics: problems with reduction of dialysis efficiency *in vivo*. J. Pharmacol. Toxicol. Methods **32**, 149–154 (1994)

R. Schaller, F. Feichtner, H. Köhler, M. Bodenlenz, J. Plank, A. Wutte, J.K. Mader, M. Ellmerer, R. Hellmich, H. Wedig, R. Hainisch, T.R. Pieber, L. Schaupp, A novel automated discontinuous venous blood monitoring system for *ex vivo* glucose determination in humans. Biosens. Bioelectron. **24**, 2239–2245 (2009)

L. Schaupp, M. Ellmerer, G.A. Brunner, A. Wutte, G. Sendlhofer, Z. Trajanoski, F. Skrabal, T.R. Pieber, P. Wach, Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion. Am. J. Physiol. Endocrinol. Metab. **276**, E401–E408 (1999)

S. Skyler, Continuous glucose monitoring: an overview of its development diabetes. Technol. Ther. **11**(Suppl 1), S5–S10 (2009)

F. Sternberg, C. Meyerhoff, F.J. Mennel, H. Mayer, F. Bischof, E.F. Pfeiffer, Does fall in tissue glucose precede fall in blood glucose? Diabetologia **39**, 609–612 (1996)

H. Stjernstrom, T. Karlsson, U. Ungerstedt, L. Hillered, Chemical monitoring of intensive care patients using intravenous microdialysis. Intensive Care Med. **19**, 423–428 (1993)

U. Ungerstedt, Microdialysis—principles and applications for studies in animals and humans. J. Intern. Med. **230**, 365–373 (1991)

G. van den Berghe, P. Wouters, F. Weekers, C. Verwaest, F. Bruyninckx, M. Schetz, D. Vlasselaers, P. Ferdinande, P. Lauwers, R. Bouillon, Intensive insulin therapy in the critically ill patients. N. Engl. J. Med. **345**, 1359–1367 (2001)

G. van den Berghe, P.J. Wouters, K. Kesteloot, D.E. Hilleman, Analysis of healthcare resource utilization with intensive insulin therapy in critically ill patients. Crit. Care Med. **34**, 612–616 (2006)

R.K. Verbeeck, Blood microdialysis in pharmacokinetic and drug metabolism studies. Adv. Drug Deliv. Rev. **45**, 217–228 (2000)

D. Vlasselaers, L. Schaupp, I. van den Heuvel, J. Mader, M. Bodenlenz, M. Suppan, P. Wouters, M. Ellmerer, G. van den Berghe, Monitoring blood glucose with microdialysis of interstitial fluid in critically ill children. Clin. Chem. **53**, 536–537 (2007)

C. Weller, M. Linder, A. Macaulay, A. Ferrari, G. Kessler, Continuous *in vivo* determination of blood glucose in human subjects. Ann. N. Y. Acad. Sci. **87**, 658–668 (1960)

I.M.E. Wentholt, A.A.M. Hart, J.B.L. Hoekstra, J.H. Devries, How to assess and compare the accuracy of continuous glucose monitors? Diabetes Technol. Ther. **10**, 57–68 (2008)

H. Yang, Q. Wang, W.F. Elmquist, The design and validation of a novel intravenous microdialysis probe: application to fluconazole pharmacokinetics in the freely-moving rat model. Pharm. Res. **14**, 1455–1460 (1997)

R.A. Yokel, D.D. Allen, D.E. Burgio, P.J. McNamara, Antipyrine as a dialyzable reference to correct differences in efficiency among and within sampling devices during *in vivo* microdialysis. J. Pharmacol. Toxicol. Methods **27**, 135–142 (1992)

Publication IV

“Use of the Site of Subcutaneous Insulin Administration for the Measurement of Glucose in Patients with Type 1 Diabetes”

Lindpointner S., Korsatko S., Köhler G., Köhler H., Schaller R., Kaidar R., Yodfat O., Schaupp L.A., Ellmerer M., Pieber T.R., Regittnig W.

Diabetes Care Vol. 33 (3); March 2010

Emerging Treatments and Technologies
ORIGINAL ARTICLE

Use of the Site of Subcutaneous Insulin Administration for the Measurement of Glucose in Patients With Type 1 Diabetes

STEFAN LINDPOINTNER, MD[1]
STEFAN KORSATKO, MD[1]
GERD KÖHLER, MD[1]
HANS KÖHLER, MSC[2]
ROLAND SCHALLER, MSC[2]
RUTHY KAIDAR, MSC[3]
OFER YODFAT, MD[3]
LUKAS SCHAUPP, PHD[1,2]
MARTIN ELLMERER, PHD[1]
THOMAS R. PIEBER, MD[1,2]
WERNER REGITTNIG, PHD[1]

OBJECTIVE — To simplify and improve the treatment of patients with type 1 diabetes, we ascertained whether the site of subcutaneous insulin infusion can be used for the measurement of glucose.

RESEARCH DESIGN AND METHODS — Three special indwelling catheters (24-gauge microperfusion [MP] catheters) were inserted into the subcutaneous adipose tissue of subjects with type 1 diabetes (n = 10; all C-peptide negative). One MP catheter was perfused with short-acting insulin (100 units/ml, Aspart) and used for insulin delivery and simultaneous glucose sampling during an overnight fast and after ingestion of a standard glucose load (75 g). As controls, the further two MP catheters were perfused with an insulin-free solution (5% mannitol) and used for glucose sampling only. Plasma glucose was measured frequently at the bedside.

RESULTS — Insulin delivery with the MP catheter was adequate to achieve and maintain normoglycemia during fasting and after glucose ingestion. Tissue glucose concentrations derived with the insulin-perfused catheter agreed well with plasma glucose levels. Median correlation coefficient and median absolute relative difference values were found to be 0.93 (interquartile range 0.91–0.97) and 10.9%, respectively. Error grid analysis indicated that the percentage number of tissue values falling in the clinically acceptable range is 99.6%. Comparable analysis results were obtained for the two mannitol-perfused catheters.

CONCLUSIONS — Our data suggest that estimation of plasma glucose concentrations from the glucose levels directly observed at the site of subcutaneous insulin infusion is feasible and its quality is comparable to that of estimating plasma glucose concentrations from glucose levels measured in insulin-unexposed subcutaneous tissue.

Diabetes Care 33:1–2, 2010

Artofn

Type 1 diabetes is presently treated by self-administration of insulin, either by a subcutaneous bolus injection using a hypodermic needle (e.g., syringe, insulin pen) or by a continuous subcutaneous infusion using an indwelling catheter connected to an insulin pump (1,2). Type 1 diabetic patients furthermore separately self-monitor glucose levels in blood obtained by finger-pricking to guide the adjustment of insulin dosage, food consumption, and physical activity (1,2). To simplify and improve the glucose management in diabetes, we sought to determine whether the site of subcutaneous insulin administration can also be used for the measurement of glucose. As a first step, we recently assessed the kinetics of insulin action on the tissue glucose concentration at the insulin delivery site in the presence of euglycemic blood plasma levels (3). Using the euglycemic clamp technique together with special indwelling catheters (microperfusion or microdialysis catheters) for coupling insulin delivery with glucose sampling at the same tissue site, we found that within 60 min after exposing adipose tissue of healthy humans to a standard 100 units/ml insulin preparation, insulin's effect on the tissue glucose concentration saturates and a stable ratio between the tissue and plasma glucose concentration is attained. This attainment of steady-state insulin action conditions at the delivery site indicates that glucose sensing and insulin delivery may be carried out simultaneously at the same adipose tissue site via a single tissue catheter. To further validate this single-port treatment approach, the objective of the present study was to ascertain in type 1 diabetic patients whether tissue glucose concentrations observed at the site of subcutaneous insulin delivery can be used to estimate plasma glucose levels. To accomplish this, microperfusion (MP) catheters were inserted in adipose tissue of type 1 diabetic subjects and used to carry out glucose sampling and simultaneous insulin delivery during an overnight fast and after ingestion of a standard glucose load (oral glucose tolerance test [OGTT]).

RESEARCH DESIGN AND METHODS

— Ten subjects with type 1 diabetes (two females and eight males; age 39.8 ± 2.9 years, range 27–57; BMI 25.3 ± 1.0 kg/m^2, range 21.1–29.5, mean ± SE) participated in this study. Their mean duration of diabetes was 22.9 ± 2.6 years (range 7–35) and their percent A1C averaged 7.6 ± 0.3% (range 5.7–8.6%, normal range 4.3–5.9%). Patients were all without residual endogenous insulin secretion, as indicated by undetectable C-peptide levels in blood plasma (i.e., <22 pmol/l). Three patients were treated with continuous subcutaneous insulin infusion and seven with multiple daily injections of insulin. At the time of the study, patients had no evi-

From the [1]Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Graz, Austria; the [2]Institute of Medical Technologies and Health Management, Joanneum Research Forschungsgesellschaft GmbH, Graz, Austria; and [3]Medingo Ltd., Yoqneam Illit, Israel.
Corresponding author: Werner Regittnig, werner.regittnig@healthsite.at.
Received 16 August 2009 and accepted 19 December 2009. Published ahead of print at http://care.diabetesjournals.org on 29 December 2009. DOI: 10.2337/dc09-1532. Clinical Trial Registry No. NCT00813410; http://clinicaltrials.gov.

rich4/zdc-dcare/zdc-dcare/zdc00310/zdc8125d10a | xppws | S=1 | 1/12/10 | 6:23 | doi: 10.2337/dc09-1532 | artno: 1532

dence of clinically overt diabetes complications and, apart from insulin, were not taking any medication known to influence carbohydrate metabolism and subcutaneous insulin absorption. Written informed consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The studies were approved by the ethics committee of the Medical University of Graz.

Study design

The diabetic subjects were admitted to the clinical research center at ~2230. Subjects treated with multiple daily injections had been instructed to leave out the injection of long-acting insulin on the evening of the study. On admission to the clinical research center, subjects with continuous subcutaneous insulin infusion treatment were asked to disconnect their own insulin pump. At ~2300, an intravenous catheter was inserted into an arm vein for blood withdrawal during the night. After catheter insertion, three 24-gauge MP catheters (4,5) were placed into the periumbilical subcutaneous adipose tissue. The distance between adjacent catheters was >35 mm. Subsequently, peristaltic pumps (Minipuls 3; Gilson, Villiers-le-Bel, France) were attached to the inflow and outflow tubing of each catheter (Fig. 1). One catheter was then perfused with a rapid-acting insulin solution (100 units/ml, Aspart; Novo Nordisk, Bagsvaerd, Denmark) to allow insulin delivery and simultaneous interstitial fluid (ISF) sampling during the experiment. The two further catheters were perfused with an insulin-free solution (5% mannitol) and used for ISF sampling only. After starting catheter perfusion, an equilibration period of 60 min elapsed before the catheter effluent samples were collected in 60-min fractions in vials kept on ice. Outflow rates of all catheters were maintained at a constant value (~0.45 μl/min) throughout the experiment. The insulin delivery rate of the insulin-perfused catheter was adjusted by simply adjusting the inflow rate of the catheter (dual pump operation mode, Fig. 1*A*). Adjustments in the inflow rate were done on the basis of frequent plasma glucose measurements (every 10–30 min) to slowly achieve and maintain normoglycemia (~6 mmol/l) overnight. For comparison purposes, the inflow rates applied in one mannitol-perfused catheter (Fig. 1*B*) were similar to the periodically adjusted inflow rates of the insulin-perfused catheter (Fig. 1*A*), whereas the inflow rate applied in the second mannitol-perfused catheter was identical to its outflow rate (Fig. 1*C*). On the next day, at ~0700, a hand or forearm vein was cannulated to allow blood withdrawal during the subsequent OGTT phase of the experiment. The forearm with this catheter was then placed in a thermoregulated box to arterialize the venous blood. At 0900, the subject ingested 75 g glucose dissolved in 300 ml of water (Glucoral; Unipack, Wr.-Neustadt, Austria). Twenty minutes before glucose ingestion, an insulin bolus was administered over a period of 15 min via the insulin-perfused MP catheter. Bolus administration periods of comparable length are used in some commercially available insulin pump models (6). The amount of insulin administered as a bolus was determined by using medical records on the subject's insulin sensitivity factor (i.e., subject's insulin-to-carbohydrate ratio). After administration of the insulin bolus, the basal insulin delivery via the insulin-perfused MP catheter was continued and periodically adjusted so as to re-establish normal plasma glucose by ~5 h after glucose ingestion. During the 8-h OGTT phase and during the 2-h period preceding initiation of the OGTT, the catheter effluent samples were collected in 30-min fractions, and plasma glucose

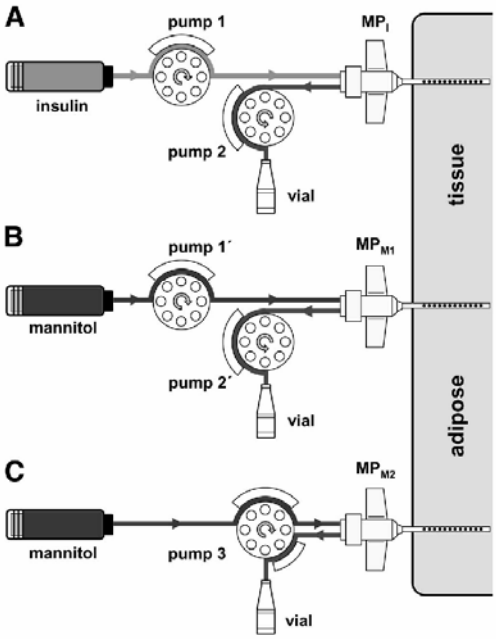


Figure 1—*Schematic of the experimental setup for assessing the feasibility of estimating plasma glucose concentrations from the ISF glucose levels observed at the insulin delivery site. A–C: Three MP catheters were inserted into subcutaneous adipose tissue of diabetic subjects (n = 10). One catheter (MP$_I$) was used for glucose sampling and simultaneous insulin delivery (A), and, as controls, two catheters (MP$_{M1}$ and MP$_{M2}$) were used for glucose sampling only (B and C). The catheter for glucose sampling and insulin delivery was perfused with a rapid-acting insulin solution (100 units/ml, Aspart) using two peristaltic pumps, with one attached to the inflow tubing and one to the outflow tubing (dual-pump operation mode). Insulin delivery rate was adjusted by adjusting the difference between the inflow and outflow rate of the catheter. The outflow conveying the extracted tissue glucose was collected in vials. The efficiency by which glucose was extracted via diffusion from the ISF of the tissue into the catheter (glucose recovery) was measured by applying the ionic reference technique (4,5). A: The catheters used for glucose sampling only (MP$_{M1}$ and MP$_{M2}$) were perfused with an insulin-free solution (5% mannitol) using either a single-pump operation mode where the inflow equaled the outflow rate (C) or a dual-pump operation mode (B) where the inflow and outflow rates equaled those applied in the insulin-perfused MP catheter (A).*

rich4/zdc-dcare/zdc-dcare/zdc00310/zdc8125d10a | xppws | S=1 | 1/12/10 | 6:23 | doi: 10.2337/dc09-1532 | artno: 1532

concentrations were determined every 5–30 min. If during experiments the plasma glucose levels decreased below 3.22 mmol/l (58 mg/dl), the subjects were asked to ingest additional glucose.

Microperfusion and analytical procedures

MP catheters applied were of concentric design with a cylindrical inner and outer tube (4,5). The outer tube consisted of a conventional intravenous 24-G cannula (shaft length: 19 mm, Neoflon; Becton Dickinson, Helsingborg, Sweden) in which 27 perforations (each 0.3 mm in diameter) were formed in the cannula wall using an Excimer Laser (LZH, Hannover, Germany). Two 750-mm lengths of Tygon tubing (inner diameter: 0.19 mm; Cole-Parmer, Vernon Hills, IL) were used to connect catheter inlet and outlet with perfusate reservoir and sampling vial, respectively.

Plasma glucose concentrations were measured at the bedside using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) with a coefficient of variation (CV) of 2%. The glucose concentrations in catheter effluents were determined using an automated CMA600 analyzer (CMA/Microdialysis, Solna, Sweden) with a within-run CV of 2%. The conductivities in the plasma, perfusate, and catheter effluent samples were measured using a contactless conductivity detector (TraceDec; I.S.T., Strasshof, Austria). The electrical conductivity was determined with a within-run CV of <1%. The plasma C-peptide concentrations were determined using a two-site enzyme immunoassay (C-peptide ELISA; Mercodia, Uppsala, Sweden) with a lower limit of quantification of 22 pmol/l. A1C was measured by high-performance liquid chromatography (HA-8160; Menarini Diagnostics, Florence, Italy).

Data analysis

The ISF glucose concentration was calculated (4,5) as the glucose concentration in the catheter effluent sample divided by the glucose recovery of the catheter (R). The recovery (or exchange efficiency) was determined for each sampling period as $R = (C_{out} - C_{in})/(C_{pl} - C_{in})$, where C_{in}, C_{out}, and C_{pl} are the measured electrical conductivity in the perfusate, the effluent sample, and the corresponding plasma sample, respectively. This method (ionic reference technique [4,5]) allowed potential recovery changes caused by changes in the catheter characteristics (e.g., changes in perfusate inflow rates) and/or undesired local variations in the tissue microenvironment (e.g., blood flow changes, accumulation of edematous fluids) to be detected. Application of this technique was possible, because the electrical conductivity in the used catheter perfusates was either negligible (mannitol) or low compared with that in blood plasma (Aspart: ~22.2% of average C_{pl}). Because each effluent sample was collected over a specified time interval (i.e., 60-min and 30-min intervals), the concentration in an effluent sample was regarded as an average concentration over the collection period. Thus, the derived ISF glucose values were considered valid at the midpoint of the interval, and an observed change in the ISF glucose concentration was considered as a time-averaged reflection of the changes that occurred in the plasma concentration during a collection interval. Estimates of plasma glucose concentration (termed "tissue glucose concentrations") were derived from the ISF glucose levels by using a prospective one-point calibration procedure (7) that consisted of dividing the ISF glucose values with the ISF-to-plasma glucose ratio calculated from the ISF glucose concentration and corresponding mean plasma glucose concentration observed during the 60-min sampling period at the beginning of each experiment. Agreement between the tissue glucose concentrations and directly measured plasma glucose concentrations was assessed by applying error grid analysis and the method of residuals (8,9). Agreement index data obtained from the application of the method of residuals and the error grid analysis were examined with Friedman's test and Fisher's exact test, respectively. Implicit in the one-point calibration method is the assumption that there is a proportional relationship ($Y = B \times X$) between the plasma glucose (X) and ISF glucose concentrations (Y). This assumption was tested by performing correlation and linear regression analysis of the full plasma and ISF glucose dataset. Correlation analysis was performed using Pearson's product-moment correlation coefficient, and linear regression analysis was performed by the least squares method. To detect potential effects of fast changes in plasma glucose and insulin concentrations on the relationship between plasma glucose levels and catheter-derived ISF glucose concentrations, linear regression analyses were additionally performed on a fasting dataset containing plasma and ISF glucose values observed during the overnight (euglycemic) phase of the experiment (−8 to 0 h), and an OGTT dataset containing plasma and ISF glucose values observed was during the hyperglycemic/hyperinsulinemic phase of the study (0–8 h). Coefficient and parameter data obtained from correlation and linear regression analysis were examined with Friedman's test or Wilcoxon's signed-rank test. A P value <0.05 was considered to indicate statistical significance. Normality of data were assessed using normal probability plots. Homogeneity of variances in the linear regression data were assessed using plots of residuals against fitted values. Data are presented as means ± SE or as median and quartile values. Data analysis was performed using MATLAB (MathWorks, Natick, MA) and SPSS (SPSS, Chicago, IL) software packages.

RESULTS

Plasma and ISF glucose time courses

Insulin delivery with the MP catheter was successful both in achieving and maintaining a stable normal plasma glucose during the overnight fasting as well as in reestablishing near-normal plasma glucose by 4–5 h after the ingestion of glucose (Fig. 2*A–C* and supplementary Fig. 1, available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc09-1532/DC1). In addition, glucose concentration time courses observed in the ISF sampled with the three MP catheters paralleled those seen in plasma (supplementary Fig. 1). The basal insulin delivery rates used during the overnight and OGTT period averaged 1.04 ± 0.11 and 0.85 ± 0.16 units/h, respectively. The amount of insulin given as a bolus averaged 7.8 ± 0.8 units (Fig. 2*A*). F2

Relationship between plasma and ISF glucose concentrations

Results from the correlation analysis of ISF glucose values against plasma glucose values are given in the supplementary Table 2. The correlation coefficient for the insulin-perfused catheters (MP_I) was found to be high (median: 0.93; interquartile range: 0.91–0.97) and did not differ from that obtained for the mannitol-perfused catheters (versus MP_{M1}: 0.94 [0.88–0.97] and MP_{M2}: 0.95 [0.91–0.98]; $P = 0.67$ with Friedman's test). In addition, regression analysis suggests that for insulin-perfused and mannitol- AQ: B

rich4/zdc-dcare/zdc-dcare/zdc00310/zdc8125d10a | xppws | S=1 | 1/12/10 | 6:23 | doi: 10.2337/dc09-1532 | artno: 1532

Glucose sampling from the insulin infusion site

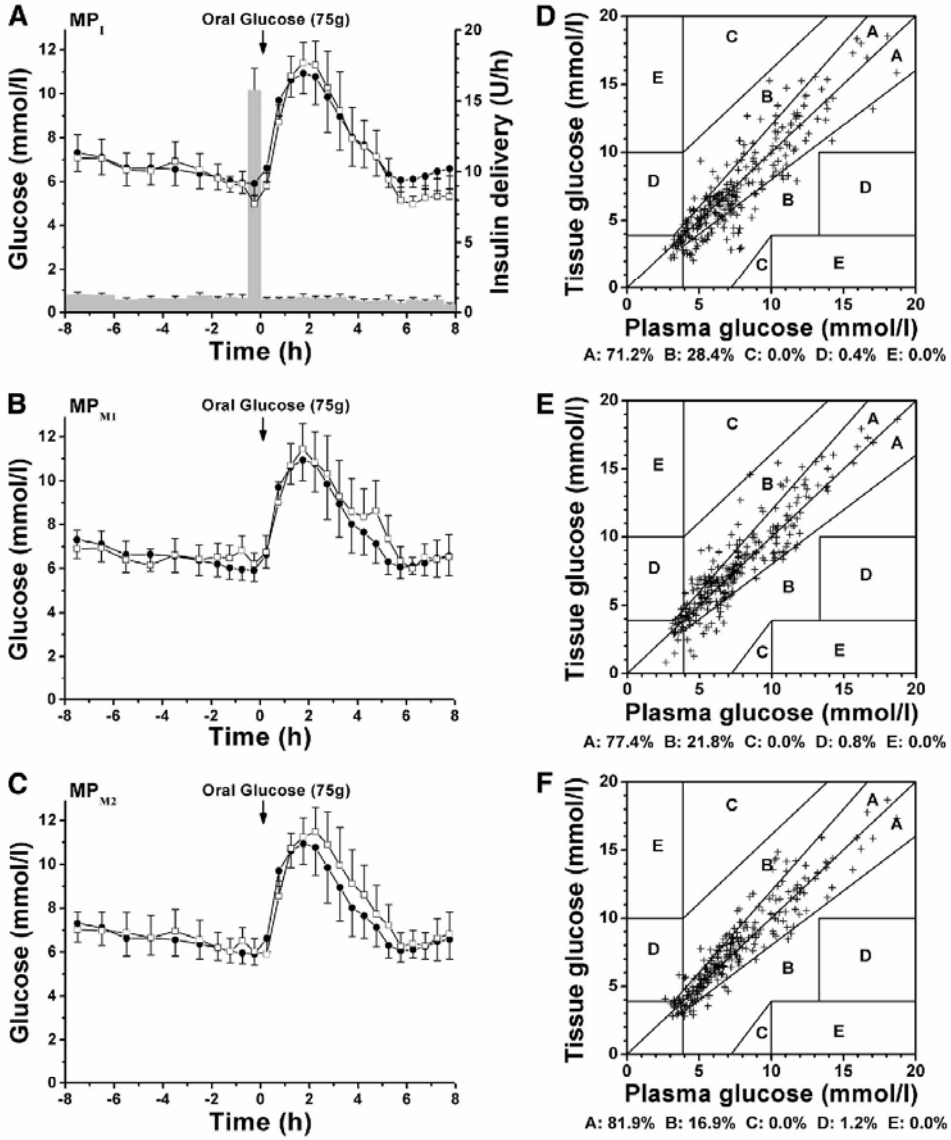


Figure 2—*Comparison of plasma and ISF-derived glucose concentrations observed during an overnight fast and OGTT in diabetic subjects. A: Average time course (n = 10, means ± SE) of plasma glucose concentration (●) and the tissue glucose concentration obtained with the MP catheter used for insulin delivery and simultaneous glucose sampling (MP_I, ○). A also shows the average time course (n = 10, means ± SE) of the insulin delivery rate (bars) used to control glucose concentration during experiments. B and C: Average time course (n = 10, means ± SE) of plasma glucose (●) and the tissue glucose obtained with the mannitol-perfused MP catheters (MP_{M1} and MP_{M2}, □). D: Error grid analysis results for the insulin-perfused MP catheter (MP_I): One of 268 data points (0.4%) fell outside of the clinically acceptable region A and B. E: Error grid analysis results for the mannitol-perfused catheter MP_{M1}: Two of 266 data points (0.8%) were outside of the clinically acceptable region A and B. F: Error grid analysis results for the mannitol-perfused catheter MP_{M2}: Three of 248 data points (1.2%) fell outside of the clinically acceptable region A and B. In A, the tendency toward lower tissue glucose concentrations than plasma glucose levels at the end of the OGTT is mainly caused by subject 8, whose tissue glucose concentration time course of the insulin-perfused catheter was an apparent outlier, with very low values during the last 4 h of the experiment (supplementary Fig. 1).*

rich4/zdc-dcare/zdc-dcare/zdc00310/zdc8125d10a | xppws | S=1 | 1/12/10 | 6:23 | doi: 10.2337/dc09-1532 | artno: 1532

perfused catheters, the derived straight-lines pass through the origin of the regression graph, i.e., the intercept values for insulin-perfused catheters (MP_1: −0.13 mmol/l [−0.71 to 0.10 mmol/l]) and mannitol-perfused catheters (MP_{M1}: 0.31 mmol/l [−0.27 to 1.19 mmol/l]; MP_{M2}: −0.03 mmol/l [−0.36 to 0.18 mmol/l]), were not different from zero ($P > 0.19$ with Wilcoxon's signed-rank test). Results from an additional regression analysis performed with the straight-line forced through the origin of regression graphs are given in supplementary Tables 3 and 4. The standard errors of the slopes as well as the root mean square errors obtained from this regression were similar for the insulin-perfused and mannitol-perfused catheters ($P > 0.49$ with Friedman's test). These results indicate that there is a strong proportional relationship between plasma glucose levels and catheter-derived ISF glucose values. Furthermore, the slope derived for the insulin-perfused catheters (MP_1: 0.726 [0.712–0.774]) was by ~16% lower than the slopes obtained for the mannitol-perfused catheters ($P = 0.002$ with Friedman's test). There was no difference between the slopes obtained for the two mannitol-perfused catheters (MP_{M1}: 0.862 [0.801–0.916] vs. MP_{M2}: 0.859 [0.833–0.977]; $P = 0.16$ with Wilcoxon's signed-rank test). Results from additional regression analyses performed on the fasting and OGTT datasets are given in supplementary Tables 5 and 6. Comparison of the analysis results showed that slopes and standard errors of the slopes obtained for insulin-perfused and mannitol-perfused catheters from the OGTT dataset are similar to those obtained for insulin-perfused and mannitol-perfused catheters from the fasting dataset (MP_1: $P > 0.48$, MP_{M1}: $P > 0.41$, and MP_{M2}: $P > 0.08$, all with Wilcoxon's signed-rank test).

Comparison between plasma and tissue glucose concentrations

Tissue glucose time courses that were derived from the ISF glucose time courses using the prospective one-point calibration procedure are shown in supplementary Fig. 1 and Fig. 2*A–C*. Error grid analysis indicated (Fig. 2*D–F*) that the percentage number of the tissue values that fall in the clinically acceptable range (zones A and B) is high for the insulin-perfused catheters (99.6%) and comparable to that obtained for the mannitol-perfused catheters (versus 99.2% for MP_{M1} and 98.8% for MP_{M2}, $P > 0.87$ with Fisher's exact test). Furthermore, applying the method of residuals, the residual means and 2 SD values obtained for the insulin-perfused catheters were 5.6% (−6.0 to 12.4%) and 31.6% (26.4 to 34.6%), respectively. These values were similar to those calculated for the mannitol-perfused catheters ($P > 0.49$ with Friedman's test; supplementary Table 1). In addition, the median absolute relative difference calculated for the insulin-perfused catheters (10.9%) was comparable to that of mannitol-perfused catheters (versus 10.5 and 10.1% for MP_{M1} and MP_{M2}, respectively). Overall, the statistical analysis suggests that the quality of estimation of plasma glucose concentrations from the ISF glucose levels directly observed at the adipose tissue site of insulin delivery is comparable to that of estimating plasma glucose concentrations from the ISF glucose levels measured in insulin-unexposed tissue.

CONCLUSIONS — The present investigation demonstrates that estimation of plasma glucose concentrations from the glucose levels directly observed at the site of subcutaneous insulin infusion is feasible in type 1 diabetic patients. Thus, glucose sensing and insulin delivery may be combined at the same adipose tissue site using a single catheter. Such a single-port treatment approach may offer several important advantages over present-time insulin replacement approaches (1) and recently proposed dual-port treatment strategies (10,11). First, it permits a significant reduction in the number of treatment-related needle-sticks, because the subcutaneously inserted catheter serves the double purpose of delivering insulin and sensing glucose and because the fingerstick testing of blood glucose is only needed to calibrate the ISF-based glucose sensing. Second, it allows a reduction in the size of the treatment system, because both the sensing and delivery components can be fully integrated into one design, and certain device components implemented in dual-port systems can be reduced in number (e.g., power supply units) or can even be eliminated (e.g., receiver-transmitter modules). Third, it increases patient convenience and possibly allows higher treatment compliance, leading to an improved glucose management. Finally, the single-port treatment approach may provide the basis for the future development of an autonomous device able to self-regulate insulin delivery (artificial pancreas).

Estimates of the plasma glucose concentration were derived from the observed ISF glucose concentrations by applying a prospective one-point calibration procedure (7) that implicitly assumes that there is a proportional relationship between plasma and ISF glucose concentrations. This assumption of proportionality may be well satisfied because correlation and linear regression analysis of plasma glucose concentrations and ISF glucose levels from insulin-exposed and insulin-unexposed tissue sites indicated that the derived intercepts are not different from zero and that glucose concentrations in the ISF correlated closely with those in plasma (supplementary Table 2). Furthermore, correlation coefficients, standard errors of the slopes, as well as the root mean square errors obtained from correlation and linear regression analysis of the full ISF and plasma glucose datasets (supplementary Tables 2–4) were similar for insulin-exposed and insulin-unexposed tissue sites. In addition, regression analyses performed individually on fasting and OGTT datasets showed that slopes and standard errors of the slopes obtained for insulin-exposed and insulin-unexposed tissue sites during the fasting were similar to those obtained for insulin-exposed and insulin-unexposed tissue sites during the OGTT, thereby indicating that changes in plasma insulin and glucose concentrations did not affect the relationship between plasma glucose concentrations and ISF glucose levels at these tissue sites (supplementary Tables 5 and 6). Overall, these results suggest that exposure of adipose tissue to standard insulin preparations did not alter the strength of the proportional relationship between plasma glucose concentrations and ISF glucose levels of adipose tissue.

Agreement between the ISF-based estimates of plasma glucose levels and directly measured plasma glucose concentrations was assessed by applying error grid analysis and the method of residuals (8,9). In the present study, values of the agreement indexes obtained for the ISF-based estimation of plasma glucose concentration from insulin-unexposed tissue sites (Fig. 2*D–F* and supplementary Table 1) were similar to or better than those previously obtained with commercial continuous glucose-sensing devices that also use the ISF of (insulin-unexposed) adipose tissue for the measurement of glucose (12–15).

rich4/zdc-dcare/zdc-dcare/zdc00310/zdc8125d10a | xppws | S=1 | 1/12/10 | 6:23 | doi: 10.2337/dc09-1532 | artno: 1532

Furthermore, values of the agreement indexes derived in the present study for the insulin-exposed adipose tissue were similar to those derived for the insulin-unexposed adipose tissue (Fig. 2*D–F*, supplementary Table 1). Thus, our data indicate that the quality of estimation of plasma glucose concentrations from the ISF glucose levels directly observed at the adipose tissue site of insulin delivery is comparable to that of estimating plasma glucose concentrations from the ISF glucose levels measured in insulin-unexposed tissue.

Recently, we assessed the kinetics of insulin action on the ISF glucose concentration at the site of subcutaneous insulin delivery (3). In this earlier study, after the start of insulin delivery, we observed an initial delay of ~60 min before insulin's effect on the ISF glucose concentration at the delivery site saturated, and the ISF-to-plasma glucose concentration ratio attained steady-state values. These values were ~20% lower than the ISF-to-plasma glucose ratio values observed during the baseline period before the start of insulin delivery. In the current study, we determined ISF glucose levels under steady-state insulin action conditions. To achieve this, the ISF sampling from the insulin delivery site was begun 60 min after the start of insulin delivery. Thus, comparison of the slopes derived for the insulin-exposed and insulin-unexposed tissue (supplementary Table 2) allowed for estimation of the magnitude of the steady-state effect of insulin on the ISF glucose concentration at the insulin delivery site in diabetic patients. We found that the slopes derived for the insulin-exposed tissue were on average ~16% lower than the slopes derived for the insulin-unexposed tissue (supplementary Table 2), thereby confirming our recent findings on the magnitude of insulin's effect on the ISF glucose concentration in human adipose tissue (3).

Besides the effect of insulin on the fat cell glucose uptake, an additional mechanism potentially influencing the glucose concentration at the site of insulin delivery may be the local dilution of the ISF by the insulin solvent. To separate and quantitate these potential effects on the glucose concentration at the insulin delivery site, one of the two mannitol catheters (MP_{M1}) was operated using also the dual-pump technique with pump speeds identical to those used in the operation of the insulin catheter (Fig. 1*B*). We reasoned that if an increase in the perfusate flow fraction directed to the tissue (due to an increase in the difference between the speeds of inflow and outflow pumps) is causing a dilution of the ISF surrounding the catheter, then there will be a decrease in the efficiency of exchange of solutes between the ISF and the perfusates of the two catheters operated with the dual-pump technique (MP_I and MP_{M1}). We found that increases in the basal delivery rates did not dilute the ISF at the insulin delivery site. In contrast, bolus administrations apparently diluted the ISF at the insulin delivery site as both exchange efficiency (recovery) and effluent glucose concentration were decreased during and shortly after the bolus administration period (i.e., between −30 and 0 min; supplementary Fig. 2). However, when accounting for this recovery change in the calculation of the tissue glucose concentration from the effluent glucose concentration (by using the ionic reference technique), the obtained tissue glucose concentrations agreed well with the corresponding plasma glucose levels also during bolus administration periods (Fig. 1*A* and *B*), thereby indicating that bolus administrations did not disturb steady-state insulin action conditions at the insulin delivery site. Thus, both changes in the basal insulin delivery rates and bolus administrations did not affect the ability of the insulin delivery catheter to predict plasma glucose concentrations.

In summary, here we have shown that glucose concentrations directly measured at the subcutaneous insulin delivery site can be used to reliably estimate blood glucose levels. Thus, glucose sensing and insulin delivery may be simultaneously performed at the same adipose tissue site via a single tissue catheter. Such a single-port treatment approach may provide the basis for the development of an autonomous treatment device able to self-regulate insulin delivery.

Acknowledgments— This work was supported in part by funding from Science Park Graz and the Federal Ministry of Economics and Labor of the Republic of Austria and by a research grant from Medingo Ltd.

T.R.P. is a cofounder and shareholder of Smart*Med GmbH and a consultant for Novo-Nordisk and sanofi-aventis. M.E. is a consultant for Sensile Medical AG and BBraun AG and a cofounder and shareholder of Smart*Med GmbH. H.K. and L.S. are cofounders and shareholders of Smart*Med GmbH. G.K., L.S., M.E., T.R.P., and W.R. have filed patent applications relating to the methodology described in this article. R.K. and O.Y. are employed by Medingo Ltd. No other potential conflicts of interest relevant to this article were reported.

We are grateful to A. Wutte, G. Bock, and B. Semlitsch for their expert assistance in conducting the study; M. Suppan for performing the C-peptide assay; A. Groselj-Strele for her assistance with statistical analysis; and all volunteers for participating in the study.

References

1. Daneman D. Type 1 diabetes. Lancet 2006;367:847–858
2. Shalitin S, Phillip M. The role of new technologies in treating children and adolescents with type 1 diabetes mellitus. Pediatric Diabetes 2007;8 (Suppl. 6):72–79
3. Lindpointner S, Korsatko S, Köhler G, Köhler H, Ellmerer M, Schaupp L, Pieber TR, Regittnig W. Glucose concentration at the subcutaneous site of insulin delivery: effect of variable insulin infusion rates. Abstract presented at the 2nd European Diabetes Technology and Transplantation Meeting, 27–29 January 2008, Innsbruck, Austria
4. Schaupp L, Ellmerer M, Brunner GA, Wutte A, Sendlhofer G, Trajanoski Z, Skrabal F, Pieber TR, Wach P. Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion. Am J Physiol 1999;276:E401–E408
5. Trajanoski Z, Brunner GA, Schaupp L, Ellmerer M, Wach P, Pieber TR, Kotanko P, Skrabal F. Open-flow microperfusion of subcutaneous adipose tissue for on-line continuous ex vivo measurement of glucose concentration. Diabetes Care 1997; 20:1114–1121
6. Diabetes Health: Insulin Pumps Reference Guide [article online], 2008. Available from http://www.diabeteshealth.com/media/pdfs/PRG1208/DH_Insulin-Pumps_08–09.pdf. Accessed 22 October 2009
7. Lodwig V, Heinemann L, Glucose Monitoring Study Group. Continuous glucose monitoring with glucose sensors: calibration and assessment criteria. Diabetes Technol Ther 2003;5:572–586
8. Trajanoski Z, Brunner GA, Schaupp L, Ellmerer M, Wach P, Pieber TR, Kotanko P, Skrabal F. Evaluating clinical accuracy of systems for self-monitoring of blood glucose. Diabetes Care 1997;20:1114–1121
9. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307–310
10. Hovorka R, Chassin LJ, Wilinska ME, Canonico V, Akwi JA, Federici MO, Massi-Benedetti M, Hutzli I, Zaugg C, Kaufmann H, Both M, Vering T, Schaller HC, Schaupp L, Bodenlenz M, Pieber TR. Closing the loop: the ADICOL experience. Diabetes Technol Ther 2004;6:307–318

AQ: C

rich4/zdc-dcare/zdc-dcare/zdc00310/zdc8125d10a | xppws | S=1 | 1/12/10 | 6:23 | doi: 10.2337/dc09-1532 | artno: 1532

11. Steil GM, Rebrin K, Darwin C, Hariri F, Saad MF. Feasibility of automating insulin delivery for the treatment of type 1 diabetes. Diabetes 2006;55:3344–3350
12. U.S. Food and Drug Administration. Summary of safety and effectiveness data: FreeStyle Navigator Continuous Glucose Monitoring System [article online], 2008. Available from http://www.accessdata.fda.gov/cdrh_docs/pdf5/P050020b.pdf. Accessed 20 June 2009
13. U.S. Food and Drug Administration. Summary of safety and effectiveness data: DexCom STS Continuous Glucose Monitoring System [article online], 2007. Available from http://www.accessdata.fda.gov/cdrh_docs/pdf5/P050012S001b.pdf. Accessed 20 June 2009
14. U.S. Food and Drug Administration. Summary of safety and effectiveness data: Guardian RT [article online], 2006. Available from http://www.accessdata.fda.gov/cdrh_docs/pdf/P980022s011b.pdf. Accessed 20 June 2009
15. Maran A, Crepaldi C, Tiengo A, Grassi G, Vitali E, Pagano G, Bistoni S, Calabrese G, Santeusanio F, Leonetti F, Ribaudo M, Di Mario U, Annuzzi G, Genovese S, Riccardi G, Previti M, Cucinotta D, Giorgino F, Bellomo A, Giorgino R, Poscia A, Varalli M. Continuous subcutaneous glucose monitoring in diabetic patients. Diabetes Care 2002;25:347–352

rich4/zdc-dcare/zdc-dcare/zdc00310/zdc8125d10a | xppws | S=1 | 1/12/10 | 6:23 | doi: 10.2337/dc09-1532 | artno: 1532

Publication V

“Glucose Levels at the Site of Subcutaneous Insulin Administration and Their Relationship to Plasma Levels”

Lindpointner S., Korsatko S., Köhler G., Köhler H., Schaller R., Ellmerer M., Pieber T.R., Regittnig W.

Diabetes Care Vol. 33 (4); April 2010

Emerging Treatment and Technology
ORIGINAL ARTICLE

Glucose Levels at the Site of Subcutaneous Insulin Administration and Their Relationship to Plasma Levels

Stefan Lindpointner, MD[1]
Stefan Korsatko, MD[1]
Gerd Köhler, MD[1]
Hans Köhler, MSC[2]
Roland Schaller, MSC[2]
Lukas Schaupp, PHD[1,2]
Martin Ellmerer, PHD[1]
Thomas R. Pieber, MD[1,2]
Werner Regittnig, PHD[1]

OBJECTIVE — To examine insulin's effect on the tissue glucose concentration at the site of subcutaneous insulin administration.

RESEARCH DESIGN AND METHODS — A CMA-60 microdialysis (MD) catheter and a 24-gauge microperfusion (MP) catheter were inserted into the subcutaneous adipose tissue of fasting, healthy subjects ($n = 5$). Both catheters were perfused with regular human insulin (100 units/ml) over a 6-h period and used for glucose sampling and simultaneous administration of insulin at sequential rates of 0.33, 0.66, and 1.00 units/h (each rate was used for 2 h). Before and after the insulin delivery period, both catheters were perfused with an insulin-free solution (5% mannitol) for 2 h and used for glucose sampling only. Blood plasma glucose was clamped at euglycemic levels during insulin delivery.

RESULTS — Start of insulin delivery with MD and MP catheters resulted in a decline of the tissue glucose concentration and the tissue-to-plasma glucose ratio (TPR) for ~60 min ($P < 0.05$). However, during the rest of the 6-h period of variable insulin delivery, tissue glucose concentration paralleled the plasma glucose concentration, and the TPR for MD and MP catheters remained unchanged at 83.2 ± 3.1 and 77.1 ± 4.8%, respectively. After subsequent switch to insulin-free perfusate, tissue glucose concentration and TPR increased slowly and reattained preinsulin delivery levels by the end of the experiments.

CONCLUSIONS — The results show the attainment of a stable TTP glucose ratio at the site of insulin administration, thus indicating that insulin delivery and glucose sensing may be performed simultaneously at the same adipose tissue site.

Diabetes Care 33:1–2, 2010

Artofin

When insulin is secreted from the pancreas, the resulting rise in the blood insulin level acts to lower the blood glucose concentration by both suppressing glucose production in the liver and enhancing glucose uptake in insulin-sensitive tissues (mainly muscle and adipose tissue) (1). Studies (2,3) investigating insulin's effect on glucose uptake in isolated muscle and fat cells have shown that the cellular response to increasing insulin concentration is a continuous increase in glucose uptake until a maximal response is reached. Further increases in the prevailing insulin concentrations beyond this threshold level does not further increase glucose uptake. Similar saturation-type concentration-response characteristics were found for insulin-stimulated arteriovenous glucose difference, blood flow, and glucose uptake (4,5) in the forearm and leg as well as for insulin-stimulated whole-body glucose uptake (1,4,5). In these in vivo studies, the maximal response to insulin in healthy humans was observed to occur at a blood plasma concentration of 300–1,000 μU/ml (1,4,5). Most notably, this maximally effective insulin level is ~100,000 times lower than the insulin concentration in preparations currently used in the replacement therapy of diabetic patients (100 units/ml) (6). Thus, given the observed saturation-type relationship between insulin concentration and insulin's effect on arteriovenous glucose difference, blood flow, and glucose uptake in human peripheral tissues, we reasoned that a similar saturation-type concentration-response characteristic for the insulin-stimulated difference between arterial blood glucose levels and interstitial fluid (ISF) glucose concentrations might exist in these tissues (online appendix Fig. 1 [available at http://care.diabetesjournals.org/cgi/content/full/dc09-1531/DC1]). An interstitial infusion of insulin into adipose tissue at rates used in the replacement therapy of diabetic patients might therefore evoke insulin concentrations at the infusion site that are much higher than the maximally effective insulin level of this tissue and, in turn, might ensure an essentially maximal and stable blood-to-ISF glucose concentration gradient in the tissue at this infusion site. If this were the case, then despite the presence of variable insulin delivery rates, reliable estimation of blood glucose concentrations from glucose concentrations measured in the ISF at the insulin delivery site would be possible.

The present study was undertaken to investigate this possibility in the subcutaneous adipose tissue of healthy subjects. Microperfusion and microdialysis techniques were utilized to perform glucose sampling and simultaneous insulin delivery at the same adipose tissue site. Using these techniques, ISF glucose samples from the site of insulin delivery were obtained in the presence of variable insulin delivery rates. The observed ISF glucose levels were then compared with plasma glucose concentrations as well as to glucose levels in ISF samples from insulin-unexposed adipose tissue.

From the [1]Department of Internal Medicine, Division of Endocrinology and Nuclear Medicine, Medical University of Graz, Graz, Austria; and the [2]Institute of Medical Technologies and Health Management, Joanneum Research Forschungsgesellschaft, Graz, Austria.
Corresponding author: Werner Regittnig, werner.regittnig@healthsite.at.
Received 16 August 2009 and accepted 14 January 2010. Published ahead of print at http://care.diabetesjournals.org on 22 January 2010. DOI: 10.2337/dc09-1531. Clinical trials reg. no. NCT00812591, www.clinicaltrials.gov.

rich4/zdc-dcare/zdc-dcare/zdc00410/zdc8182d10a | xppws | S=1 | 2/5/10 | 7:25 | doi: 10.2337/dc09-1531 | artno: 1531

RESEARCH DESIGN AND METHODS

— The study was conducted in five healthy male adult volunteers. The age of the subjects was 29.8 ± 4.0 years (range 19–44) and the BMI was 23.0 ± 0.9 kg/m^2 (20.7–26.0). Written informed consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The study was approved by the ethics committee of the Medical University of Graz.

In the morning after an overnight fast, study subjects were admitted to the clinical research center. At ~7:30 A.M., an intravenous catheter was placed into an arm vein for glucose infusion. Another catheter was inserted into a vein of the contralateral hand for blood sampling. The forearm with the sampling catheter was then placed in a thermoregulated box (55°C) to ensure arterialization of the venous samples. Subsequently, a 24-gauge microperfusion (MP) catheter (7,8) and a microdialysis (MD) catheter (CMA60, modified as described below; CMA/Microdialysis, Solna, Sweden) were inserted into the periumbilical subcutaneous adipose tissue, and peristaltic pumps (Minipuls 3; Gilson, Villiers-le-Bel, France) were attached to the inflow and outflow tubing of each catheter (Fig. 1). Both catheters were then sequentially perfused with an insulin-free solution (5% mannitol; Fresenius Kabi, Graz; Austria) for 2 h, a standard human insulin preparation (100 units/ml Actrapid; Novo Nordisk, Bagsvaerd, Denmark) for 6 h, and, again, an insulin-free solution (5% mannitol) for 2 h. During the 6-h insulin perfusion period, the insulin delivered to the tissue by the MP catheter was at a rate of ~0.33 units/h over the first 2 h, ~0.66 units/h over the subsequent 2 h, and ~1.00 units/h over the final 2 h. Similar insulin delivery rates, but in reverse chronological order, were administered via the MD catheters. As the outflow rates of the two catheters were maintained at a constant value (~0.45 μl/min) throughout the experiment, the insulin delivery rate of each catheter was adjusted by simply adjusting the inflow rate of the catheter (dual-pump operation mode) (9) (Fig. 1). During the experiment, catheter effluents were continuously collected in 30-min fractions in vials (Microvial; CMA/Microdialysis) kept on ice and covered to avoid fluid evaporation. In parallel, glucose concentrations in blood plasma were measured frequently (i.e., every 5–30 min), and, to prevent a fall in the blood glucose concentration during and after the subcutaneous insulin delivery period, glucose (0.1 g/ml; Fresenius Kabi) was intravenously infused at a variable rate.

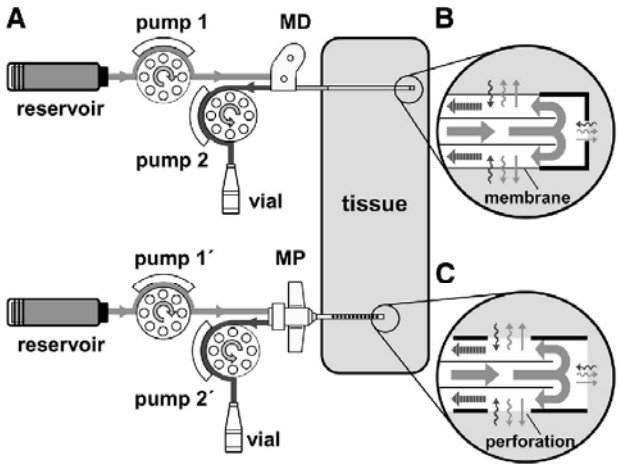


Figure 1—*Schematic of the experimental set-up for the assessment of insulin's effect on the glucose concentration at the tissue site of insulin delivery. A: A microperfusion (MP) and a microdialysis (MD) catheter were inserted into subcutaneous adipose tissue of healthy subjects (n = 5). Both catheters were perfused sequentially with an insulin-free solution (mannitol) for 2 h, a standard human insulin preparation (100 units/ml, Actrapid) for 6 h, and, again, an insulin-free solution (mannitol) for 2 h. Perfusion of each catheter was accomplished by applying two peristaltic pumps, with one pump (1, 1′) attached to the inflow tubing and another (2, 2′) to the outflow tubing (dual-pump operation mode [9]). B and C: The perfusate streamed through the inflow tubing and the inner cannula to the tip of the catheter and then entered the space between inner and outer cannula of the catheter (bold arrows). By operating the inflow pump at a higher speed than the outflow pump, a fraction of the perfusate entering the space between inner and outer cannula was forced to flow through the perforations and membrane pores of the MP and MD catheter, respectively, to the surrounding tissue (tissue-directed flows indicated by thin straight arrows). The magnitude of this flow fraction and, thus, the amount of perfusate solutes (e.g., insulin) convectively transported to the tissue was controlled by controlling the difference between the inflow and outflow rates of the catheter. In parallel to this convective tissue-directed solute transport, diffusive bidirectional solute transport took place across the perforations and membrane pores of the MP and MD catheter, respectively (wavy arrows), thereby causing ISF solutes (e.g., glucose) to enter the fluid fraction that flowed in the space between inner and outer cannula of the catheter (bold striped arrows). Along with the permeated ISF solutes, this fluid fraction was pumped continuously through catheter outlet and outflow tubing to the collecting vial. The efficiency by which glucose was transported via diffusion from the ISF of the tissue into the catheter (glucose recovery) was measured by applying the ionic reference technique (7,8).*

Microperfusion, microdialysis, and analytical procedures

MP and MD catheters applied were of concentric design with a cylindrical inner and outer tube (Fig. 1*B* and *C*). The outer tube of the MP catheter (7,8) consisted of a conventional, intravenous 24G cannula (shaft length: 19 mm, Neoflon; Becton Dickinson, Helsingborg, Sweden) in which 27 perforations (each 0.3 mm in diameter) were formed in the cannula wall using an Excimer laser (LZH, Hannover, Germany). Two 750-mm lengths of Tygon tubing (outer diameter: 2.0 mm, inner diameter: 0.19 mm; Cole-Parmer, Vernon Hills, IL) were used to connect catheter inlet and outlet with perfusate reservoir and sampling vial, respectively. In the case of the MD catheter (CMA60), part of the outer tube was made from a polyamide membrane with a molecular weight cutoff of 20 kDa (membrane length: 30 mm, pore sizes: ~1 μm). To operate MD catheters in the dual pump mode (Fig. 1), the original inflow and outflow tubing of the catheters were replaced by two 750-mm lengths of Tygon tubing. In addition, to increase the insulin delivery efficiency of MD catheters, a fluid communication between the distal inner tube end and the outer surface of the cath-

rich4/zdc-dcare/zdc-dcare/zdc00410/zdc8182d10a | xppws | S=1 | 2/5/10 | 7:25 | doi: 10.2337/dc09-1531 | artno: 1531

eter tip was established by forming a perforation into the catheter tip using a 30G syringe needle (point length: 1.2 mm; Microlance, Becton Dickinson, Drogheda, Ireland). To monitor the outflow rate of the MP and MD catheters and to determine the exact sampled effluent volume, the sample vials were weighed before and after sample collection. The effluent sampling delay time introduced by the dead space volume of the catheter outflow tubing was taken into account when the sample collection was begun.

Plasma glucose concentrations were measured at the bedside using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) with a coefficient of variation (CV) of 2%. The glucose concentrations in catheter effluents were determined using an automated CMA600 analyzer (CMA/Microdialysis) with a within-run CV of 2%. Separate in vitro experiments have shown that preservatives contained in the regular human insulin solution used to perfuse the catheters (Actrapid) do not compromise the function of the CMA600 glucose analyzer. The conductivities in the plasma, perfusate, and catheter effluent samples were measured using a contactless conductivity detector (TraceDec; I.S.T., Strasshof, Austria). The electrical conductivity was determined with a within-run CV of <1%.

Data analysis

The ISF glucose concentration was calculated (7,8) as the glucose concentration in the catheter effluent sample divided by the glucose recovery of the catheter (*R*). The recovery was determined for each sampling period as $R = (C_{out} - C_{in})/(C_{pl} - C_{in})$, where C_{in}, C_{out}, and C_{pl} are the measured electrical conductivity in the perfusate, the effluent sample, and the corresponding plasma sample, respectively. Application of this technique (ionic reference technique) (7,8) was possible because the electrical conductivity in the used catheter perfusates was either negligible (mannitol) or low compared with that in blood plasma (Actrapid: ~1.7% of average C_{pl}). To facilitate comparison of glucose time courses observed in the tissue ISF with those determined in the blood plasma, we calibrated the MP-derived and MD-derived ISF glucose values against the mean plasma glucose concentration observed during the 2-h baseline period (basal calibration). These glucose values (termed tissue glucose concentrations) and the corresponding plasma glucose levels were then used to calculate the time courses of the tissue-to-plasma glucose ratio (TPR). The differences of mean glucose and TPR results were examined by one-factor repeated-measures ANOVA. If significance was achieved, post hoc comparison of means with Dunnett's test was performed. A *P* value <0.05 was considered to indicate statistical significance. Normality of data were assessed using normal probability plots. Data are presented as means ± SE. Data analysis was performed using MATLAB (MathWorks, Natick, MA) and SPSS (SPSS, Chicago, IL) software packages.

RESULTS

Glucose dynamics at the site of subcutaneous insulin administration

Fig. 2*A* shows the time courses of the glucose concentration in arterialized blood plasma and in the tissue surrounding the MP and MD catheters. Commencement of insulin delivery with the MD and MP catheters induced a fall in the tissue glucose concentration for ~60 min ($P < 0.05$). However, during the rest of the 6-h period of variable insulin delivery, the tissue glucose concentration paralleled the glucose concentration observed in plasma, thereby indicating that insulin's effect on the tissue glucose concentration saturated within 60 min after the start of insulin administration. After switching back from insulin to mannitol perfusates, tissue glucose concentration increased slowly and reattained plasma glucose levels at the end of the experiments (Fig. 2*A*).

Figures 2*B* and *C* depict the TPR for the MD and MP catheters as a function of time. As can be seen, during the first ~60 min of insulin delivery the TPR declined to a level of 83.8 ± 3.6 and 78.5 ± 5.0% for MD and MP catheters, respectively, and then remained at these levels until the end of the 6 h period of variable insulin delivery. During the final 5 h of the insulin delivery period, the TPR levels for MD and MP catheters averaged 83.2 ± 3.1 and 77.1 ± 4.8%, respectively.

Taken together, the temporal pattern of change in tissue and plasma glucose and TPR values suggests that within 60 min after exposing adipose tissue to a standard 100 units/ml insulin preparation, insulin's effect on the tissue glucose concentration saturates and a stable ratio between the tissue and plasma glucose concentration is attained.

Glucose recoveries and insulin infusion rates

At the used outflow rate of ~0.47 μl/min, the transport efficiency (recovery) of glucose for the MP catheter was found to be 25.1 ± 5.4% during the first 2 h of insulin delivery and remained unchanged during the subsequent 2 h and final 2 h of insulin delivery (24.6 ± 4.4 and 24.8 ± 4.9%, respectively; $P = 0.93$, one-factor repeated-measures ANOVA). Similarly, recovery for the MD catheter was 96.8 ± 1.2% during the first 2 h of insulin delivery and remained at this level during the subsequent 2 h (98.0 ± 1.3%) and final 2 h (98.1 ± 1.3%) of insulin delivery ($P = 0.20$, one-factor repeated-measures ANOVA). The lower glucose recovery for MP catheters compared with MD catheters may be mainly attributable to the different size of the exchange area of the applied catheters (MP perforations distributed over an 11-mm shaft length versus MD membrane length of 30 mm). Figure 2*B* and *C* show the average time course of the total insulin delivery rate (i.e., the sum of the convective and diffusive portions of the insulin delivery rates) for the MD and MP catheters (see also online appendix supplementary methods). F2

Glucose infusion rates

The time course of the intravenous glucose infusion rate is shown in online appendix Fig. 2. As can be seen, the glucose infusion rate needed to maintain euglycemia gradually increased during the second half of the 6-h insulin delivery period and reached a peak value of 1.75 ± 0.31 $mg \cdot kg^{-1} \cdot min^{-1}$ 1 hour after termination of insulin delivery. Glucose infusion was required until the end of the experiments.

CONCLUSIONS — The present investigation was undertaken to assess insulin's effect on the tissue glucose concentration at the site of subcutaneous insulin administration. For this reason, we subcutaneously administered insulin at variable rates and simultaneously sampled ISF glucose directly from the administration site. To carry out ISF glucose sampling and simultaneous insulin administration at the same adipose tissue site, we utlized MP and MD catheters together with standard insulin preparations as catheter perfusates and used two peristaltic pumps to operate each catheter (Fig. 1). This technique of catheter operation allowed the withdrawal of ISF at a

rich4/zdc-dcare/zdc-dcare/zdc00410/zdc8182d10a | xppws | S=1 | 2/5/10 | 7:25 | doi: 10.2337/dc09-1531 | artno: 1531

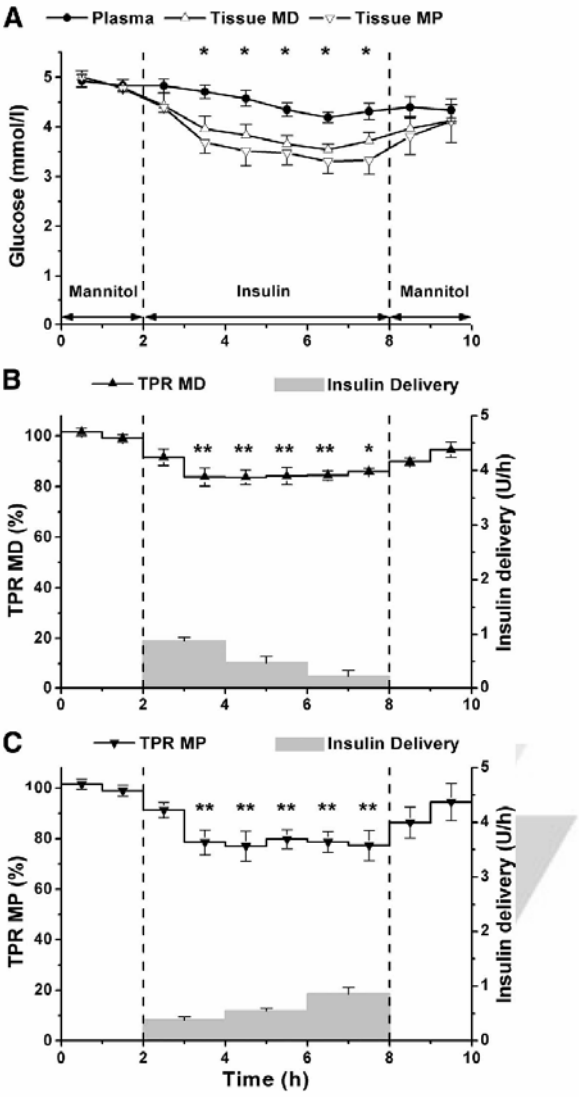


Figure 2—*Glucose dynamics at the subcutaneous tissue site of insulin delivery. A: Average time course (n = 5, means ± SE) of plasma glucose concentration (●) as well as tissue glucose concentration determined with MD (△) and MP (▽) catheters. *P < 0.05 tissue glucose vs. corresponding plasma glucose values, one-factor repeated-measures ANOVA and Dunnett's post hoc test. B and C: Average time course (n = 5, means ± SE) of the tissue-to-plasma glucose ratio (TPR) obtained with MD (▲) and MP (▼) catheters. *P < 0.05; **P < 0.01 vs. the first basal TPR value, one-factor repeated-measures ANOVA (Huynh-Feldt corrected) and Dunnett's post hoc test. Panels also show the average time course (n = 5, means ± SE) of the insulin delivery rates (bars) of the MD (B) and MP (C) catheters.*

constant rate as well as the simultaneous adjustment of the insulin delivery rate during the experiments.

When insulin delivery to the adipose tissue of the healthy humans was started at basal rates similar to those used in the replacement therapy of diabetic patients, there was an initial delay of ~60 min before the effect of insulin on the tissue-to-blood glucose concentration gradient at this delivery site reached its maximum (Fig. 2). This observed delay in insulin action (activation time) may be a reflection of the time required for the convectional and diffusional transport of insulin from the catheter to the surface of the insulin-sensitive fat cells surrounding the catheter as well as for the subsequent recruitment and activation of glucose transport proteins stimulating cell glucose uptake (10). After this activation time, despite changes in the insulin delivery rates, the tissue-to-blood glucose concentration gradient remained stable, thereby supporting our hypothesis that insulin's effect on the tissue glucose concentration at the insulin delivery site is saturated and attains steady-state values. This steady-state condition in insulin action at the insulin delivery site in turn allowed us to reliably estimate blood glucose concentrations from glucose concentrations measured in the ISF of this tissue site (11). After switching back from insulin to mannitol perfusates, the tissue glucose concentration and the tissue-to-blood glucose concentration gradient increased slowly and reattained preinsulin delivery levels by the end of the 2-h mannitol perfusion period (Fig. 2), thereby indicating that insulin's effect on the tissue glucose concentration vanished within 2 h after termination of the insulin delivery. Overall, the observed activation and deactivation kinetics of insulin action on the tissue-to-blood glucose concentration gradient in adipose tissue are compatible with previous findings in humans showing similar activation and deactivation kinetics of insulin's action on the forearm (12), leg (4), and peripheral (13) glucose uptake.

Conclusions in the present study differ from the recent study of Hermanides et al. (14), who reported no influence of insulin on the glucose concentration in the adipose tissue located near the insulin infusion site. The disparity between our results and those of Hermanides et al. (14) may be attributable to the different experimental procedure used to assess the effect of insulin on the tissue glucose at the insulin administration site. Whereas the MP and MD catheters used in the present study functioned simultaneously as insulin delivery and ISF sampling instruments (single-port approaches), Hermanides et al. (14) used a dual-port approach con-

rich4/zdc-dcare/zdc-dcare/zdc00410/zdc8182d10a | xppws | S=1 | 2/5/10 | 7:25 | doi: 10.2337/dc09-1531 | artno: 1531

sisting of a subcutaneously inserted insulin infusion catheter and an MD catheter, which had a membrane length of 25 mm (14,15) and which was inserted in subcutaneous tissue at a closest mean distance of 9 ± 2 mm from the infusion catheter. Thus, in contrast to our study in which ISF glucose was withdrawn from the insulin-exposed tissue layer surrounding the MD and MP catheter, Hermanides et al. (14) performed ISF glucose sampling from tissue regions that were at considerable distances from the insulin administration site. A previous study by Linde and Philip (16), using radiography of the radioactivity distribution of subcutaneously injected ^{125}I-insulin, indicated that when a bolus amount of 0.25 ml of a 40 unit/ml insulin solution (= 10 units) is administered subcutaneously, the maximal volume of distribution of the administered insulin may have an average cross-sectional area of ~170 mm^2 or an average radius of ~7 mm (when a near-spherical shape is assumed) (17). Furthermore, our recent study in diabetic subjects (11), using MP catheters for insulin delivery and simultaneous glucose sampling, showed that a subcutaneous administration of a bolus amount of ~0.08 ml of a 100 unit/ml insulin solution (~8 units) is causing a dilution of the ISF at the insulin delivery site, thereby decreasing the glucose concentration around the insulin delivery catheter during and shortly after the bolus delivery period. In comparison, in the study of Hermanides et al. (14), a bolus amount of ~0.11 ml of a 100 unit/ml insulin solution (~11 units) was administered. Apparently, no decrease in the effluent glucose during the bolus administrations and, thus, no dilution effect of the insulin solvent were observed. Therefore, a possible reason for not observing a significant effect of insulin and/or insulin solvent on the local tissue glucose concentration in their study may be that, due to the considerable length of the microdialysis membrane used (25 mm), the whole or most parts of the glucose-exchanging membrane, especially the membrane part near the catheter outlet, may not have been positioned in the insulin-exposed tissue layer surrounding the insulin infusion catheter. There have been other studies evaluating the effect of insulin on the glucose concentration in human adipose tissue by using either continuous glucose sensors (18,19) or microdialysis-based glucose sensing (19–21). However, whereas we and Hermanides et al. (14) assessed the effect of supraphysiological insulin levels (i.e., 100 units/ml) on the adipose tissue glucose concentration, these studies evaluated the effect of physiological insulin levels (<150 μU/ml) on this glucose concentration. Furthermore, in contrast to the study of Hermanides et al. (14) and our study, where insulin was locally introduced into the ISF of subcutaneous adipose tissue, these studies invariably increased the insulin levels in the central circulation by intravenous insulin infusions (18–21) and/or by enhancing endogenous insulin secretion using intravenous glucose infusions (18,19). The results of these studies indicated that physiological changes in the insulin levels affect (19,21) or do not affect (18,20) the ISF-to-plasma gradient in human adipose tissue. The divergent findings may be partially attributable to technical and procedural differences in the performance of the studies (18).

Besides the effect of insulin on the fat cell glucose uptake, an additional mechanism potentially influencing the glucose concentration at the site of insulin delivery may be the local dilution of the ISF by the insulin solvent. We reasoned that if an increase in the perfusate flow fraction directed to the tissue (Fig. 1, thin straight arrows) is causing a dilution of the ISF surrounding the catheter, then there will be a decrease in the efficiency of exchange of solutes between the ISF and the perfusates of the two catheters. We found that changes in the tissue-directed flow rates did not cause changes in the exchange efficiency (recovery) of the two catheters, thereby suggesting that insulin solvent delivery at basal rates is not diluting the ISF in the tissue surrounding the catheters. This observation is in agreement with the results of our recent study in diabetic subjects (11), in which after changes in the basal delivery rates no changes in the catheter recovery were observed. The relatively high blood flow per unit adipose tissue weight (30–65 μl · min^{-1} · g^{-1}) (22) compared with the amount of insulin solution infused during basal delivery (0.1–0.2 μl/min) may be the likely reason for not observing a local dilution of the ISF when basal insulin is delivered.

Our present and recent study (11) may also provide the basis for the pursuit of a principal concept to the design of a single-port treatment system. This concept involves the integration of a continuous glucose sensor directly onto the shaft wall of an insulin infusion catheter. When connected to an insulin pump, the system may then permit simultaneous insulin infusion and glucose sensing. Available information on solute transport in ISF surrounding infusion catheters (which is governed by a combination of convection, diffusion, and tissue clearance) (17,23,24) may be used to guide the exact placement of the glucose sensor onto the shaft of an insulin infusion catheter. For example, when a typical bolus amount of 0.1–0.2 ml (= 10–20 units) of a standard insulin solution is administered through a conventional infusion catheter (diameter: ~0.5 mm; shaft length: ~10 mm), there may be a significant backflow of infusate from the catheter tip along the catheter shaft (24) toward the skin, so that the initial area of distribution of infused insulin (initial depot volume) (17,23) may correspond to a cylindrical tissue layer surrounding the catheter shaft (24) and having a width of a few millimetres (17). Shortly after bolus administration, the relative rates of diffusion and clearance may then produce first an expansion of the insulin-exposed tissue layer about the catheter shaft to a width of several millimetres (e.g., ~7 mm) (16) and, as soon as clearance prevails, a decrease of the size of this layer, so that 6–10 h after bolus administration all the insulin is cleared from the tissue site of insulin administration (17,21). Furthermore, in the case of insulin delivery at basal rates (~0.01 ml/h = ~1 unit/h, usually administered as microboluses with 0.025–0.1 units per pump stroke) (25), backflow distances may be shorter and hence the volume of insulin-exposed tissue about the catheter shaft may be smaller than after bolus administrations (24). Therefore, when a basal-bolus insulin regimen is applied, the best placement location of a continuous glucose sensor on the catheter shaft may be close to the catheter tip because at this site the tissue surrounding the sensor may be permanently exposed to insulin. In the case of suspension of the insulin pump treatment, a recalibration of the glucose sensor may be required when the length of pump suspension exceeds the deactivation time of insulin action. The deactivation time, however, may largely depend on the actual insulin depot size at the delivery site, which can be very high after bolus administrations or lower after prolonged basal insulin delivery (two to five times the hourly infusion rates) (25).

In summary, our results show that within 60 min after exposing adipose tissue to a standard 100 units/ml insulin

rich4/zdc-dcare/zdc-dcare/zdc00410/zdc8182d10a | xppws | S=1 | 2/5/10 | 7:25 | doi: 10.2337/dc09-1531 | artno: 1531

preparation, insulin's effect on the tissue glucose concentration saturates and a stable ratio between the tissue and plasma glucose concentration is attained, thus indicating that insulin delivery and glucose sensing may be performed simultaneously at the same adipose tissue site via a single tissue catheter. This single-port treatment approach may hold great promise to simplify and improve glucose management in diabetes.

Acknowledgments— This work was supported in part by funding from Science Park Graz and the Federal Ministry of Economics and Labor of the Republic of Austria.

T.P. is a cofounder and shareholder of Smart*Med and a consultant of Novo Nordisk and Sanofi-Aventis. M.E. is a consultant of Sensile Medical and BBraun and a cofounder and shareholder of Smart*Med. L.S. and H.K. are cofounders and shareholders of Smart*Med. W.R., L.S., G.K., M.E., and T.P. have filed patent applications relating to the methodology described in this report. No other potential conflicts of interest relevant to this article were reported.

We are grateful to A. Wutte for her expert assistance in conducting the study, M. Suppan for performing insulin assays, A. Groselj-Strele for her assistance with statistical analysis, and all volunteers for participating in the study.

Parts of this study have been presented in abstract form at the 2nd European Diabetes Technology and Transplantation Meeting, Innsbruck, Austria, 27–29 January 2008.

References

1. Rizza RA, Mandarino LJ, Gerich JE. Dose-response characteristics for effects of insulin on production and utilization of glucose in man. Am J Physiol 1981;240:E630–E639
2. Kono T, Barham FW. The relationship between the insulin-binding capacity of fat cells and the cellular response to insulin: studies with intact and trypsin-treated fat cells. J Biol Chem 1971;246:6210–6216
3. Nesher R, Karl IE, Kipnis DM. Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. Am J Physiol 1985;249:C226–C232
4. Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man: a novel mechanism for insulin resistance. J Clin Invest 1990;85:1844–1852
5. Bonadonna RC, Saccomani MP, Seely L, Zych KS, Ferrannini E, Cobelli C, DeFronzo RA. Glucose transport in human skeletal muscle: the in vivo response to insulin. Diabetes 1993;42:191–198
6. Hirsch IB. Insulin analogues. N Engl J Med 2005;352:174–183
7. Schaupp L, Ellmerer M, Brunner GA, Wutte A, Sendlhofer G, Trajanoski Z, Skrabal F, Pieber TR, Wach P. Direct access to interstitial fluid in adipose tissue in humans by use of open-flow microperfusion. Am J Physiol 1999;276:E401–E408
8. Trajanoski Z, Brunner GA, Schaupp L, Ellmerer M, Wach P, Pieber TR, Kotanko P, Skrabal F. Open-flow microperfusion of subcutaneous adipose tissue for on-line continuous ex vivo measurement of glucose concentration. Diabetes Care 1997;20:1114–1121
9. Regittnig W, Koehler G, Bodenlenz M, Schaller HC, Koehler H, Ellmerer M, Schaupp L, Pieber TR. Coupling of subcutaneous insulin delivery and subcutaneous glucose sensing by means of a microperfusion or microdialysis probe. Abstract presented at the 5th Annual Diabetes Technology Meeting, San Francisco, CA, 10–12 November 2005
10. Carruthers A. Facilitated diffusion of glucose. Physiol Rev 1990;70:1135–1176
11. Regittnig W, Lindpointner S, Korsatko S, Kohler G, Kaidar R, Yodfat O, Kohler H, Ellmerer M, Pieber TR. Glucose concentrations at the site of subcutaneous insulin delivery in patients with type 1 diabetes. Diabetologia 52(Suppl. 1):S42, 2009
12. Utriainen T, Malmström R, Mäkimattila S, Yki-Järvinen H. Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. Diabetologia 1995;38:555–564
13. Prager R, Wallace P, Olefsky JM. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. J Clin Invest 1986;78:472–481
14. Hermanides J, Wentholt IM, Hart AA, Hoekstra JB, DeVries JH. No apparent effect of insulin on microdialysis continuous glucose-monitoring measurements. Diabetes Care 2008;31:1120–1122
15. Maran A, Crepaldi C, Tiengo A, Grassi G, Vitali E, Pagano G, Bistoni S, Calabrese G, Santeusanio F, Leonetti F, Ribaudo M, Di Mario U, Annuzzi G, Genovese S, Riccardi G, Previti M, Cucinotta D, Giorgino F, Bellomo A, Giorgino R, Poscia A, Varalli M. Continuous subcutaneous glucose monitoring in diabetic patients. Diabetes Care 2002;25:347–352
16. Linde B, Philip A. Massage-enhanced insulin absorption: increased distribution or dissociation of insulin? Diabetes Res 1989;11:191–194
17. Trajanoski Z, Wach P, Kotanko P, Ott A, Skrabal F. Pharmacokinetic model for the absorption of subcutaneously injected soluble insulin and monomeric insulin analogues. Biomed Technik 1993;38:224–231
18. Steil GM, Rebrin K, Hariri F, Jinagonda S, Tadros S, Darwin C, Saad MF. Interstitial fluid glucose dynamics during insulin-induced hypoglycaemia. Diabetologia 2005;48:1833–1840
19. Monsod TP, Flanagan DE, Rife F, Saenz R, Caprio S, Sherwin RS, Tamborlane WV. Do sensor glucose levels accurately predict plasma glucose concentrations during hypoglycemia and hyperinsulinemia? Diabetes Care 2002;25:889–893
20. Rosdahl H, Lind L, Millgard J, Lithell H, Ungerstedt U, Henriksson J. Effect of physiological hyperinsulinemia on blood flow and interstitial glucose concentration in human skeletal muscle and adipose tissue studied by microdialysis. Diabetes 1998;47:1296–301
21. Moberg E, Hagström-Toft E, Arner P, Bolinder J. Protracted glucose fall in subcutaneous adipose tissue and skeletal muscle compared with blood during insulin-induced hypoglycaemia. Diabetologia 1997;40:1320–1326
22. Rosell S, Belfrage E. Blood circulation in adipose tissue. Physiol Rev 1979;59:1078–1104
23. Mosekilde E, Jensen KS, Binder C, Pramming S, Thorsteinsson B. Modelling absorption kinetics of subcutaneous injected soluble insulin. J Pharmakokinet Biopharm 1989;17:67–87
24. Morrison PF, Chen MY, Chadwick RS, Lonser RR, Oldfield EH. Focal delivery during direct infusion to brain: role of flow rate, catheter diameter, and tissue mechanics. Am J Physiol 1999;277:R1218–R1229
25. Kreagen EW, Chisholm DJ. Pharmacokinetics of insulin: implications for continuous subcutaneous insulin infusion therapy. Clin Pharmacokinet 1985;10:303–314

rich4/zdc-dcare/zdc-dcare/zdc00410/zdc8182d10a | xppws | S=1 | 2/5/10 | 7:25 | doi: 10.2337/dc09-1531 | artno: 1531

CURRICULUM VITAE

Roland Schaller

PERSONAL DATA

Date of Birth:	3rd of October 1974
Nationality:	Austria
Address:	Merangasse 42,
	A-8010 Graz
E-mail:	fastbeat@brainity.com

EDUCATION

2004 – 2010	Doctoral study at the Graz University of Technology, Graz, Austria Doctoral thesis in cooperation with Joanneum Research, Graz, Austria “New methods for glucose monitoring in humans”
1994 - 2002	Study of Biomedical Engineering at the Graz University of Technology Diploma thesis in cooperation with Bosch, Stuttgart, Germany “Development of a force sensor for the airbag control unit”
1989 - 1994	HTBLA, LiTec, Linz, Austria

POSITIONS

Since 2004	Scientist at Joanneum Research, Graz, Austria
2002 - 2010	Volunteer at women’s shelter “Haus Elisabeth“, Caritas, Graz, Austria
1999 - 2004	Medizintechnik Kozak, Gratwein, Austria Assistant for recurrent testing of medical electrical equipment
2002	Study assistant at Joanneum Resarch; Projects Adicol and Lungsound