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Automated Blood Glucose Control

Development and Testing of an Artificial Endocrine Pancreas Using a Novel Intravascular Glucose Monitor and a New Approach to Insulin Pharmacology

Thesis for the degree of Philosophiae Doctor

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Norsk sammendrag

Automatisert blod glukose kontroll – utvikling og uttesting av en kunstig endokrin pankreas bestående av en intravaskulær glukose monitor og en ny tilnærming til insulin farmakologi

Denne PhD-avhandlingen omhandler utviklingen og testingen av en ny kunstig endokrin pankreas. Bestanddelene i en kunstig endokrin pankreas er en glukose-sensor, en insulin infusjons sprøyte og en kontroll-algoritme. Vårt utgangspunkt var at eksisterende tilnærminger til denne problemstillingen ikke fungerer, og vi identifiserte noen av de bakenforliggende biologiske forklaringene til hvorfor så er tilfelle. Vi satte så ned en rekke forskningsspørsmål/-problemstillinger med det endelige og overordene mål å lage en fungerende kunstig endokrin pankreas.

Den første artikkelen beskriver utprøvingen av en ny intravaskulær glukose-sensor – IvS-1 – i en levende grise-modell. Studiet viste at verdier målt med IvS-1 hadde god overensstemmelse med glukose-verdier fra samtidige blodprøver, og at respons-tiden i apparatet var rask. Den andre artikkelen studerte farmakologiske egenskaper ved intravenøs administrering av insulin. Hovedfokus var å finne tids-forskyvningen fra gitt insulin til endringer i grisenes blod glukose nivå. Den tredje artikkelen viste at blod glukose verdien var den samme i arterielt og venøst blod, selv under særdeles ustabile sirkulatoriske forhold. I den fjerde artikkelen satte vi sammen all vår akkumulerte kunnskap og laget en kunstig endokrin pankreas. Vi brukte IvS-1 som glukose-sensor og regulerte ned blod glukosenivået i diabetiske griser vha. vår nyutviklete insulin-algoritme med repeterte intravenøse insulin bolus.

Avhandlingen konkluderes med å besvare forskningsspørsmålene, og slår fast at en kunstig endokrin pankreas er biologisk mulig. Men det vil være særdeles teknisk krevende å lage et stabilt system som kan fungere i en klinisk setting..

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Table of contents

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I first met with Petter Aadahl as a medical student back in 2001, when I was writing an undergraduate thesis on oxygen physiology with him as my primary supervisor. I think that that work was the starting point for my subsequent deep fascination with physiology, and vital in my later choice to become both an anesthesiologist as well as a scientist. Petter has been my mentor ever since, and I attribute much of my success to his everlasting faith in me and my ideas. You have never given me simple and straightforward assignments, but encouraged me to follow my own ideas – this is a

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List of papers

Paper I: Skjaervold NK, Solligård E, Hjelme DR, Aadahl P. Continuous measurement of blood glucose: Validation of a new intravascular sensor. Anesthesiology 2011; 114: 120-125.

Paper II: Skjaervold NK, Lyng O, Spigset O, Aadahl P. Pharmacology of intravenous insulin administration: Implications for future closed-loop glycemic control by the intravenous/intravenous route. Diabetes Technology & Therapeutics 2011, 13(11): 1-7.

Paper III: Skjaervold NK, Aadahl P. Comparison of arterial and mixed venous blood glucose levels in hemodynamically unstable pigs: Implications for location of a continuous glucose sensor. Acta Diabetologica 2012 [Epub ahead of print]

Paper IV: Skjaervold NK, Ostling D, Lyng O, Spigset O, Hjelme DR, Aadahl P. Blood glucose control in diabetic pigs using a novel continuous blood glucose monitor and repetitive intravenous insulin boluses: Exploiting natural insulin pulsatility as a pharmacological principle for a future artificial pancreas. Manuscript

Summary

This thesis describes the stepwise development and testing of the components of a novel artificial endocrine pancreas, consisting of a continuous glucose monitor, an insulin infusion device and a control algorithm. The starting point was the belief that current approaches to the challenges posed by creating an artificial endocrine pancreas do not work, and we identified some of the biological mechanisms that can explain this lack of success. We further established a set of research questions with the overall goal of constructing a working artificial endocrine pancreas.

The first paper describes the *in vivo* (pig model) testing of a prototype of a new intravascular continuous glucose monitor, the IvS-1. The study determined that the monitor was able to produce readings with a reasonably fast response time that highly agreed with simultaneously drawn blood samples. The second study examined the pharmacological properties of intravenous insulin boluses, and estimated the time delays from insulin administration until changes in the blood glucose level. The third study demonstrated that blood glucose levels were the same in the arterial and venous systems, even at times of great circulatory stress. The fourth study relied on our accumulated knowledge to enable us to construct an artificial endocrine pancreas using the IvS-1 and a novel insulin regulatory algorithm based on insulin boluses. We then regulated the blood glucose level in diabetic pigs in a proof-of-concept study.

The thesis concludes by answering the research questions, and asserts that the construction of an artificial endocrine pancreas is possible from a biological standpoint.

However, there are several serious technological challenges that have to be overcome in order to make a stable system for in-hospital – and later outpatient – use.

Introduction

Hyperglycemia

Diabetes mellitus is a highly prevalent disease worldwide. According to the World Health Organization, diabetes affected approximately 346 million people in 2011, caused an estimated 3.4 million deaths in 2004, and is often described as an epidemic (1). Type 1 diabetes is a condition where pancreatic beta cells are destroyed as a result of mechanisms that are not fully understood. The illness usually has its debut in childhood, and renders the patient absolutely dependent on insulin. Type 2 diabetes is considered a "lifestyle" disease, although much evidence points to a genetic disposition. The illness probably combines increased insulin resistance (in liver, muscles and fat) with decreased insulin production. In addition to classical type 1 and type 2 diabetes, there are several clinical syndromes of dysglycemia such as: mature onset diabetes of the young (MODY), late-onset type 1 diabetes, gestational diabetes, etc. (2).

Critically ill patients in intensive care units (ICU) of modern hospitals tend to develop hyperglycemia as a result of a series of mechanisms involving general organ dysfunction, their catabolic state and iatrogenic factors such as steroid therapy and high doses of dietary sugars (3,4). At the turn of the millennium, several studies showed beneficial outcomes in ICU patients – a shorter ICU stay, fewer complications and lower mortality – if their blood glucose levels (BGL) were kept within normal values (4.5 – 6.0 mmol/l) with the use of insulin infusions (5-7). However, subsequent studies have failed to replicate these promising findings (8,9). This has led to what could be

called a *blood glucose controversy*, where different research groups are fighting over the pros and cons of tight glycemic control.

Glucose/insulin (patho-) physiology

The insulin/glucose regulatory system is complex and not fully understood. Insulin is secreted from the beta cells of the pancreas into the portal blood stream. During a sudden increase in BGLs there will be a biphasic increase in plasma insulin concentration; an initial rapid surge with a peak concentration within minutes is caused by the release of stored insulin from granules, while a second steadily continuing increase is caused by the instant secretion of newly synthetized insulin (2). However, studies dating back as far as the 1920s have shown that BGLs in resting mammalians are not stable, but display tiny oscillations with a periodicity of 10 – 15 minutes (10-13). Recent research has attributed this to the pulsatile nature of pancreatic beta cells (14,15). Insulin is released into the portal blood stream in synchronized bursts with a periodicity of approximately five minutes (16-19). The amount of insulin released with each burst is adjusted in conjunction with the existing BGL and the direction of change of the BGL at that time (20). Even in periods with a stable demand and supply of glucose, insulin pulses are always changing, resulting in small variations in BGLs (16,19).

Glucagon is the system's main counter-regulatory hormone and has an effect that is opposite that of insulin. Interestingly, glucagon is released from the pancreas anti-synchronally with insulin, and with the same periodicity (21,22).

The pulsatility of pancreatic beta cells and hence the oscillatory insulin levels seem to diminish in type 2 diabetes (23,24). Plasma insulin or BGL oscillations in ICU patients have not been studied. However, a recent study found gross decomplexification in the variation in BGL time series in ICU non-survivors (25). This indicates a vital failure in their insulin/glucose regulatory system, meaning that their hyperglycemia is not merely caused by glucose overload.

Blood glucose control and blood glucose measurement

The traditional methods for BGL control in diabetic patients are dietary regulation, subcutaneous injections of insulin, and/or (in type 2 diabetes) oral medications (biguanides, sulfonylurea derivatives, and others) (26).

Insulin was first isolated in 1922, and very soon afterwards was used in the treatment of diabetes mellitus, which resulted in a dramatic improvement in the lives of diabetic patients (27). In the first decades after WW II, the focus was to prolong the acting time of insulin to limit the number of injections, leading to the first “intermediate-acting” families of insulin in the 1940s and 1950s, and “long-acting” families starting in the 1980s (28). In recent years, “short-” and “ultra-short acting” insulin have been produced to meet the needs of current multiple daily insulin injection regimes and insulin pump therapy (29). Earlier forms of insulin were extracted from bovine and porcine pancreas, while most current insulin for medical use is produced with modern recombinant techniques. Most recently, subcutaneous insulin pumps have emerged for diabetic

control in type 1 diabetes patients, gaining widespread popularity in the USA and in northern Europe (30).

Intravenous insulin infusion, on the other hand, is used only in hospitals for control in complicated diabetes (such as in diabetic ketoacidosis) and in glycemic control in ICU patients (31). It is generally accepted that intravenous insulin administration yields the fastest onset, but there are very few studies that examine the pharmacology of intravenous insulin administration. The elimination half-life of insulin is reported to be approximately 6 minutes (32). This means that by using an ordinary "rule of thumb," it will take approximately 30 minutes for an intravenous insulin infusion to reach steady-state plasma levels (5 times its elimination half-life). However, there is probably an unknown time delay in the pharmacodynamic action of insulin, meaning that the time from a change in the stable plasma values of insulin until a concurrent change in BGL is not known. We have not found any studies that examine the time lags between changes in intravenous insulin administration and changes in BGL.

The lives of diabetic patients have been very much improved since the introduction of lightweight easy-to-use blood glucose monitors for home use. These devices require a small blood specimen obtained by a lancet, and enable multiple daily insulin injection regimes and a more normalized diet (33). Over the last two decades, systems for continuous blood glucose measurement (CGM) have been developed. These instruments use an electrochemical sensing unit based on the same principle as the glucose oxidase sensors found in standard blood gas analyzers. Glucose is converted to gluconic acid and hydrogen peroxide, where the latter delivers electrons to the electrode and creates

an electric current proportional to the concentration of glucose. The sensing unit is located in a needle for minimally invasive *subcutaneous* positioning, and a box with the processing unit, power supply and output display is carried attached to the body (34-38).

There has been some research activity on *intravascular* CGM as well. One study has described the feasibility of enzymatic sensors (essentially the same technology as a subcutaneous CGM) in dogs. The sensors were implanted in the central caval vein for 1 – 15 weeks and demonstrated acceptable agreement with blood glucose values and high bio-compatibility (39). As recently as 2006, a study was published describing the use of an enzymatic intravascular CGM in combination with intra-peritoneal insulin delivery (40). However, a recent review from (among others) the same author suggests that both intravenous CGM as well as intra-peritoneal insulin delivery have been abandoned (41). However, today there appear to be several commercial groups working with intravascular CGM using various technological approaches. The motivation is probably the introduction of tight glycemic control in ICUs, where the invasive approach of intravascular positioning is more acceptable than in outpatient care. It is complicated to get a comprehensive overview of all of the developments this field, since these groups rarely publish their results in scientific journals, and there are few review articles. A recent report from the NTNU Technology Transfer Office identified and classified 11 companies/products that were working on intravascular CGM (Table 1), none of which have yet reached the commercial market.

Classification	Product (Company)	Technology
Intravascular	GlySure	Fluorescence and fiber optic sensor
	GluCath (Glumetrics)	Fluorescence and fiber optic sensor
	iGlyko	Fluorescence and fiber optic sensor
	Glucoclear (DexCom & Edwards Lifescience)	Electrochemical sensor inside an IV catheter
Intravascular microdialysis	Eirus CGM (Dipylon Medical)	Microdialysis
	MicroEye (Probe Scientific)	Microdialysis
Periodic blood samples	Proxima (Sphere Medical)	Microchip
	Cascade Metrix	Electrochemical
	OptiScanner (Optiscan Biomedical)	IR spectroscopy
	GlucoScout (Via Medical)	Electrochemical
	STG-22 (Nikkiso)	Electrochemical

Table 1: Overview of different commercial approaches to intravascular continuous glucose measurement (supplied by and with permission from Nicolas Elvemo at NTNU Technology Transfer Office)

Artificial endocrine pancreas (AEP)

The ultimate solution to BGL regulation in diabetic patients is generally seen as the artificial endocrine pancreas (AEP). It consists of a CGM, an insulin infusion system and a control algorithm, assembled as a fully automated closed loop regulation system with no need for manual adjustment (41-47) .

In the late 1950s, Technicon Instruments developed the Autoanalyzer, which was capable of online continuous measurements of glucose in whole blood. This instrument was the ancestor of a series of experiments that used similar techniques: Blood was withdrawn from a dual-lumen intravenous catheter and continuously analyzed *ex vivo,* usually using the glucose-oxidase method (48). The time from when blood was drawn until the output reading was available was approximately five minutes. This type of system could be used both as a diagnostic tool in the management of diabetic patients (49), and as helpful guidance during surgical resection of insulinomas (50). These instruments were used as the input link in the first generation AEPs, which were extensively studied during the 1970s, and which led to the development of the Biostator (51) and later the STG-22 (52-55). In these systems, insulin is infused into the patient through an intravenous cannula, and the insulin infusion rate is regulated by a simple algorithm. These systems are able to regulate blood glucose levels quite well. However, they are hampered by their large size, their invasiveness and the need to immobilize the patient, making them suitable only for monitoring during surgery, intensive care and possibly as research tools.

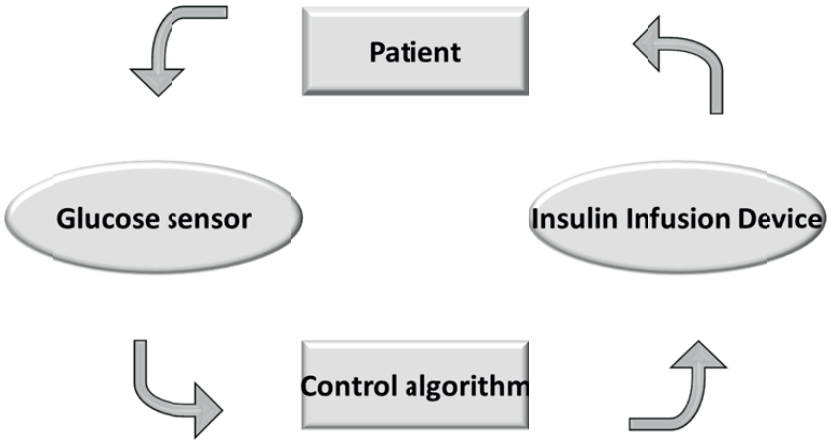

Figure 1: Outline of an artificial endocrine pancreas.

During the past decade, most of the research on AEP in Europe and North America has been focused on combining the use of off-the-shelf subcutaneous CGM and subcutaneous insulin pumps (43,44). These systems are designed for outpatient use and clearly have some advantages as a result of their minimal invasiveness and use of standard components, which should yield relatively low costs. Several systems have been tested in clinical trials, but few have been tested for more than a few hours to one day, and always with a manual backup system to avoid unwanted hypoglycemia (56-62).

A serious challenge to the construction of an AEP is the insulin control algorithm. The traditional method used by the early Biostator and probably by the STG-22 is the classical proportional-integral-derivate (PID) controller. PID controllers do not try to model the insulin/glucose system at all, but merely address the accumulated past errors in BGL, the current BGL and its direction of change in order to calculate the appropriate

insulin dose (63-65). PID controllers have now been basically abandoned but are still used in a few AEP studies (62,66,67).

The main problem posed by PID controllers is the serious time-delay issue, which affects all aspects of the AEP, from the time delay in BGL between the blood compartment and the subcutaneous tissue where the CGM is located to the time delay from subcutaneously administered insulin until a change in BGL (68-70). Several researchers have tried to mathematically model the natural glucose/insulin regulatory system, and the early "minimal model" by Bergman constitutes the foundation of most of these models (71). Adapting these models to a control algorithm results in what is called model-predictive-control (MPC) (72-75). Most research groups working on AEP use MPC, but a group led by Roman Hovorka at the University of Cambridge clearly has the lead. The current models are undoubtedly impressive, but using MPC in working AEP systems is subject to several limitations. First of all, as stated above, current AEP systems only work for a few hours. In a recent review, Hovorka downgrades expectations regarding the future of AEP, stating:

"Nowadays, early generations of the artificial pancreas are setting less ambitious but realistic and clinically important goals to prevent hypoglycemia or to reduce its duration and severity whilst improving overall glucose control" (44)

Other leading researchers are more optimistic regarding future subcutaneous-based AEP, stating:

"Finally, fully automated closed-loop is expected to deliver safe and efficacious glucose control at home for a prolonged period of time" (41)

Our group doubts whether a fully automated AEP is possible using the subcutaneous route (given that technical issues such as long-term stability and bio-compatibility must be addressed), because we think there are inherent limitations in the overall approach. Some critical issues are: 1) insulin sensitivity in the natural insulin/glucose regulatory system, which is constantly changing (76) and constitutes a serious challenge in forecasting BGL values; 2) the oscillatory nature of resting BGL and the pulsatility of insulin secretion; 3) the possibility, suggested by some authors, that the insulin/glucose regulatory system exhibits deterministic chaotic components (77), meaning that arbitrary small changes in the initial condition cause large future errors so that it is not possible to foresee the effect of insulin over time, no matter how complicated the pharmacokinetic model is; 4) and finally, the roles of glucagon and other counter-regulatory hormones are not included.

This thesis takes a new look at the physiology of glucose control by *in vivo* testing a novel intravascular CGM and by re-investigating an intravenous mode of insulin administration. We propose a new approach to combining these aspects into a new AEP, concluding with *in vivo* test of the system.

Aims of the study

The overall aim was to develop a possible technological and biological platform for future automated blood glucose regulation by the intravenous/intravenous route, and to test the feasibility of this system in an animal model.

Aim 1: To test whether a novel intravascular continuous glucose monitor could measure blood glucose in good agreement with state-of-the-art blood sample analyses of glucose, and with a minimal time delay. To further develop the system to provide real-time readouts of the current blood glucose level.

Aim 2: To determine the inherent lag time in the insulin/blood glucose system. To use this information to make a new algorithm for rapid and predictable blood glucose control.

Aim 3: To get a firm idea of the pros and cons of glucose monitor positioning in different intravascular locations.

Aim 4: To establish a diabetic pig model.

Aim 5: To put all of this information together in the newly adapted glucose control algorithm and the intravascular continuous glucose monitor to regulate the blood glucose level in diabetic pigs.

Methodological considerations

The IvS-1 CGM

The IvS-1 sensor (Invivosense, Trondheim, Norway) is a biosensor with a hydrogel matrix incorporated with 3-phenylboronic acid. The gel contracts with rising glucose concentrations as a consequence of glucose-induced cross binding of the phenylboronic molecules. The hydrogel is fabricated on the tip of an optical fiber, and the whole sensor is covered with a partly heparinized semi-permeable coating. The diameter of the gel is measured using an interferometric technique. Changes in glucose concentrations cause the volume of the gel to change, causing its diameter to change accordingly (78). The sensor only measures a relative change in glucose concentration, and needs to be calibrated against some other method of glucose measurement.

In the original version (used in Paper I) the output unit and user interface of the IvS-1 only displayed the continuous length measurement of the gel. The glucose values were calculated afterwards; the length measurement data had to be calibrated with simultaneously sampled blood sample glucose measurements (from a Radiometer ABL 720 blood gas analyzer; Radiometer, Brønshøj, Denmark). Additionally, the data were corrected based on the pH measurements from the blood samples, and a linear drift constant was added to correct for the constant background swelling of the gel.

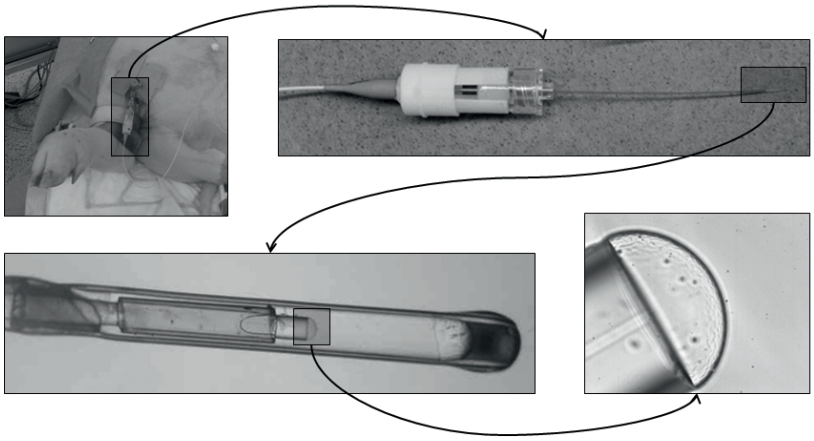

Figure 2: Pictures that show the hydrogel part of the IvS-1 continuous blood glucose monitor at increasing magnification.

In order to use the IvS-1 data as part of the AEP trials in Paper IV, we needed the system to yield real-time BGL data. This was achieved by an *in vitro* calibration procedure where the sensors were exposed to three buffer solutions of 0.0, 2.0 and 10.0 mmol/l glucose. After insertion and stabilization of the sensor, one-point calibration was achieved by adjusting the IvS-1 output in accordance with blood samples. This was very challenging when there were great oscillations in values; because the time from blood is withdrawn until the glucose value is obtained is approximately two minutes. By this time, the animals' real BGL could have changed by several decimals mmol/l. The software for the sensor system (LabView application; National Instruments, Austin, TX)

therefore had to adjust the current output in accordance with the output at the time of blood withdrawal, which was not easily accomplished.

The animal model

Our group has a long tradition in the use of pigs in the laboratory. The advantages of pigs are their physiological similarity to humans; the size of the animal, including its organs and blood vessels are such that standard instruments and equipment used in humans can be used; and their ease of handling and relative inexpensiveness and easy availability (79). Handling, fasting, anesthesia, surgical preparation and the killing of the animals is described in the papers (the most complete details can be found in Paper I).

In Study IV we had to construct a diabetic pig model, and we chose to do this using streptozotocin, which induces beta cell destruction. This is a well-known method, mostly used in rodents but also to some extent in swine (80-82). The available literature regarding streptozotocin-induced diabetes in swine is based on the construction of lasting diabetic models in mini-pigs. Our approach was a little different from those described previously, as we only wanted partly diabetic individuals and we only needed the animals for a short acute experiment. As the drug is very expensive, we had to find the smallest working dose with the correct time interval from drug introduction until the day of the experiment, while unable to afford to conduct any trials before the real experiment. That was indeed a challenging task, and resulted in the fact that not all

animals became as diabetic as we wanted. Nevertheless, the model was fair enough for use in this proof-of-concept study.

All animal experiments were conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (83) and were approved by the Norwegian State Commission for Animal Experimentation. In all animal experimentation the "three Rs" must be considered (84):

Replacement: The studies reported in this thesis had to be performed on an intact organism, because we were seeking information about the complicated and not fully understood interplay of the insulin glucose regulatory system. In addition, the validation of the IvS-1 reported in Paper I warranted the use of an animal model.

Reduction: We always analyzed the data continuously as the studies went on to reduce the workload, the cost and the use of animals, and I do not think that we performed any unnecessary animal sacrifices. One could ask whether the same animals could have been used several times. This is not so easy, both because of the animal welfare issues (multiple trials/anesthesia, postoperative care) as well as the fact that domestic pigs grow very fast. In the future, it might be more reasonable to create a long-time diabetic pig model by using some type of miniature pig, instrumenting them with permanent catheters and using only light sedation for multiple studies.

Refinement: We believe that our general knowledge regarding pigs in the laboratory as well as our experience with human anesthesia made it possible to keep the animal

suffering at an absolute minimum. In addition, most of the experiments were acute experiments. We always strived to keep our techniques and measurements at the highest possible level to yield good and trustworthy results.

Data handling and statistics

Paper I involved comparing the performance of the IvS-1 with standard blood gas analyses obtained from the Radiometer ABL 720 machine. Ordinary statistics, such as correlation and regression, are not very useful in assessing the performance of the ability of different medical technological instruments to measure the same physiological variable. This is so because there is no null hypothesis, the two sets of measurements should ideally to be the same (85-87). We therefore compared the measurements using a Bland-Altman analysis, calculating the mean bias and the *95 % limits of agreement*. In addition, since the measurements were obtained from a set of probes in a set of pigs, they were not independent. We therefore performed analyses of variance as recently described by Bland and Altman to check for possible clustering at the animal and sensor level (88). A problem with the latter method is that it does not allow for adjusting for clustering at more than two levels (i.e. hierarchical measurements; here: pigs < probes < measurement). However, since we did not find any clustering of importance on any of the levels (we performed the analysis twice), we found the method to be sufficient.

In Paper II we analyzed the effect of three different bolus doses of insulin on a series of pre-defined variables in four pigs: The time until the first detectable decrease in BGL (T_{decr}), the maximum rate of decrease (MRD), the time until MRD ($T_{decrmax}$), the

maximum decrease in BGL (ΔC_{max}), the time until the lowest measured BGL (T_{min}) and the area corresponding to the drop in the BGL curve for the first 30 minutes after the insulin bolus administration (the integral of $BGL_{30} - BGL_0$ =area over the curve from 0 to 30 minutes; $AOC_{0\text{-}30}$); see Figure 1, Paper II. To account for the clustering in animals we used linear mixed effect models with insulin dose as the fixed effect, the animal as the random effect and the defined variables as the outcome variables (89).

In Paper III we made a point of showing the importance of treating clustered variables as non-independent. We compared arterial and mixed venous BGLs from a set of pigs from two different studies (study < pig < sample). We obtained a small mean difference between the measurements, and considered this difference using both the ordinary Student's t-test and mixed effect models.

In Paper IV we were able to use ordinary descriptive statistics.

All the statistical analyses were conducted using R software, version 2.10.1 (the R Foundation for Statistical Computing, Vienna, Austria).

Summary of results (from papers)

Paper I

The aim of the study was to validate the performance of the IvS-1 in a pig model that involved the use of 20 different probes in seven animals (one to four probes in each animal), which resulted in 807 paired IvS-1/blood gas readings. Fluctuations in the BGL curve were achieved by intravenous insulin and glucose. The sensors were tested over a range of blood glucose values with the lowest value at 0.63 mmol/l and the highest at 15.75 mmol/l. The mean bias between the measurements (IvS-1 – ABL) was 0.0131 mmol/l with a standard deviation of 0.470 mmol/l and a *95 % limits of agreements* of (-0.908, 0.934) mmol/l (Figure 1, Paper I). Among the 121 paired measurements in the hypoglycemic area below 2.2 mmol/l, the mean bias was – 0.0435 mmol/l with a standard deviation of 0.260 mmol/lol/l and a *95% limits of agreements* of (– 0.553, 0.466) mmol/l.

Paper II

The aim of the study was to predict the lag time in the insulin/blood glucose system, i.e. to find the time from intravenous insulin administration until predefined changes in the BGL were observed. We reported findings from studies in 12 pigs. We found that intravenous insulin *infusions* at "clinical" rates led the BGL to decline slowly. We therefore introduced intravenous bolus *injections* (IB) as an alternative, using three different insulin doses of 0.01, 0.02 and 0.04 IU/kg. IB led to a first detectable decrease

(T_{decr}) in 2 – 6 minutes, a maximum rate of decrease ($T_{decrmax}$) shortly thereafter, and a nadir value (T_{min}) in 15 – 20 minutes before the BGL started to rise again. These time variables were independent of the IB dose. We found the maximum rate of decrease (MRD) to vary from 0.10 to 0.14 mmol/l/min dependent on the IB dose. The maximum decrease (ΔC_{max}) as well as the drop in the BGL curve for the first 30 minutes (AOC_{0-30}) were also dependent on the IB dose.

Paper III

The aim of this study was to compare BGL values obtained from simultaneously drawn arterial and mixed venous blood samples in a pig model of gross circulatory stress. We used previously collected material from an ischemia/reperfusion model that consisted of 116 paired data sets from 11 pigs in two studies. We found a mean difference between the BGL values of 0.065 mmol/l, with the mixed venous value slightly higher than the arterial value. However, when the values were compared using a mixed effect model where the experiments and the subjects were included as random effects, we found no difference between the groups. We concluded that BGL values obtained from the two locations were the same.

Paper IV

In this study we constructed an insulin bolus algorithm by using our knowledge from Paper II. We constructed a diabetic pig model, and reprogrammed the IvS-1 to be able to display real-time BGL values by introducing calibration procedures and automated

compensation for sensor drift. The aim was to adjust the BGL of the diabetic animals by lowering the BGL to target values (4.5 – 6.0 mmol/l) in one to two hours, and to keep their BGL within those values for the rest of the study period. The animals developed a mildly diabetic state from streptozotocin pre-treatment (starting BGL, BGL_0 from 7.46 to 14.06 mmol/l). They were steadily brought into the target range in 21 to 121 min, depending on their BGL_0. The animals were then kept within the target for 128 to 238 min; the time at hypoglycemic levels below 4.5 mmol/l varied from 2.9 to 51.1 min. The main problems were the drift of the IvS-1 sensor and difficulties with the calibration procedure, which led to incorrect real-time BGL values. The study confirmed our hypotheses regarding this new principle for blood glucose control, and the algorithm was constantly improved during the study to produce the best results in the last animals.

Results and discussion

Validation of the IvS-1 CGM

In Paper I we concluded that the performance of the IvS-1 was promising with measurements in high *agreement* with the Radiometer ABL 720 analyzer, and especially that the sensor performed well in the low glucose range. It should be noted that the much-used term *accuracy* is somewhat problematic, as this requires a comparison with a true value that cannot be obtained when it comes to physiological measurements such as BGL (85). At best, the agreement can be compared with the best available method (the gold standard). Nevertheless, the term accuracy is so frequently a part of any discussion of CGMs that I too will use it to some extent[1]. In the published article we stated that the variance was equally spread throughout the range of measurements (Figure 1, Paper I). However, it is surely possible to argue that the values in the highest range have somewhat higher variances. The reason for this is probably that we included paired data taken in the first few minutes after the glucose boluses, and as a consequence of the unstable physiology of the model at this time and the fact that

[1] To complicate the issue further, it is possible to argue that when one instrument is calibrated with another, the first is *defined* as the "gold standard", for which the term *accuracy* would be more suitable. This means that in Paper I, when the properties of the IvS-1 were tested, the word *agreement* should be used, while in Paper IV, when the IvS-1 was calibrated with the ABL 720, the word *accuracy* would be most correct. In addition to accuracy/agreement the concept of *precision* is important when it comes to examining the variations in quantitative laboratory data. If the same biochemical variable (e.g. glucose) is measured repeatedly from the same biological sample (e.g. blood) using the same laboratory instrument (e.g. IvS-1), there will be small differences. The magnitude of this variation is the precision of the instrument. In the future, the accuracy/agreement and the precision of the IvS-1 (the analytical variance) will have to be measured in order to create an AEP, because it is necessary to know when a change in BGL readings corresponds to a real change in the subject's BGL and when it could be attributed to variance in the instrument.

the response time of the sensor is about two minutes (see next paragraph), these paired data would naturally have high variances.

We did not originally aim to measure *response time*, as the fundamental importance of this parameter (especially in the development of the artificial pancreas) was not clear to us at the time of the study. The reviewers of the paper suggested we re-examine our material to look for opportunities to assess response time. We then were able to construct a figure (Figure 2, Paper I), showing that the IvS-1 readings followed the rise in BGL after an IV glucose bolus, and by the time of the next blood sample five minutes later, the sensor readings were already falling. Later, and especially in the work for Paper IV, we performed glucose bolus tests of the sensors to look specifically at response time (as well as the total response). These tests showed that when a large glucose bolus was given, the time from bolus until the sensor reached its maximum value was approximately 2 minutes (Figure 3).

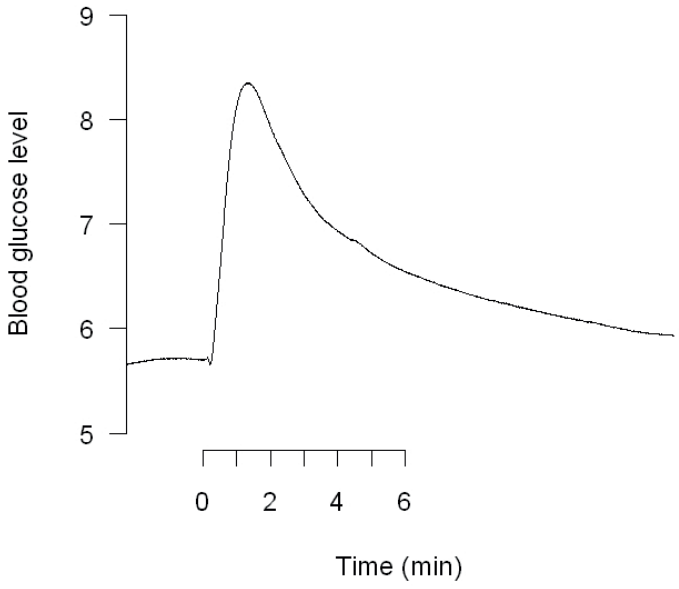

Figure 3: Example of IV glucose bolus test of an IvS-1 sensor. It is reasonable to believe that the "true" BGL will reach its maximum value almost instantaneously at the time of bolus injection, followed by an immediate exponential decay. The maximum IvS-1 reading is 2 minutes after the bolus time, indicating a response time of 2 minutes.

In Paper I some sensors broke down, which resulted in increased drifting and eventually failure. The sensors used were fabricated with different types of coating, although most of them were heparinized. We believe that *bio-incompatibility,* which caused clotting, triggered a chemical degradation mechanism in the sensor material. Another explanation for increased drift during clotting is that a biofilm on the sensor might

constitute some kind of microenvironment where the glucose content might be different than in the overall blood compartment (90). We also believe the intra*venous* positioning enhanced the clotting problem due to low pressures and flows. In subsequent experiments in Paper IV, we switched to peripheral intra*arterial* positioning only, using the femoral artery. In these experiments the bio-compatibility issue seemed to have almost vanished, although this remains to be confirmed in long-term experiments. Pigs do hyper-coagulate (79), and this was a problem especially in the early experiments. However, we found that the clotting tendencies of the animals were highly dependent upon surgical stress, and in Paper IV this was kept at an absolute minimum.

Stability issues caused by measurement drifting independent of bio-incompatibility are also a problem, and constitute the biggest challenge both during the studies as well as in the future work with the sensor. The problem is caused by a slow background swelling of the hydrogel in most of the sensors. For the most part, this drifting seemed to be linear, and in Paper I (where the IvS-1 readings were interpreted in retrospect) we could adjust for the drift. Sometimes the drifting increased abruptly, which posed serious problems. We interpreted this as a bio-compatibility/clotting problem and usually considered the actual sensor to be broken. The background swelling became a serious obstacle in the work with Paper IV when we were trying to get real-time BGL values from the IvS-1 and implement the artificial pancreas algorithm. It was very difficult to find the correct linear drift from the calibration procedures, and to implement this in the LabView program to display the "correct" real-time BGL measurement. (See: *Results and discussion/Putting it all together – blood glucose regulation in diabetic pigs*)

Another possible problem of all *in vivo* biosensors is *interference* from other compounds. The IvS-1 interferes with pH, and this leads to drifting, in this case from both swelling and shrinking. An internal unpublished *in vitro* study by InvivoSense examined the interference effect of 26 substances (drugs, nutrition and endogenous compounds) most relevant in an ICU setting. Of these, only pH was found to be clinically relevant. Currently, Dag Roar Hjelme and postdoc Sondre Volden are trying to construct a pH sensor based on the same smart-gel technology as the glucose sensor. The idea is to create a system that allows for auto-correction by putting the pH sensor and the glucose sensor in the same assembly.

Studies of insulin pharmacology – a new approach to regulation

The original idea for study II was to find an appropriate method for insulin administration that could take advantage of the sophisticated IvS-1 to regulate the BGL at the desired values. However, we soon realized that the traditional method using constant infusions of insulin was not leading us anywhere, as the time delays were substantial (Figure 2, Paper II). It did not make any sense to use a glucose sensor with a reaction time in the range from seconds to minutes and a regulation method with a time delay in the range from half-an-hour to two hours. In our despair, we realized that the only possible method that could lower the time delay to its minimum was the use of

intravenous boluses. From that moment, the sole perspective of the study was to characterize the attributes of intravenous insulin boluses. [2]

Thus, the primary aim of Paper II was to predict the time lags in the insulin/blood glucose system to find the appropriate times from an insulin bolus (IB) until predefined changes in BGL were observed. We defined a series of time variables (Figure 1, Paper II) and calculated their values with respect to different IB doses. Of most importance was the time from IB until first detectable decrease (T_{decr}) and the time from IB until maximum rate of decrease ($T_{decrmax}$). Since the BGL values were measured only in blood samples at an interval of one minute and with the ABL-720 analyzer only displaying the BGL values with one decimal place, there was some uncertainty regarding the absolute values. However, we reported in the paper that the T_{decr} was approximately 3 to 4 minutes and the $T_{decrmax}$ was approximately 3.5 to 4.5 minutes. At this time, our group was convinced that a working AEP *had* to take advantage of IB as an alternative to constant infusions of insulin. Further work on the insulin algorithm is described in the next section.

[2] The choice to later pulse the insulin administration in the AEP was therefore a result of the physiology we observed in the laboratory. Only later did we learn that this is the method by which the pancreas regulates BGL, and in fact is a prominent principle in several regulatory systems in the intact organism.

Putting it all together – blood glucose regulation in diabetic pigs

The primary aim of the last study was to apply our theories of BGL regulation with IB in a more realistic model, i.e. diabetic pigs. In order to perform the experiment three preliminary tasks had to be accomplished:

1) The insulin algorithm: Based on our experience from Paper II, we started to work on an insulin regulation algorithm. It was not at all clear what this should be like. The natural pancreatic beta cells display a regulatory rhythm, while the amount of insulin released by each pulse varies. We knew that an IB starts to lower the BGL in approximately five minutes, reaching a nadir in approximately 15 minutes. This meant that by using a fixed interval of five minutes (like a natural pancreas), several IBs would affect the BGL at a given time – which would be far too complicated at this stage. We therefore constructed a simplified system where the effect of each IB was followed throughout its effect period before a new IB was administered. An early theoretical sketch clarifying the idea is shown in Figure 4.

The fundamental principles (stated in Paper IV) were clear to us at that time (first establish glycemic control by constant insulin stress and then keep the BGL within target values to exploit the effect of consecutive IBs, the starting IB dose, the principle of halving and doubling based on the effect of the previous IB dose, timing by means of the IvS-1 monitor to allow the appropriate time until effect, etc.), and we made preliminary versions of the algorithm as flow charts. The flow charts were amended as the studies went on, and the current version is displayed in Figure 2, Paper IV.

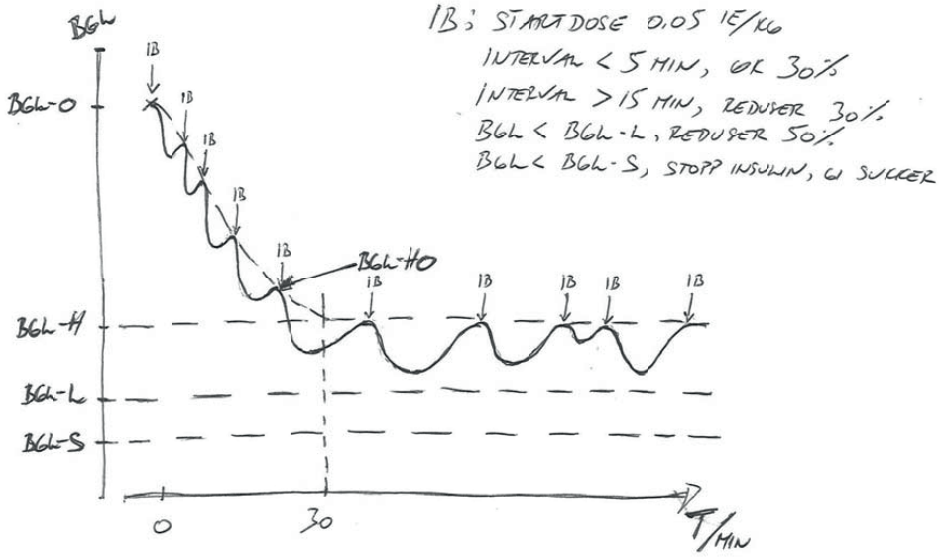

Figure 4: Early theoretical sketch demonstrating some of the ideas in our control system before it had been constructed. These ideas were: Consecutive IBs timed in accordance with the CGM-readings, IB doses calculated on basis of the effect of the previous IB dose, using fluctuation of BGL within a range instead of a fixed BGL target value, and the need to first establish glycemic control and then to maintain control. (The figure should be compared with Figure 3, Paper IV)

2) *The diabetic model:* We needed pigs that exhibited some diabetic properties, and chose to use streptozotocin overdoses as a mean to destroy the pancreatic beta cells. This procedure is explained in the *Methodological considerations/The animal model* section.

3) *Real-time BGL display:* In order to conduct this study we needed the IvS-1 to display real-time BGL values, which was accomplished using the calibration procedures described in the *Methodological considerations/The IvS-1 CGM* section. This turned out to be very challenging for the engineers due to limitations in the original LabView application, the inherent instability/drift in the sensors, and very limited time and resources for software development before the study took place. DO was responsible for the hardware arrangement and software development, with some assistance from DRH, and the details of these complicated procedures are outside the scope of this thesis.

Overall, the last study was successful in demonstrating the potential of our method of regulation. It was possible to reduce the BGL until it reached the target corridor rapidly and predictably, and to maintain the BGL within the corridor during most of the study period. The insulin algorithm was continuously refined as the studies went on, producing the best results in the last animals. The main problem in the study was difficulty with the real-time BGL display, which caused most of the inaccuracies in the results reported. The regulatory algorithm relies on very small changes in BGL, which means that the accuracy of the CGM is critical. Most of all, it is imperative that the CGM displays the correct direction of change in BGL at all times (i.e. whether the BGL is rising or falling) as a mistake in this parameter will result in incorrect conclusions. As such, our results indicate that the insulin algorithm is robust and has a high margin of safety. However, the algorithm is very demanding for the CGM.

We chose to administer IBs as needed on the basis of the existing BGL value as an alternative to insulin pulses with constant timing, which would be closer to the actual

physiological situation (as described above). At the time, this was the only way to make the system work. The flip side of this method is that it probably results in larger amplitudes in BGL fluctuations than natural pancreatic oscillations do, because the carry-over effect of consecutive endogenous insulin pulses will smooth the BGL curve. However, the most important issue at the time was to determine whether this new regulatory principle could work at all. In the future, a model able to correct for, and take advantage of, this carry-over effect might be constructed, either by fixed interval IBs and/or IBs with a combination of a small continuous insulin infusion. The amplitude in BGL oscillations could probably be decreased, which would enable it to approach the physiological pattern found in intact organisms.

During this project we did not have any formal collaboration with anyone who had cybernetic expertise. We therefore had to explore the complicated world of control systems by ourselves and to try to understand how complex non-linear biological systems can be controlled. Our control method has little in common with the control systems most used by other groups, such as the PID and MPC controllers (See section *Introduction/ Artificial endocrine pancreas*). Our approach might be described as a *heuristic control system,* where our clinical understanding of the problem at hand coupled with common-sense understanding of the problem formed the basis for decision making. On the other hand, the system is more PID-like than MPC-like, because we did not try to model the system as such (we believe this is not possible, as described in the *Introduction/glucose/insulin (patho-) physiology* section), but only tried to respond to changes in BGL as they were coming from a black box. The major advantage of the heuristic approach over the PDI approach is that it eliminates uncertainties due to

parameter variations inherent in a PDI approach that operates with fixed control parameters. Thus, the heuristic approach is more robust and can likely be applied to any group of patients once the control objectives are properly set. Another possible way to optimize a PID system is to use adaptive control where the control parameters are not fixed but can adapt to changing situations.

Conclusions – answers to the aims

I will conclude this thesis by addressing the aims stated in the beginning of the paper.

Aim 1: *To test whether a novel intravascular continuous glucose monitor could measure blood glucose with highly accurate results and almost no time delay. To further develop the system to provide real-time readouts of the current blood glucose level.*

Paper I showed that the IvS-1 measurements were in high agreement with the blood glucose values obtained from the ABL 720 machine, and that the sensor reacted rapidly within minutes. Later experiments with the sensor and unpublished measurements of the sensor performance have shown us that the reaction time is on the order of seconds to minutes in a clinical setting of blood glucose control. In practical use, the IvS-1 seems to be able to detect very small changes in BGL, much smaller than the 0.1 mmol/l cut-off value provided by the blood gas machine. The principles for the pre-insertion *in vitro* calibration and the repetitive *in vivo* one-point calibration of the IvS-1 sensors were developed and applied in Paper IV. However, the system did not perform satisfactorily, and merits further research. The problems could be caused by technical issues related to the pre-insertion calibration procedure, software issues, the need for continuous pH correction, or issues regarding the background swelling (stability) of the gel.

Aim 2: *To determine the inherent lag time in the insulin/blood glucose system. To use this information to make a new algorithm for rapid and predictable blood glucose control.*

In Paper II we found that insulin bolus injections in previously healthy pigs yielded a first detectable decrease in BGL in 2 to 6 minutes, a maximum rate of decrease shortly thereafter and a nadir value in 15 – 20 minutes. In Paper IV we confirmed these finding in diabetic pigs. The time intervals seemed to be consistent while the overall response was naturally dependent upon the insulin dose. We used this information and coupled it with the knowledge of physiological pulsed insulin secretion from the endocrine pancreas to make a new algorithm for insulin control. The principle ideas behind the algorithm were born during the experiments leading to Paper II, while the details were constantly refined during the experiments for Paper IV up to and including the current version.

Aim 3: *To get a firm idea of the pros and cons of glucose monitor positioning in different intravascular locations.*

Paper I showed the benefit of using intravascular monitor positioning. In Paper III we found that the blood glucose value is the same in mixed venous and arterial blood. In our first studies (Paper I) we mostly used central venous positioning, while in later work we preferred intra-arterial positioning. We considered the latter approach to be preferable, as it seemed to lead to enhanced sensor performance with less clotting.

Future studies using this model will therefore rely on intra-arterial sensor positioning only.

Aim 4: *To establish a diabetic pig model.*

We were able to establish a satisfactory diabetic model for our purpose by using streptozotocin. However, this method is expensive when the animal is only used once (acute experiment), and we will therefore continue to search for simpler and cheaper methods to block endogenous insulin production during acute experiments. An alternative is to construct robust long-lasting models by inducing diabetes in miniature pigs and use these in repetitive less-invasive studies.

Aim 5: *Putting it all together: using the newly adapted glucose control algorithm and the intravascular continuous glucose monitor to regulate blood glucose levels in diabetic pigs.*

The entire effort that led to this thesis, with studies in a variety of scientific fields within physiology, pharmacology, *in vivo* biosensing, material physics and control theory, combined with a range of technical challenges and difficulties, merely served to answer one question: Is it really possible to construct a fully automatic artificial pancreas? As a result of my research, I would say that in theory, it is. However, there are several obstacles. First of all, it will require an absolutely reliable, accurate biosensor that reacts rapidly, and that has the properties described in the Results and discussion/Validation of the IvS-1 section. Secondly, this sensor has to be positioned in an intravascular

compartment to avoid delays in the readings of the actual glucose levels. To minimize any kind of bio-incompatibility and bio-fouling, we believe the best site is intra-arterial (at least with the current technology). Thirdly, the insulin has to be delivered intravenously (peripherally, in the central caval or probably best in the portal vein). Fourthly, the insulin delivery has to be pulsed either by our method, a more physiological "correct" rhythm with stable pulses, or alternatively in a combination of constant infusion and pulses. Paper IV showed that these principles do work in a short-term experiment, as we were able to control the BGL (almost) as intended. Nevertheless, producing a stable long-term implantable AEP remains a great challenge.

Issues for further research

As described in the *Results and discussion/Validation of the IvS-1 sensor* section, there are five attributes of the CGM that seem to be important for its optimal performance: accuracy, response time, bio-compatibility, stability and interference. As described in this thesis, the current challenge relates to issues of stability and interference. Current and future research should focus on pH stability (possibly with the addition of a pH sensor) and minimizing the drift by using alternative hydrogels (or at least to make the drift more linear and predictable). In addition, both the hardware and the software that run the IvS-1 system have to be upgraded. The data acquisition software was not optimized for real time calibration and compensation. This became evident while the engineers were trying to twist the program to display real-time BGL for the experiments in Paper IV; reprogramming of the LabView application is a necessary first step for this. We also need to take a closer look at the whole calibration procedure and the set-up for pre-insertion *in vitro* calibration.

The next step is a total assembly of the AEP system. In the studies presented here, a syringe pump administered the IBs, which were manually adjusted in accordance with the insulin algorithms. The algorithm should be programmed using the same LabView application that runs the IvS-1 system and should be hard-wired to a syringe pump controlled by the software. From this point we will have a nice working system to perform new animal studies, the algorithms could then be further refined and the long-time stability and bio-compatibility of the glucose sensors could be tested.

From this point there are at least two possible roads to follow: 1) assembling and testing of the currently developed and/or a similar system in humans and 2) minimizing the size of the hardware approaching an implantable AEP to be tested in animals.

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Paper I

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Paper II

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Original Article

Pharmacology of Intravenous Insulin Administration: Implications for Future Closed-Loop Glycemic Control by the Intravenous/Intravenous Route

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Abstract

Background: Our group is attempting to construct an artificial pancreas based on intravenous glucose monitoring and intravenous insulin delivery. To do so, the pharmacology of intravenous insulin administration must be studied. We used a pig model to determine the inherent lag time in the insulin/blood glucose system. The goal was to suggest a method that reduces the blood glucose level in a rapid and yet predictable manner.
Methods: Six pigs received continuous intravenous insulin infusions at 0.04, 0.08, or 0.4 IU/kg/h for 60 min. Two pigs received short-term intravenous infusions at 0.4 IU/kg/h for 2 min, repeated five times at 60-min intervals. Four animals received five intravenous insulin bolus injections at 60-min intervals, two at 0.01 IU/kg and two 0.02 IU/kg, with a final dose of 0.04 IU/kg. The blood glucose level was measured every 1–5 min.
Results: A high rate of intravenous insulin infusion led to rapid declines in blood glucose levels. The same rapid decline was achieved when the infusion was halted after 2 min. Using the latter method and with intravenous insulin boluses, blood glucose levels started to rise again after approximately 15–20 min. Insulin boluses led to a first detectable decrease in blood glucose level after 2–6 min and to a maximum rate of decrease shortly thereafter.
Conclusions: We found that intravenous bolus injections of insulin lowered blood glucose levels rapidly and predictably. Repetitive small intravenous insulin boluses together with an accurate and fast-responding intravascular continuous glucose monitor should be studied as a method of closed-loop glycemic control.

Introduction

The development of an artificial endocrine pancreas has been a major research area in diabetes care for over 50 years. It consists of a continuous glucose monitor, an insulin infusion system, and a control algorithm, assembled as a closed-loop regulatory system with no need for manual adjustments. The Biostator® (Miles Laboratories, Elkhart, IN) was constructed in the late 1970s, as a system that combined continuous blood sampling analyzed ex vivo with automated intravenous insulin infusion.[1] The system performed very well but was hampered by its large, bulky size, its invasiveness, and the need to immobilize the patient. In the past 20 years most research has been focused on subcutaneous continuous glucose monitoring and subcutaneous insulin administration.[2] Despite considerable effort and abundant use of resources on establishing fully automated blood glucose regulation by this route, success has been elusive. At the turn of the century, blood glucose control in the intensive care setting awakened substantial interest because of studies describing its beneficial role on intensive patient outcome.[3–5] This led to a renewed interest in more invasive and aggressive methods of glucose control, reopening the intravenous route to both insulin administration as well as glucose monitoring.

We have previously described a novel intravascular continuous glucose monitor ideal for intensive care use.[6] Our goal is to further develop this technology into a fully automatic closed-loop regulatory system, primarily for improved blood glucose control in intensive care patients and subsequently as a solution for outpatient diabetes care. An obvious advantage of using intravenous monitoring and insulin delivery—as opposed to the subcutaneous route—is to minimize the time lag between glucose monitoring and insulin delivery and between insulin delivery and the time a new blood glucose level (BGL) is reached. The time lag problem is a serious challenge in subcutaneous/subcutaneous closed-loop regulatory systems, calling for complicated mathematical modeling of the insulin control algorithm.[7] Even so, there is also a time lag issue with

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the intravenous route. We have observed—both clinically and in animal models—that continuous intravenous insulin infusions reduce the BGL in a rather slow and unpredictable way. It is difficult notably to rapidly lower the BGL without drifting into hypoglycemia. The elimination half-life of insulin is reported to be approximately 6 min;[8] however, there are no reports on the lag time from intravenous insulin administration until a decrease in BGL is observed.

To take full advantage of the intravenous/intravenous route of blood glucose control, the pharmacology of intravenous insulin administration must be studied. The aims of this study were to use a pig model to determine the inherent lag time in the insulin/blood glucose system and to suggest a method of insulin administration that reduces BGLs in a rapid and yet predictable manner.

Materials and Methods

Animals

The study was approved by the Norwegian State Commission for Animal Experimentation. Twelve pigs (weighing 24–32 kg) were acclimatized and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The pigs were premedicated with intramuscular diazepam (0.4 mg/kg) (Stesolid®, Dumex-Alpharma, Copenhagen, Denmark) and azaperon (12 mg/kg) (Stresnil®, Janssen-Cilag, Vienna, Austria). Anesthesia was induced with intravenous atropine (0.04 mg/kg) (Nycomed Pharma AS, Oslo, Norway), ketamine HCl (10 mg/kg) (Parke-Davis, Solna, Sweden), and thiopental sodium (5 mg/kg) (Pentothal®, Abbott Scandinavia AB, Solna, Sweden).

The animals were tracheotomized through a midline surgical cut-down and mechanically ventilated and monitored on an anesthesia machine (Aisys, GE Healthcare Technologies, Oslo). The fraction of inspired oxygen was kept at 0.3, the tidal volume was kept at 10 mL/kg, and minute ventilation was adjusted to maintain an arterial partial pressure of CO_2 of 4.5–5.5 kPa. Anesthesia was maintained by isoflurane (0.5–1.0%) (Forene®, Abbott Scandinavia AB) and an infusion of intravenous fentanyl (at 7 μg/kg/h) (Pharmalink, Spanga, Sweden). Fluid balance was achieved using a continuous infusion of heated (37°C) Ringer's acetate at 5 mL/kg/h. The insulin used in the study was recombinant human insulin (Actrapid®, Novo Nordisk, Bagsværd, Denmark). The animals were euthanized with an overdose of pentobarbital (pentobarbital NAF, Apotek, Lørenskog, Norway) at the end of the study.

An intra-arterial line was placed in the right carotid artery via the tracheotomy wound for monitoring and blood sampling. An intravenous line was placed in the right internal jugular vein through a separate surgical cut-down. The bladder was exposed through a small laparotomy incision for the insertion of a bladder catheter. Glucose was measured in whole arterial blood with a blood gas analyzer (ABL 725, Radiometer, Brønshøy, Denmark). All animals were allowed to rest for at least 60 min to stabilize their BGL before the administration of insulin.

Study protocol

Continuous intravenous insulin infusions. Six pigs were assigned to receive a continuous intravenous insulin infusion of 0.04, 0.08 ("clinical" doses), or 0.4 (a high dose) IU/kg/h (two pigs in each group) for 1 h. Glucose measurements were performed every 5 min throughout the 1-h study period.

Short-term intravenous insulin infusion. After a preliminary analysis of the continuous insulin infusion group, two animals were assigned to receive a continuous intravenous insulin infusion of 0.4 IU/kg/h insulin for 2 min (a total of 0.013 IU/kg). This infusion was repeated five times at 60-min intervals. Glucose measurements were performed every 2–5 min throughout the 5-h study period.

Intravenous insulin boluses. After preliminary analyses of the continuous and short-term intravenous insulin infusion groups, four animals were assigned to receive repetitive intravenous insulin bolus injections. Based on findings from the studies with insulin infusions and pilot animals, we chose 0.01, 0.02, and 0.04 IU/kg in alternate doses with 60-min intervals. Five intravenous insulin boluses were given to each animal, two at 0.01 IU/kg and two at 0.02 IU/kg in a randomized order and then a final dose of 0.04 IU/kg. Glucose measurements were performed every 1–5 min throughout the 5-h study period, with the most frequent sampling during the first 10 min after each bolus.

Blood glucose analysis

To analyze BGL we used arterial blood samples on a Radiometer ABL 725 blood gas analyzer. In this instrument, glucose is transported across the outer membrane of a multilayer glucose electrode. The glucose oxidase that is immobilized between the inner and outer membrane layers converts glucose to hydrogen peroxide (glucose + O_2 → gluconic acid + H_2O_2), which crosses the inner membrane toward the electrode's anode. The oxidation of hydrogen peroxide creates an electric current that is proportional to the amount of hydrogen peroxide available and hence is proportional to the amount of glucose in the sample. In addition, the analyzer also uses different electrodes with similar techniques to measure partial pressure of O_2, partial pressure of CO_2, pH, and lactate.[9]

Calculations and statistics

Glucose measurements from the continuous and short-term insulin infusion groups were collected and plotted against time. No further statistical analyses were performed on these data. Six variables were derived from the bolus injection part of the study (Fig. 1). The time until the first detectable decrease in BGL (T_{decr}) was defined as the time between the intravenous insulin bolus administration and a reduction in BGL of ≥ 0.1 mmol/L that was not followed by a rise in BGL above this value within the next 5 min. The maximum rate of decrease (MRD) was calculated using linear regression as follows: individual slopes were constructed using all possible sets of five consecutive data points in the time series, and then the steepest line was chosen and reported as MRD. The time until MRD ($T_{decrmax}$) was defined as the time between intravenous insulin bolus administration and the first data point used when calculating MRD. The maximum decrease in BGL (ΔC_{max}) was defined as the difference in BGL between baseline and the lowest BGL measured within the next 55 min. The time until the lowest measured BGL (T_{min}) was defined as the time from the intravenous insulin bolus administration until ΔC_{max} was achieved.

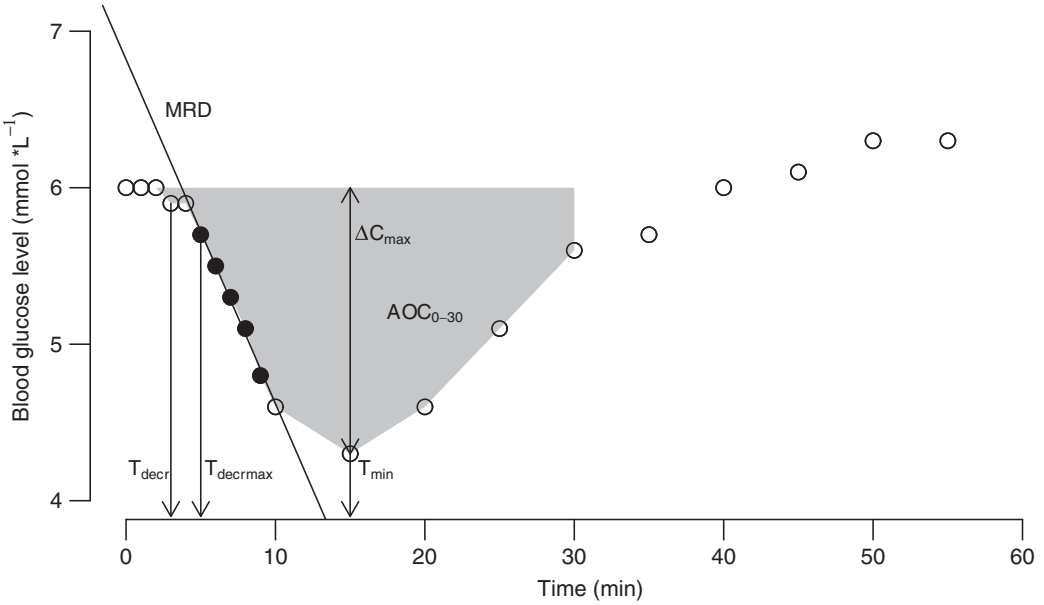

FIG. 1. The six variables derived from intravenous insulin bolus injections at time = 0. ΔC_{max}, maximum decrease in blood glucose level; MRD, maximum rate of decrease (linear regression using five consecutive observations [black circles] for all possible combinations and choosing the steepest regression line); T_{decr}, time until the first detectable decrease in blood glucose level; $T_{decrmax}$, time until the maximum rate of decrease; T_{min}, time until the lowest measured blood glucose level. Gray shading marks the area of the drop in the blood glucose curve for the first 30 min after insulin bolus injection (AOC_{0-30}).

As an overall measure of the effect of insulin on BGL we also calculated the area corresponding to the drop in the BGL curve for the first 30 min after the intravenous insulin bolus administration (area over the curve from 0 to 30 min [AOC_{0-30}]) using the trapezoidal rule (gray shading in Fig. 1). Thirty minutes was chosen as the time frame because the insulin effect seemed to fade after this period, an observation that is in accordance with the previously reported elimination half-life of insulin of approximately 6 min.[8] Thus, further changes in BGL (increases or decreases) after 30 min would be expected to be a result of the general oscillations of the BGL curve rather than a result of the previous insulin dose.

The statistical analysis was conducted using R software, version 2.10.1 (R Foundation for Statistical Computing, Vienna, Austria). We compared the effect of the three dose levels of insulin as fixed factors on the six defined outcome variables using linear mixed-effect models (the *lme* function from the *nlme* package), thereby adjusting for nested sampling from four different animals.

Results

Continuous intravenous insulin infusion

Intravenous insulin infusion led to a dose-dependent sigmoid-shaped decrease of the BGL (Fig. 2). The BGLs decreased throughout the study time of 60 min.

Short-term intravenous insulin infusions

Short-term intravenous insulin infusions produced a rapid BGL decline similar to that caused by high-rate continuous intravenous insulin infusions. The BGLs reached a nadir at approximately 15–20 min before returning to their baseline value (Fig. 3).

Intravenous insulin boluses

The BGL curves of each of the four animals are shown in Figure 4. All insulin doses led to a detectable fall in BGL. The BGL of one animal fell to hypoglycemic values after the fourth bolus dose, making the animal unsuitable for further study. This exclusion left us with the following data: eight boluses in four animals of 0.01 IU/kg, seven boluses in four animals of 0.02 IU/kg, and three boluses in three animals of 0.04 IU/kg.

Results for the six outcome variables are presented in Table 1. T_{decr} appeared within 5 min for all but one of the boluses, for which the change was apparent after 6 min. There was no significant difference in T_{decr} between the various doses. MRD was high at all doses and was also significantly dose-dependent. $T_{decrmax}$ was not found to be dose-dependent. ΔC_{max} was found to be highly and significantly dose-dependent, whereas there was no significant difference between the different doses for T_{min}. AOC_{0-30} was highly and significantly dose-dependent.

To provide a visual comparison of the various bolus and continuous infusion doses, the values were converted to ratios by dividing each BGL value with its respective baseline BGL value (Fig. 5).

Discussion

The principal finding of this study is that intravenous insulin infusion at "clinical" rates (i.e., 0.04–0.08 IU/kg/h) led

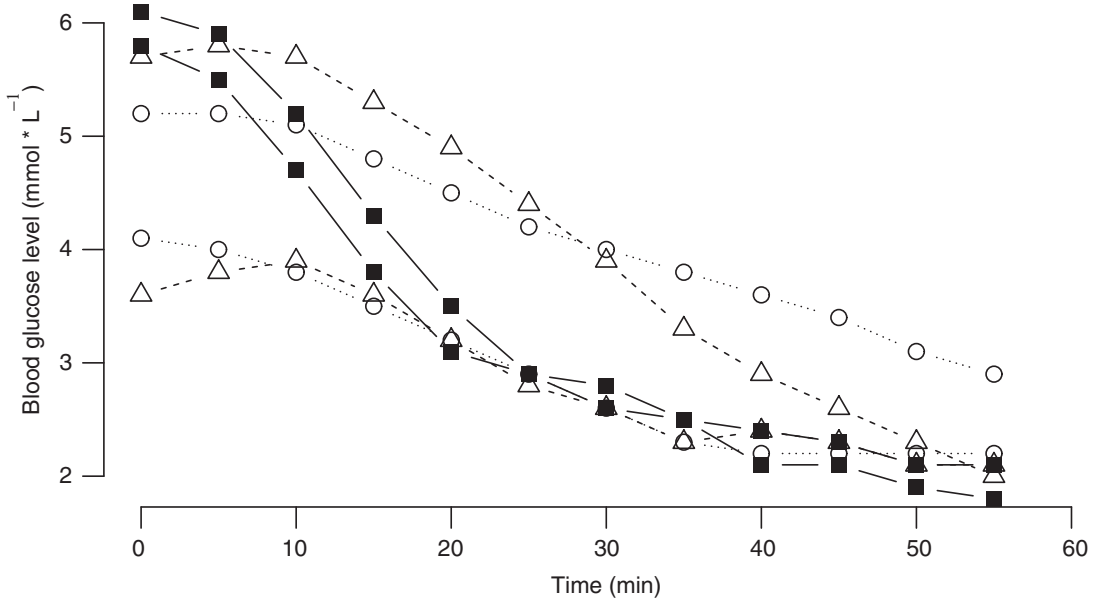

FIG. 2. Blood glucose levels after continuous intravenous insulin infusion in six pigs. Each of the six animals is depicted with a separate curve. Two animals were given a dose of 0.04 IU/kg/h (open circles), two animals were given 0.08 IU/kg/h (open triangles), and two animals were given 0.4 IU/kg/h (closed squares).

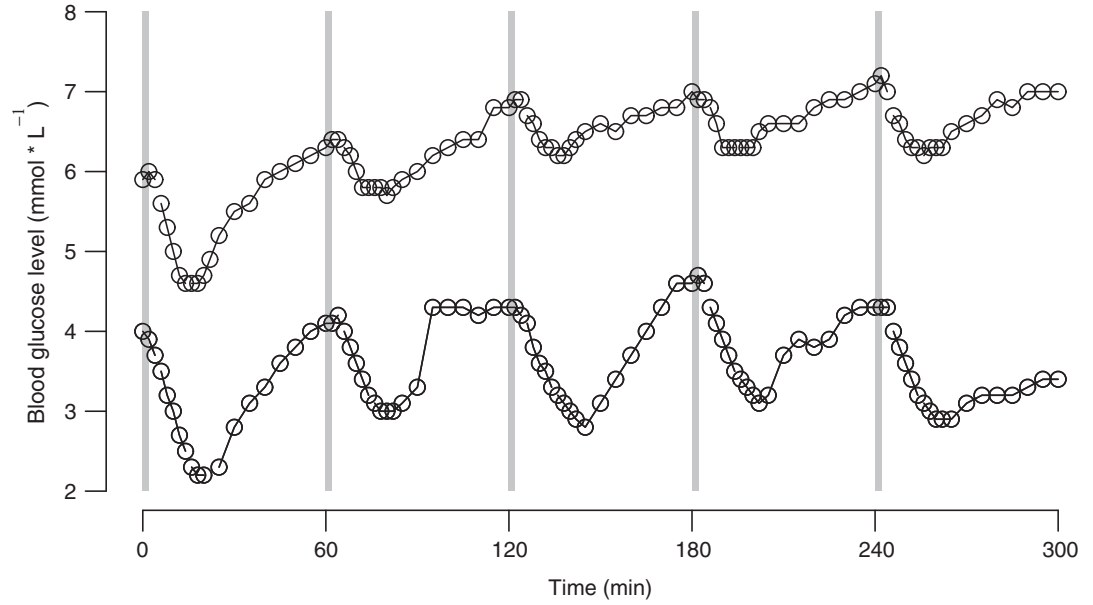

FIG. 3. Repetitive short-term intravenous insulin infusions in two pigs. Each animal received 0.4 IU/kg/h for 2 min (a total of 0.013 IU/kg) six times with 60-min intervals between each infusion. The periods of insulin infusions are marked with gray shading.

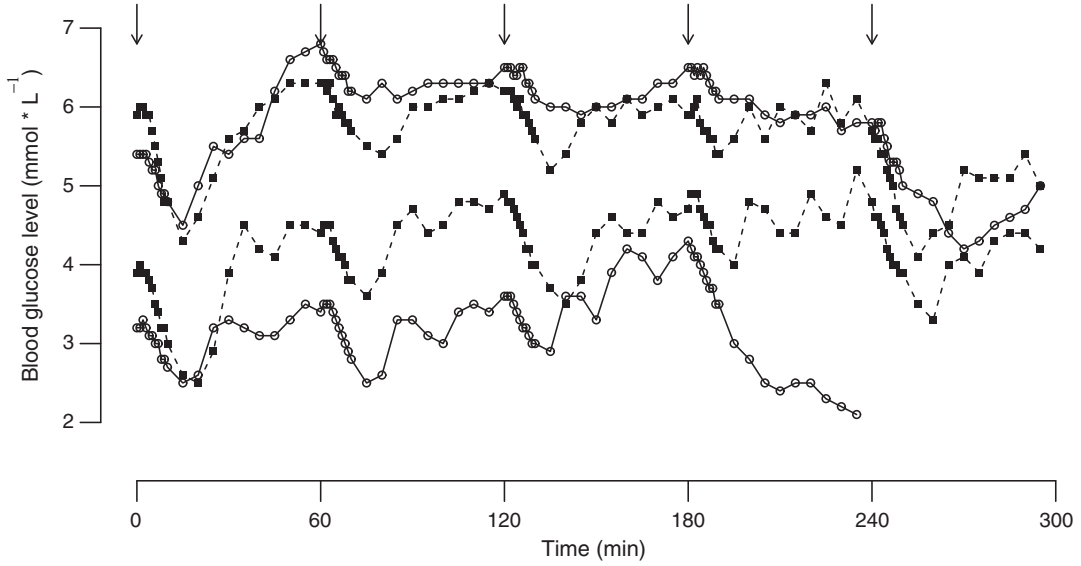

FIG. 4. Blood glucose level curves in four pigs receiving repetitive bolus doses of intravenous insulin. The timing of the insulin boluses is indicated with arrows. The insulin doses were given in the following sequence: (0.01 + 0.02 + 0.01 + 0.02 + 0.04) IU/kg in two animals (open circles) and (0.02 + 0.01 + 0.02 + 0.01 + 0.04) IU/kg in two animals (closed squares).

BGLs to decline slowly. To achieve more rapid changes, the infusion rate had to be increased to very high levels, which led to a sustained depression of the BGL. By halting the high rate infusions after 2 min we achieved the same rapid decline, with BGL reaching a nadir within 15–20 min, and then a rise in the BGL toward baseline values without drifting into hypoglycemia. We hypothesized that a 2-min infusion would not be very different from a bolus injection, and so we continued working with boluses. This has the advantage that one only needs to manipulate one variable (the dose), compared with short-term infusions where there are two (the dose and the infusion time).

Intravenous insulin bolus administration led to a detectable decrease in BGL after a time interval of 2–6 min and to a maximum rate of decrease shortly thereafter. Neither the T_{decr} nor the $T_{decrmax}$ was found to be dose-dependent. However, this finding could be due to a type II error caused by the low number of animals in the study, and both Table 1 and Figure 5 indicate a trend toward a shorter time until the maximum rate of decrease for the highest bolus dose. Intravenous insulin boluses resulted in a dose-dependent reduction in BGLs with regard to both the maximum decrease and the area of the decline under the BGL curve. Figure 5 shows the effect of an intravenous bolus injection of insulin on the relative changes in BGL compared with that of intravenous insulin infusion. The rapid onset and the predictable and short-lasting effect of the boluses at the doses chosen are clearly evident. In particular, the difference between administering 0.04 IU/kg as a bolus (closed squares) and as a 1-h infusion (upper margin of the gray shading) is striking.

Table 1. The Six Outcome Variables from the Intravenous Insulin Boluses

	Insulin dose (IU/kg)			
	0.01	*0.02*	*0.04*	P *value*
T_{decr} (min)	3.88 (0.64)	4.00 (1.41)	3.33 (0.58)	0.32
MRD (mmol/L/min)	0.098 (0.010)	0.10 (0.030)	0.14 (0.017)	0.027
$T_{decrmax}$ (min)	4.50 (1.41)	4.57 (1.33)	3.67 (1.15)	0.74
ΔC_{max} (mmol/L)	0.73 (0.14)	1.10 (0.37)	1.57 (0.058)	< 0.001
T_{min} (min)	16.1 (4.6)	18.6 (5.6)	21.7 (7.6)	0.46
$AOC_{0–30}$ (mmol/L/min)	9.42 (3.63)	18.51 (6.47)	27.82 (2.48)	< 0.001

All data are mean (SD) values.

P values are from comparisons using linear mixed-effect models.

$AOC_{0–30}$, area over the curve from 0 to 30 min; ΔC_{max}, maximum decrease in blood glucose level; MRD, maximum rate of decrease; T_{decr}, time until the first detectable decrease in blood glucose level; $T_{decrmax}$, time until maximum rate of decrease; T_{min}, time until the lowest measured blood glucose level.

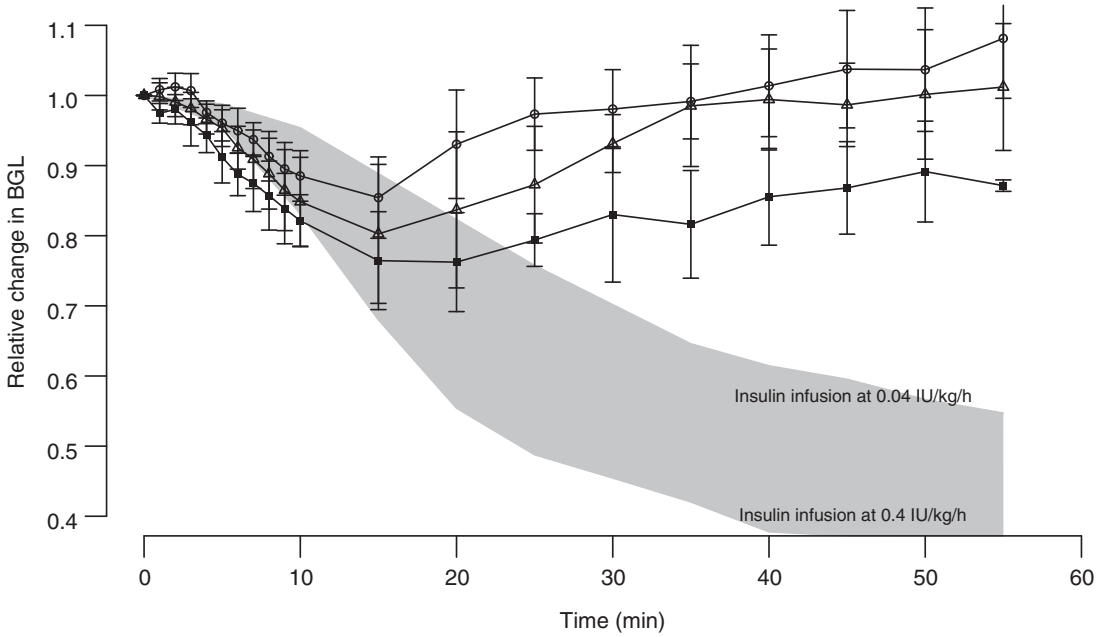

FIG. 5. Effect of intravenous insulin boluses on blood glucose level (BGL) after repeated injections in four pigs. The doses were 0.01 IU/kg (open circles), 0.02 IU/kg (open triangles), and 0.04 IU/kg (closed squares). The effect of continuous intravenous insulin infusions is shown as a gray area, with a dose of 0.04 IU/kg/h at the upper margin and a dose of 0.4 IU/kg/h at the lower margin. The boluses were plotted as the mean of each dose, with error bars depicting their respective SD.

The pancreas does not secrete insulin into the portal bloodstream continuously, but rather in pulses at 5-min intervals. The amount of insulin secreted in each pulse is primarily regulated by changes in the blood glucose concentration; when the BGL is rising, more insulin is secreted with each pulse, and when the BGL is falling, less insulin is secreted with each pulse.[10,11] This principle causes tiny fluctuations of the normal blood glucose curve.[12] If a closed-loop system were to resemble nature as closely as possible, it would therefore secrete insulin in pulses at 5-min intervals, as described above and as proposed in a recent article.[13] With the current knowledge, we still find this to be too complex. However, we do believe it should be possible to construct a closed-loop system using repetitive small boluses of intravenous insulin together with an accurate and fast-responding intravascular continuous glucose monitor. The timing of the bolus injections would be determined by the BGL curve, and the dose of the intravenous insulin boluses would be calculated based on the history of the previous boluses and their effect on the BGL curve, thereby allowing tiny oscillations in BGL, ideally with amplitudes of approximately 0.5 mmol/L.

The strength of this study lies in the use of a model with large animals that resemble humans, which allowed us to obtain numerous consecutive blood samples from each animal and to manipulate BGLs without fear of the implications of hypoglycemia. The major weaknesses include our use of a pig model and the fact that the animals were healthy and did not have diabetes. However, we believe that the fundamental principles from our findings will be applicable to humans with diabetes, although this issue merits further examination. Another limitation was the choice to give the largest bolus dose only at the end of each experimental course. This procedure was chosen because we were uncertain whether such a high dose would influence the effect of subsequent insulin doses. Finally, the number of pigs examined was low, leading to a risk of type II errors when comparing the different doses. Nevertheless, we believe that this study provides sufficient data to form the basis for performing carefully controlled studies with bolus doses in humans.

In conclusion, we found that continuous intravenous infusion of insulin at normal "clinical" doses leads to slow changes in the BGLs due to the inherent lag time in the insulin/blood glucose system. In contrast, bolus injections of insulin lowered BGLs rapidly and predictably. In the future, closed-loop glycemic control might be achieved by using repetitive small boluses of intravenous insulin together with an accurate and fast-responding intravascular continuous glucose monitor.

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Author Disclosure Statement

All authors declare that they do not have any competing financial interests.

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Intravenous Bolus Insulin Delivery: Implications for Closed-Loop Control and Hospital Care

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Intravenous insulin infusion to treat diabetic ketoacidosis has been one of the cornerstones of therapy since the 1920s. Few appreciate that this route of insulin delivery was not approved by the U.S. Food and Drug Association until 2005. Even more surprising are the minimal published data on the kinetics of intravenous insulin, by either intravenous infusion or intravenous bolus. Most appreciate that the actual pharmacokinetics of intravenous insulin are quite short, but it was quite surprising to learn the pharmacodynamics may be longer than appreciated.[1] However, those who previously worked with the Biostator® (Ames Division, Miles Laboratories, Elkhart, IN) in the late 1970s and 1980s saw firsthand how slow intravenous insulin could be.[2]

The precise action times of intravenous insulin have taken on greater interest with the goal of developing an artificial pancreas. The real problem is the painfully slow pharmacodynamics of our current "rapid-acting" subcutaneous insulin.[3] If a safe and effective system using intravenous insulin could be developed for an artificial pancreas, waiting for a faster subcutaneous insulin analog wouldn't be required.

The article by Skjaervold et al.[4] provides us with a better understanding about the time–action profile of intravenous insulin. In some respects, it is surprising it has taken so many decades for this type of work to be completed. On the other hand, intravenous insulin is still rarely used today, although given the more common use of intravenous insulin infusion in the hospital this route of therapy is more often used now than in past decades.[5] It should also be appreciated that the infusions and boluses done by Skjaervold and co-workers were performed in a pig model, and thus human studies should be considered before serious attention is paid to the use of intravenous insulin in a closed-loop system.

The findings by the study by Skjaervold et al.[4] can be summarized as follows: (1) Despite the short half-life of intravenous insulin, when it is infused at rates up to 0.08 IU/kg/h blood glucose declines slowly. (2) Higher rates lead to more rapid decreases in blood glucose, but eventually hypoglycemia will ensue. (3) Hypoglycemia can be prevented with these higher rates of infusion leading to quicker reduction of blood glucose if the infusion lasts for only 2 min. (4) Intravenous bolus insulin at doses of 0.01–0.04 IU/kg results in a decrease in blood glucose on average at 4 min. (5) With intravenous bolus insulin there appears to be a clear dose dependency for degree and length of glucose level lowering. (6) Because of the short glucose response of the intravenous bolus, hypoglycemia as seen with the intravenous infusion after 30 min does not occur.

What are the major implications of these results? First, it is unlikely that subcutaneous insulin will ever have effects as quick as intravenous insulin. For a closed-loop system, intravenous bolus insulin appears to have desirable kinetics, although many future studies in humans will need to confirm these initial findings. Although we are obviously a long way from the use of outpatient intravenous bolus insulin in a true "artificial pancreas," insulin provided by this route could possibly be used in a standardized manner for inpatients. Intraoperative blood glucose control by anesthesiologists is frequently performed with intravenous bolus insulin, yet I am not aware of insulin kinetic data, safety, or efficacy studies using insulin in this manner. In fact, my main concern with the use of intravenous bolus insulin in any part of the hospital is that these patients often have poorly controlled diabetes with resultant potassium, magnesium, and phosphate deficiency. The use of intravenous bolus insulin in this population may actually be quite dangerous. At the very least, intravenous bolus insulin should be specifically studied in different patient populations (specifically, levels of glycemic control) in the hospital, including the operating room.

Next, assuming the data from Skjaervold and colleagues can be repeated and confirmed in humans, for a closed-loop system having such a quick and effective insulin delivery will require an equally rapid glucose sensor without a lag time. This is unlikely to occur with interstitial glucose measurement, and thus an intravascular glucose sensor will be required. Like the intravenous bolus insulin delivery, besides a closed-loop implication for type 1 diabetes, there are also inpatient implications if this type of sensing device can be perfected. Indeed, it is likely an *inpatient* intravascular sensor used in combination with intravenous bolus insulin is a more realistic initial goal, depending on the duration the sensor lasts and the ease of delivering the insulin.

Finally, besides repeating these intravenous bolus insulin studies in humans, it will be interesting to perform these studies specifically in individuals with type 1 diabetes. Besides assessing fluxes of potassium, magnesium, and phosphate, close attention to electrocardiogram changes will be required. In particular, patients with long-standing diabetes with coronary artery disease should be studied to ensure safety. Other

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metabolites such as free fatty acids and β-hydroxybutyrate should also be assessed. We would expect complete suppression of lipolysis with intravenous bolus insulin, but it likely depends on the frequency with which the insulin is provided.

Although it is too soon to know if intravenous bolus insulin will someday be used for a closed-loop system, the study by Skjaervold et al.[4] gives us initial and important information about the glycemic impact of this type of insulin delivery. Hopefully these studies can be repeated in humans with a true real-time intravascular sensor so that the elusive goal of an artificial pancreas can be achieved. Although not mentioned by the authors, these data could have important impact for the use of insulin in the hospital. As we approach the centennial birthday of the discovery of insulin, it seems appropriate to finally understand the best way to use intravenous insulin.

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Paper III

Is not included due to copyright

Paper IV

TITLE PAGE

Title:

Blood glucose control using a novel continuous blood glucose monitor and repetitive intravenous insulin boluses – exploiting natural insulin pulsatility as a principle for a future artificial pancreas

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Dag Östling and Dag R Hjelme are both shareholders in Invivosense Ltd Norway, the inventor and manufacturer of the IvS-1 intravasal

continuous glucose monitor used in this study. The rest of the authors have no financial interests to disclose.

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ABSTRACT

Objective: The goal of our group is to construct an artificial endocrine pancreas based on intravenous glucose monitoring and intravenous insulin administration. The intravenous approach minimizes time delays, which makes the regulatory algorithm much easier. The aim of this study was to construct a glucose regulatory algorithm by employing the natural pulsatile pattern of insulin secretion and the oscillatory pattern of resting blood glucose levels and further to regulate the blood glucose level in diabetic pigs by this method.

Design: An intervention study in pigs.

Setting: Animal laboratory in a university hospital.

Subjects: Four healthy pigs.

Interventions: We developed a control algorithm based on repetitive intravenous bolus injections of insulin and combined this with an intravascular blood glucose monitor. Four anesthetized diabetic pigs were used in the study, instrumented with the intravascular blood glucose monitor and regulated according to our algorithm.

Measurements and Main Results: The animals developed a mildly diabetic state from streptozotocin pre-treatment (with a starting blood glucose level

7.46 to 14.06 mmol/l). They were steadily brought within the target range of 4.5 – 6.0 mmol/l in 21 to 121 min and kept within that range for 128 to 238 min. The period with hypoglycemic values varied from 2.9 to 51.1 min, which put the levels in the correct range from 73 to 99%.

Conclusions: The study confirmed our hypotheses regarding the feasibility of this new principle for blood glucose control, and the algorithm was constantly improved during the study to produce the best results in the last animals. The main obstacles were the drift of the IvS-1 sensor and problems with the calibration procedure, which calls for an improvement in the sensor stability before this method can be applied fully in new studies in animals and humans.

INTRODUCTION

The development of an artificial endocrine pancreas (AEP), composed of a system of continuous blood glucose monitoring and automated insulin infusion, has been a long sought-after solution in diabetic care (1). Current research is focused on subcutaneous glucose measurements and subcutaneous insulin administration (2). However, the performance of these systems are still less than satisfactory, and the reality of a working AEP remains elusive.

A novel intravascular continuous glucose sensor, the IvS-1 (Invivosense, Trondheim, Norway), has been constructed using smart-gel technology. The sensor material consists of a hydrogel that incorporates boronic acid molecules that shrink and swell in response to the surrounding glucose concentration. The gel is assembled on the tip of an optical fiber, and the monitor employs optical interferometry to measure very small changes in gel size (3). Our group has tested the IvS-1 in preclinical *in vivo* studies in pigs, and found that it demonstrated high accuracy and a rapid response time (4).

(Figure 1 here)

The sensor was originally developed to meet the need for better control of blood glucose levels (BGL) in patients in intensive care units. However, when we discovered the potential of our technology we started to work towards the development of an AEP. We realized that the sensor was able to

instantaneously detect very small changes in BGL, and we found small oscillations in BGL with a period of approximately 10 minutes.

Recent research has demonstrated the pulsatile nature of pancreatic beta cells. A convincing body of evidence indicates that insulin is secreted in synchronized bursts from the entire pancreas into the portal blood stream (5)(6)(7). Likewise, multiple studies in humans and animals have described the oscillatory nature of systemic levels of blood glucose and insulin (8)(9)(10)(11). Pulsatile pancreatic activity seems to be lost in advanced type 2 diabetes (12). Pulsed intravenous insulin delivery has been shown to be more effective in lowering BGL compared to equal doses of continuously infused insulin (13)(14), and the pulsatile nature of endogenous insulin secretion has been mimicked for therapeutic reasons as pulsatile intravenous insulin therapy. Compared to standard therapy, pulsed therapy has shown better metabolic control, less end-organ damage and restoration of normal pulsatile pancreatic function in type 2 diabetes (15)(16)(17).

When we first started to infuse insulin intravenously to regulate BGL, we found that the time from start of the infusion until a new steady state BGL was reached to be two hours or longer, which is very long. This means it would take several hours to adjust any insulin infusion to the correct rate to achieve an appropriate and stable BGL. The insulin resistance in a single individual is constantly changing (18), and some authors even suggest that the regulatory system includes deterministic chaotic components that would render it impossible to foresee the effect of insulin on BGL during a given time using

ordinary linear methods (19). A control system based on continuous infusions will therefore always be “running to catch up” and will have great difficulties in lowering a patient’s BGL to a sufficient degree without risking hypoglycemia. The use of *subcutaneous* sensors and infusions would increase the time delay and complicate the situation even more.

Given this background, we conducted a series of experiments to characterize the effects of intravenous bolus injections of insulin (IB) in a previous study (20). Here, we found the time lag from an IB until a first observed decrease in BGL to be four to six minutes. The maximum rate of decrease in BGL occurred shortly thereafter, and a nadir was reached 15 – 20 minutes after the IB. These time intervals seemed to be rather dose-independent – as long as the IB dose was sufficient to yield any change in BGL at all.

Based on these observations, we hypothesized that it should be possible to construct an AEP using the biological pancreas as a model. The IvS-1 would detect early changes in BGL, and the system should respond instantaneously with adjusted repetitive IBs. We had to construct a novel algorithm with the target of establishing and maintaining a normal fasting BGL, defined as 4.5 – 6.0 mmol/l. BGL regulation had to be a two-stage process, with the first step to establish glycemic control in a hyperglycemic subject by rapidly bringing BGL to the desired level, and thereafter to maintain the desired glucose level over time.

MATERIALS AND METHODS

The insulin algorithm and administration

We identified the following key elements that had to be incorporated in the algorithm:

1) The overall goal was to bring BGL to within a predefined range of 4.5 – 6.0 mmol/l and to keep it in this range, including small oscillations around the middle value in this range.
2) IB was administered whenever needed in accordance with the continuous BGL readings; adjusting the IB doses was kept simple with only three alternatives according to the previously administered IB: the same dose as the previous one, half the previous dose or twice the previous dose.
3) Any decrease in BGL should be observable within five minutes after an IB, otherwise a new IB should be administered.
4) Blood glucose control should be *established* by a rapid decrease in BGL from hyperglycemic levels with a series of consecutive IBs. The BGL should be lowered ≥ 1 mmol/l for each IB injected.
5) As soon as the BGL dropped below 6.0 mmol/l, blood glucose control should be *maintained* by meticulously timed IBs to allow small fluctuations in the blood glucose curve.

The insulin algorithm was constructed as a flowchart based on simple IF-THEN decisions, and the details of the algorithm evolved as the study went on. The current version of the algorithm is shown in Figure 2.

(Figure 2 here)

Human recombinant insulin 0.1 IU/ml (Actrapid, NovoNordisk, Bagsværd, Denmark) was used in the study. The drug was manually administered from a syringe pump (using the bolus function) in accordance with the insulin algorithm.

The animal model

The study was approved by the Norwegian State Commission for Animal Experimentation. Forty-eight hours prior to the main experiments, we induced diabetes in healthy pigs by destroying their pancreatic beta cells using the cytotoxic agent streptozotocin 200 mg/kg i.v. (Zanosar, Teva Parenteral Medicines, Irvine, CA, USA) (21)(22). On the day of the experiment the animals were put under general anesthesia, an arterial line was established for monitoring and blood samples, central venous access was obtained, and the animals were instrumented with two IvS-1 sensors, one in each femoral artery. Animal handling, anesthesia and surgical intervention have been described in previous studies (4)(20).

IvS-1 calibration

In order to achieve real-time continuous BGL output, the IvS-1 software had to be updated and a pre-insertion *in vitro* calibration procedure had to be determined. We applied a calibration procedure by exposing the sensor to buffer solutions with glucose concentrations of 0.0, 2.0 and 10.0 mmol/l. A nonlinear least square algorithm was used to compute the two calibration parameters describing the nonlinear calibration function. After insertion, the IvS-1-signal quickly stabilized. The time series from the IvS-1 was calibrated using the computed calibration parameters and a one-point calibration method to set the off-set parameter by adjusting the IvS-1 level with the blood glucose level achieved from a simultaneously drawn blood-sample analyzed on a bed-side Radiometer ABL 720 blood-gas analyzer (Radiometer, Brønshøy, Denmark). Throughout the study several *in vivo* calibration procedures were performed to adjust for the inherent drift in the sensor. To ensure we had redundancy, we instrumented each animal with two IvS-1 sensors hard-wired to the same monitor. After the first *in vivo* calibration we chose whichever one had the most stable signal, and used data from this sensor for the rest of the experiment.

Data handling, analyzes and statistics

After the experiment, the IvS-1 data was retrieved by using data from the repetitive blood samples as calibration parameters. To transform the interferometric length measurement data into glucose concentration data, we used the nonlinear two-parameter calibration function described above. We compensated for baseline drift by using a fixed baseline drift rate, and compensated for pH interference by using the pH values from the blood-gas

analyzer to compute pH corrected calibration parameters. The pH dependence of the calibration parameters was found from a set of *in vitro* experiments.

RESULTS

This paper presents data from four animals, with the details shown in Figure 3 and Table 1. The effect of the streptozotocin pre-treatment varied between animals; at the time of the first insulin bolus the starting BGL value (BGL_0) ranged from 7.46 to 14.06 mmol/l. We were able to establish glycemic control by bringing the BGL below 6.0 mmol/l (T_{est}) in 21 to 121 minutes, with the longest time in animals with a high BGL_0 value. In animals 2-4, the rate of decrease (RD) from the first insulin bolus until the 6.0 mmol/l limit was reached was relatively high, from 0.064 – 0.077 (mmol/l)/min. (As a comparison, we found the maximum RD to be approximately 0.1 (mmol/l)/min after a single bolus dose of insulin in our earlier experiments (20).) After reaching the target range, the animals were kept under glycemic control (T_{ctrl}) from 128 to 238 minutes. The total time with BGL values below the lower limit of the range, i.e. 4.5 mmol/l (T_{low}), varied from 3 to 51 min in the four animals. The lowest BGL measured in the four animals (BGL_{low}) varied from 3.81 to 4.44 mmol/l.

(Figure 3 here)

(Table 1 here)

DISCUSSION

The intuitive interpretation of Figure 3 is that the fundamental principles of this AEP model work. BGL was rapidly and safely brought within the predefined range, and kept within the range during the study time. We tried to administer IB as best we could according to the algorithm; however, small details in the control algorithm had to be updated and changed as the experiments went on. We found that the animals behaved very differently in terms of their insulin needs. However, the time lag between IB and effect was predictable and in accordance with our previous research.

The amplitude in BGL variations during established control was somewhat larger than desired. We believe the amplitude can be substantially decreased by further improvement in blood glucose sensor stability and improvement in the insulin regulatory algorithm. As explained in the introduction, actual pancreatic beta cells oscillate with a fixed time interval of approximately five minutes, whereas the amount of insulin released with each pulse is constantly changing. As the effect of a single IB starts to lower BGL in four to six minutes, and the nadir BGL values are reached after approximately 15 minutes, consecutive insulin bursts from the pancreas with an interval of five minutes will yield a carryover effect where several bursts interfere with BGL at a given time. We found this regulation system to be too complicated to model at this stage, and therefore constructed a simplified system where the effect of each IB was followed throughout its effect period before a new IB was administered. In the future, a model able to correct for, and take advantage of,

this carryover effect might be constructed, either through the use of fixed interval IBs and/or IBs combined with a small continuous infusion of insulin.

Current AEP models are based on subcutaneous glucose measurement and subcutaneous insulin administration. The insulin infusion algorithms are based on what is called model predictive control, where the pharmacokinetic properties of insulin are modeled in a series of equations that are used to calculate the correct insulin dose at a given time. Several studies have shown the feasibility of such systems in controlling BGL overnight in diabetic subjects. However, it is very challenging to calculate the correct insulin dose when the subject is eating, exercising or ill, and a fully automatic AEP thus has yet to be constructed (23)(24). We believe a more empirical system like ours could tackle such obstacles, as it allows for the correct insulin dose to be calculated and timed continuously based on the effect of the previous IB. In this system it is very unlikely that BGL would drift into hypoglycemic levels, or that it would rise into gross hyperglycemia.

The main challenge during the study was that the IvS-1 was not optimally calibrated at all times, which led to small errors in the real-time BGL display. We therefore to some extent had to rely on a combination of the IvS-1 output and the blood samples to estimate the correct real-time BGL in order to use the insulin algorithm properly. The observed periods of BGL below the range's lower limit were caused by a discrepancy between the observed real-time BGL and the correct BGL calculated after the experiment (the reported BGL). As such, the major limitation of the current technology is a background

swelling of the glucose-sensing hydrogel, which resulted in a drift in the IvS-1 output. Our current work is focused on enhancing the hydrogel.

There will always be some doubt as to whether results from animal studies are valid in a human population; on the other hand, the model makes it possible to manipulate BGL and to instrument study subjects without fear of any iatrogenic damage to healthy volunteers or patients. The IvS-1 probes used are all handcrafted and the set-up of the animal experiment is complicated, which is why the number of animals used in the study is low. However, all of the animals studied (both the final four as well as animals in our earlier studies) displayed the same behavior. The main point of this paper has been to illustrate the physiological principle of using nature's own regulatory system in an artificial control system, without fine-tuning the details.

CONCLUSIONS

We conclude that the use of real-time accurate intravasal glucose monitoring in combination with repetitive bolus injections of insulin, administered to mimic the natural pulsatility of endogenous insulin, is a promising method for a future artificial pancreas.

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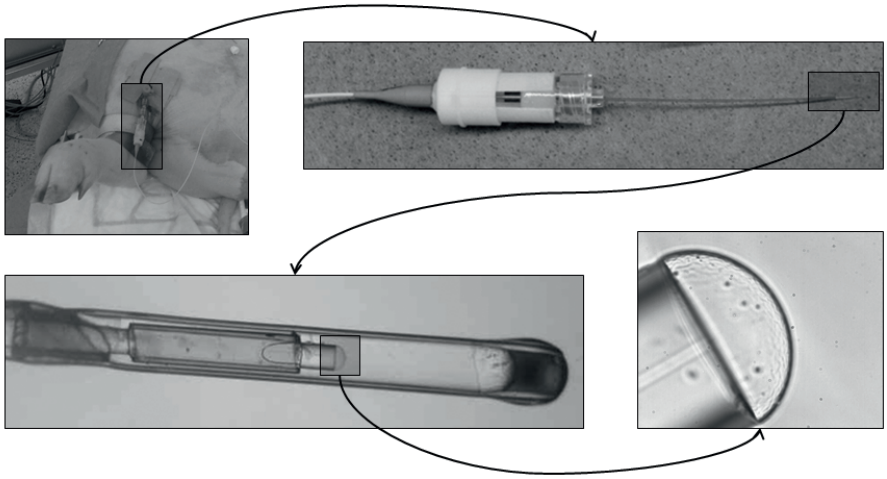
FIGURES



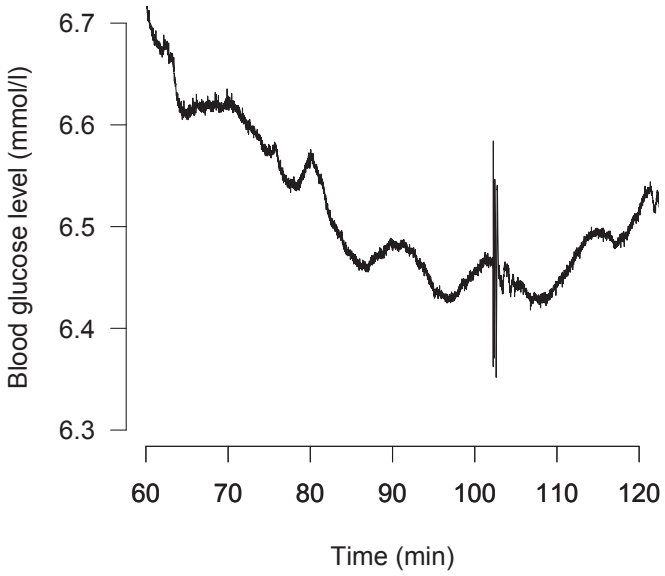


Figure 1A: Pictures that show the hydrogel part of the IvS-1 continuous blood glucose monitor prototype at increasing magnification; B: a classic example of oscillating blood glucose levels with a periodicity of approximately 10 minutes and an amplitude of approximately 0.05 mmol/l (from animal 2, which was only mildly affected by the streptozotocin pretreatment; measurements from 60 to 120 minutes in the actual experiment after IvS-1 stabilization but before the first insulin bolus; artifact seen at 103 minutes).

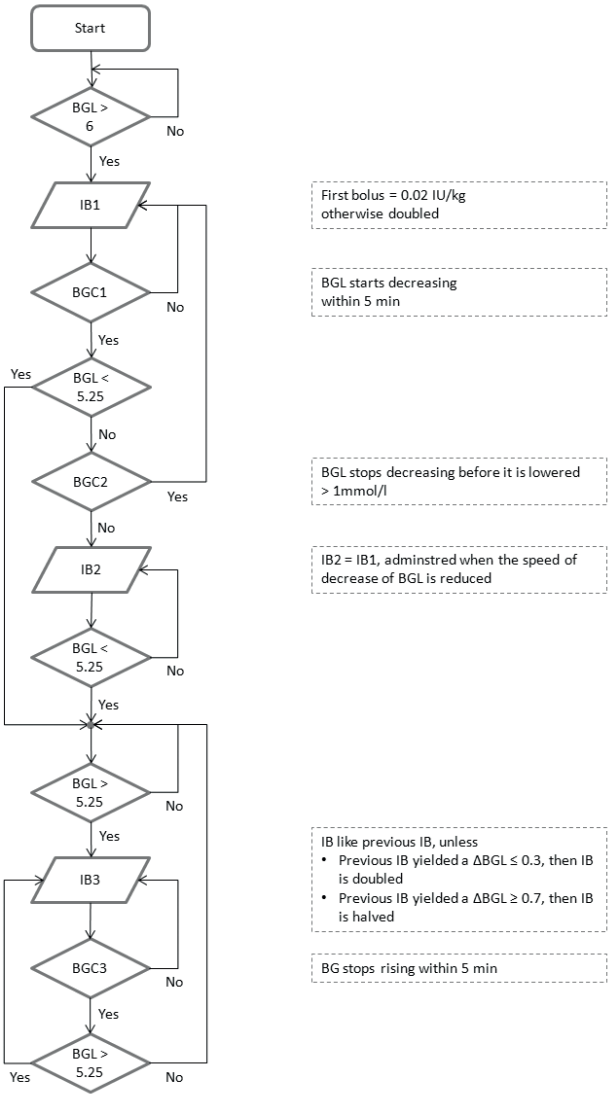


Figure 2: The current insulin algorithm; BGL: blood glucose level with the value in mmol/l; IB1-3: insulin bolus 1 to 3; BGC1-3 blood glucose control 1-3; ΔBGL the total amplitude in blood glucose level between two consecutive insulin boluses.

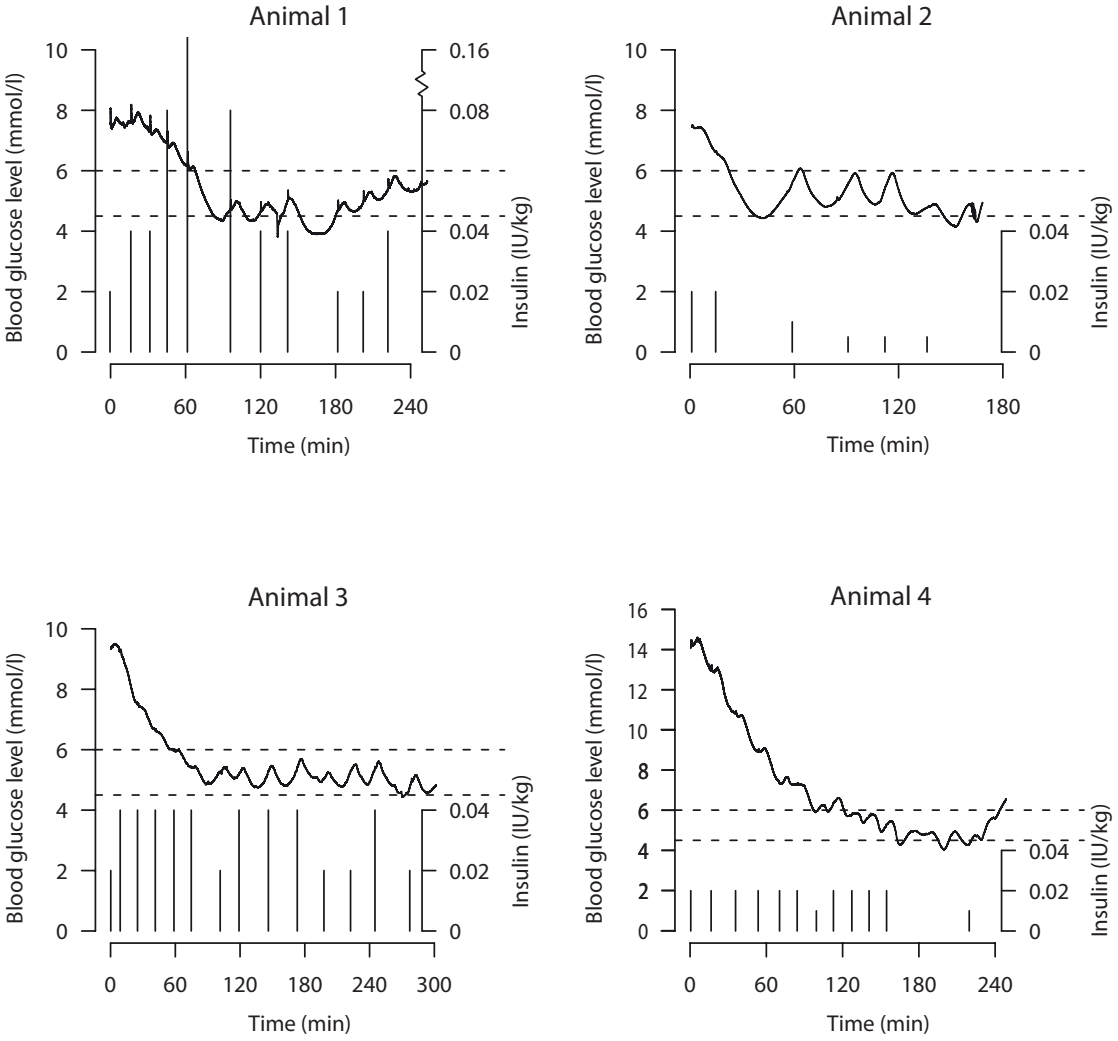


Figure 3: Blood glucose control in four animals; the curves depict the blood glucose level, the histograms depict the insulin boluses and the horizontal dashed lines depict the ideal blood glucose target interval of 4.5 to 6.0 mmol/l.

TABLES

Animal	BGL_0 (mmol/l)	BGL_{low} (mmol/l)	T_{est} (min)	RD ((mmol/l)/min)	T_{ctrl} (min)	T_{low} (min)	T_{range} (%)
1	7.53	3.81	68	0.025	186	51	73
2	7.46	4.14	21	0.073	146	20	86
3	9.33	4.44	63	0.064	238	3	99
4	14.06	4.04	121	0.077	128	22	83

Table 1: The seven outcome variables from the four animals; BGL_0: the blood glucose level at the time of the first insulin bolus; BGL_{low}: the lowest blood glucose level recording during the experiment; T_{est}: time to establish glycemic control from the first insulin bolus until the blood glucose level went below 6.0 mmol/l; RD: the rate of blood glucose level decrease during T_{est}; T_{ctrl}: time with glycemic control from blood glucose level went below 6.0 mmol/l until end of experiment; T_{low}: total time with blood glucose levels below 4.5 mmol/l during T_{ctrl}; T_{range}: percentage time with BGL in the correct range between 4.5 and 6.0 mmol/l during T_{ctrl}

Dissertations at the Faculty of Medicine, NTNU

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1978

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199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

2002

201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

2003

216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.

217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES
223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

2004

235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA

244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

2005

248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS
251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

2006

269. Torstein Baade Rø: EFFECTS OF BONE MORPHOGENETIC PROTEINS, HEPATOCYTE GROWTH FACTOR AND INTERLEUKIN-21 IN MULTIPLE MYELOMA
270. May-Britt Tessem: METABOLIC EFFECTS OF ULTRAVIOLET RADIATION ON THE ANTERIOR PART OF THE EYE
271. Anne-Sofie Helvik: COPING AND EVERYDAY LIFE IN A POPULATION OF ADULTS WITH HEARING IMPAIRMENT

272. Therese Standal: MULTIPLE MYELOMA: THE INTERPLAY BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MARROW MICROENVIRONMENT
273. Ingvild Saltvedt: TREATMENT OF ACUTELY SICK, FRAIL ELDERLY PATIENTS IN A GERIATRIC EVALUATION AND MANAGEMENT UNIT – RESULTS FROM A PROSPECTIVE RANDOMISED TRIAL
274. Birger Henning Endreseth: STRATEGIES IN RECTAL CANCER TREATMENT – FOCUS ON EARLY RECTAL CANCER AND THE INFLUENCE OF AGE ON PROGNOSIS
275. Anne Mari Aukan Rokstad: ALGINATE CAPSULES AS BIOREACTORS FOR CELL THERAPY
276. Mansour Akbari: HUMAN BASE EXCISION REPAIR FOR PRESERVATION OF GENOMIC STABILITY
277. Stein Sundstrøm: IMPROVING TREATMENT IN PATIENTS WITH LUNG CANCER – RESULTS FROM TWO MULITCENTRE RANDOMISED STUDIES
278. Hilde Pleym: BLEEDING AFTER CORONARY ARTERY BYPASS SURGERY - STUDIES ON HEMOSTATIC MECHANISMS, PROPHYLACTIC DRUG TREATMENT AND EFFECTS OF AUTOTRANSFUSION
279. Line Merethe Oldervoll: PHYSICAL ACTIVITY AND EXERCISE INTERVENTIONS IN CANCER PATIENTS
280. Boye Welde: THE SIGNIFICANCE OF ENDURANCE TRAINING, RESISTANCE TRAINING AND MOTIVATIONAL STYLES IN ATHLETIC PERFORMANCE AMONG ELITE JUNIOR CROSS-COUNTRY SKIERS
281. Per Olav Vandvik: IRRITABLE BOWEL SYNDROME IN NORWAY, STUDIES OF PREVALENCE, DIAGNOSIS AND CHARACTERISTICS IN GENERAL PRACTICE AND IN THE POPULATION
282. Idar Kirkeby-Garstad: CLINICAL PHYSIOLOGY OF EARLY MOBILIZATION AFTER CARDIAC SURGERY
283. Linn Getz: SUSTAINABLE AND RESPONSIBLE PREVENTIVE MEDICINE. CONCEPTUALISING ETHICAL DILEMMAS ARISING FROM CLINICAL IMPLEMENTATION OF ADVANCING MEDICAL TECHNOLOGY
284. Eva Tegnander: DETECTION OF CONGENITAL HEART DEFECTS IN A NON-SELECTED POPULATION OF 42,381 FETUSES
285. Kristin Gabestad Nørsett: GENE EXPRESSION STUDIES IN GASTROINTESTINAL PATHOPHYSIOLOGY AND NEOPLASIA
286. Per Magnus Haram: GENETIC VS. AQUIRED FITNESS: METABOLIC, VASCULAR AND CARDIOMYOCYTE ADAPTATIONS
287. Agneta Johansson: GENERAL RISK FACTORS FOR GAMBLING PROBLEMS AND THE PREVALENCE OF PATHOLOGICAL GAMBLING IN NORWAY
288. Svein Artur Jensen: THE PREVALENCE OF SYMPTOMATIC ARTERIAL DISEASE OF THE LOWER LIMB
289. Charlotte Björk Ingul: QUANITIFICATION OF REGIONAL MYOCARDIAL FUNCTION BY STRAIN RATE AND STRAIN FOR EVALUATION OF CORONARY ARTERY DISEASE. AUTOMATED VERSUS MANUAL ANALYSIS DURING ACUTE MYOCARDIAL INFARCTION AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY
290. Jakob Nakling: RESULTS AND CONSEQUENCES OF ROUTINE ULTRASOUND SCREENING IN PREGNANCY – A GEOGRAPHIC BASED POPULATION STUDY
291. Anne Engum: DEPRESSION AND ANXIETY – THEIR RELATIONS TO THYROID DYSFUNCTION AND DIABETES IN A LARGE EPIDEMIOLOGICAL STUDY
292. Ottar Bjerkeset: ANXIETY AND DEPRESSION IN THE GENERAL POPULATION: RISK FACTORS, INTERVENTION AND OUTCOME – THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
293. Jon Olav Drogset: RESULTS AFTER SURGICAL TREATMENT OF ANTERIOR CRUCIATE LIGAMENT INJURIES – A CLINICAL STUDY
294. Lars Fosse: MECHANICAL BEHAVIOUR OF COMPACTED MORSELLISED BONE – AN EXPERIMENTAL IN VITRO STUDY
295. Gunilla Klensmeden Fosse: MENTAL HEALTH OF PSYCHIATRIC OUTPATIENTS BULLIED IN CHILDHOOD
296. Paul Jarle Mork: MUSCLE ACTIVITY IN WORK AND LEISURE AND ITS ASSOCIATION TO MUSCULOSKELETAL PAIN

297. Björn Stenström: LESSONS FROM RODENTS: I: MECHANISMS OF OBESITY SURGERY – ROLE OF STOMACH. II: CARCINOGENIC EFFECTS OF *HELICOBACTER PYLORI* AND SNUS IN THE STOMACH

2007

298. Haakon R. Skogseth: INVASIVE PROPERTIES OF CANCER – A TREATMENT TARGET ? IN VITRO STUDIES IN HUMAN PROSTATE CANCER CELL LINES
299. Janniche Hammer: GLUTAMATE METABOLISM AND CYCLING IN MESIAL TEMPORAL LOBE EPILEPSY
300. May Britt Drugli: YOUNG CHILDREN TREATED BECAUSE OF ODD/CD: CONDUCT PROBLEMS AND SOCIAL COMPETENCIES IN DAY-CARE AND SCHOOL SETTINGS
301. Arne Skjold: MAGNETIC RESONANCE KINETICS OF MANGANESE DIPYRIDOXYL DIPHOSPHATE (MnDPDP) IN HUMAN MYOCARDIUM. STUDIES IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION
302. Siri Malm: LEFT VENTRICULAR SYSTOLIC FUNCTION AND MYOCARDIAL PERFUSION ASSESSED BY CONTRAST ECHOCARDIOGRAPHY
303. Valentina Maria do Rosario Cabral Iversen: MENTAL HEALTH AND PSYCHOLOGICAL ADAPTATION OF CLINICAL AND NON-CLINICAL MIGRANT GROUPS
304. Lasse Løvstakken: SIGNAL PROCESSING IN DIAGNOSTIC ULTRASOUND: ALGORITHMS FOR REAL-TIME ESTIMATION AND VISUALIZATION OF BLOOD FLOW VELOCITY
305. Elisabeth Olstad: GLUTAMATE AND GABA: MAJOR PLAYERS IN NEURONAL METABOLISM
306. Lilian Leistad: THE ROLE OF CYTOKINES AND PHOSPHOLIPASE A_{2}S IN ARTICULAR CARTILAGE CHONDROCYTES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS
307. Arne Vaaler: EFFECTS OF PSYCHIATRIC INTENSIVE CARE UNIT IN AN ACUTE PSYCIATHRIC WARD
308. Mathias Toft: GENETIC STUDIES OF LRRK2 AND PINK1 IN PARKINSON'S DISEASE
309. Ingrid Løvold Mostad: IMPACT OF DIETARY FAT QUANTITY AND QUALITY IN TYPE 2 DIABETES WITH EMPHASIS ON MARINE N-3 FATTY ACIDS
310. Torill Eidhammer Sjøbakk: MR DETERMINED BRAIN METABOLIC PATTERN IN PATIENTS WITH BRAIN METASTASES AND ADOLESCENTS WITH LOW BIRTH WEIGHT
311. Vidar Beisvåg: PHYSIOLOGICAL GENOMICS OF HEART FAILURE: FROM TECHNOLOGY TO PHYSIOLOGY
312. Olav Magnus Søndenå Fredheim: HEALTH RELATED QUALITY OF LIFE ASSESSMENT AND ASPECTS OF THE CLINICAL PHARMACOLOGY OF METHADONE IN PATIENTS WITH CHRONIC NON-MALIGNANT PAIN
313. Anne Brantberg: FETAL AND PERINATAL IMPLICATIONS OF ANOMALIES IN THE GASTROINTESTINAL TRACT AND THE ABDOMINAL WALL
314. Erik Solligård: GUT LUMINAL MICRODIALYSIS
315. Elin Tollefsen: RESPIRATORY SYMPTOMS IN A COMPREHENSIVE POPULATION BASED STUDY AMONG ADOLESCENTS 13-19 YEARS. YOUNG-HUNT 1995-97 AND 2000-01; THE NORD-TRØNDELAG HEALTH STUDIES (HUNT)
316. Anne-Tove Brenne: GROWTH REGULATION OF MYELOMA CELLS
317. Heidi Knobel: FATIGUE IN CANCER TREATMENT – ASSESSMENT, COURSE AND ETIOLOGY
318. Torbjørn Dahl: CAROTID ARTERY STENOSIS. DIAGNOSTIC AND THERAPEUTIC ASPECTS
319. Inge-Andre Rasmussen jr.: FUNCTIONAL AND DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN NEUROSURGICAL PATIENTS
320. Grete Helen Bratberg: PUBERTAL TIMING – ANTECEDENT TO RISK OR RESILIENCE ? EPIDEMIOLOGICAL STUDIES ON GROWTH, MATURATION AND HEALTH RISK BEHAVIOURS; THE YOUNG HUNT STUDY, NORD-TRØNDELAG, NORWAY
321. Sveinung Sørhaug: THE PULMONARY NEUROENDOCRINE SYSTEM. PHYSIOLOGICAL, PATHOLOGICAL AND TUMOURIGENIC ASPECTS
322. Olav Sande Eftedal: ULTRASONIC DETECTION OF DECOMPRESSION INDUCED VASCULAR MICROBUBBLES
323. Rune Bang Leistad: PAIN, AUTONOMIC ACTIVATION AND MUSCULAR ACTIVITY RELATED TO EXPERIMENTALLY-INDUCED COGNITIVE STRESS IN HEADACHE PATIENTS

324. Svein Brekke: TECHNIQUES FOR ENHANCEMENT OF TEMPORAL RESOLUTION IN THREE-DIMENSIONAL ECHOCARDIOGRAPHY
325. Kristian Bernhard Nilsen: AUTONOMIC ACTIVATION AND MUSCLE ACTIVITY IN RELATION TO MUSCULOSKELETAL PAIN
326. Anne Irene Hagen: HEREDITARY BREAST CANCER IN NORWAY. DETECTION AND PROGNOSIS OF BREAST CANCER IN FAMILIES WITH *BRCA1*GENE MUTATION
327. Ingebjørg S. Juel : INTESTINAL INJURY AND RECOVERY AFTER ISCHEMIA. AN EXPERIMENTAL STUDY ON RESTITUTION OF THE SURFACE EPITHELIUM, INTESTINAL PERMEABILITY, AND RELEASE OF BIOMARKERS FROM THE MUCOSA
328. Runa Heimstad: POST-TERM PREGNANCY
329. Jan Egil Afset: ROLE OF ENTEROPATHOGENIC *ESCHERICHIA COLI* IN CHILDHOOD DIARRHOEA IN NORWAY
330. Bent Håvard Hellum: *IN VITRO* INTERACTIONS BETWEEN MEDICINAL DRUGS AND HERBS ON CYTOCHROME P-450 METABOLISM AND P-GLYCOPROTEIN TRANSPORT
331. Morten André Høydal: CARDIAC DYSFUNCTION AND MAXIMAL OXYGEN UPTAKE MYOCARDIAL ADAPTATION TO ENDURANCE TRAINING

2008

332. Andreas Møllerløkken: REDUCTION OF VASCULAR BUBBLES: METHODS TO PREVENT THE ADVERSE EFFECTS OF DECOMPRESSION
333. Anne Hege Aamodt: COMORBIDITY OF HEADACHE AND MIGRAINE IN THE NORD-TRØNDELAG HEALTH STUDY 1995-97
334. Brage Høyem Amundsen: MYOCARDIAL FUNCTION QUANTIFIED BY SPECKLE TRACKING AND TISSUE DOPPLER ECHOCARDIOGRAPHY – VALIDATION AND APPLICATION IN EXERCISE TESTING AND TRAINING
335. Inger Anne Næss: INCIDENCE, MORTALITY AND RISK FACTORS OF FIRST VENOUS THROMBOSIS IN A GENERAL POPULATION. RESULTS FROM THE SECOND NORD-TRØNDELAG HEALTH STUDY (HUNT2)
336. Vegard Bugten: EFFECTS OF POSTOPERATIVE MEASURES AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY
337. Morten Bruvold: MANGANESE AND WATER IN CARDIAC MAGNETIC RESONANCE IMAGING
338. Miroslav Fris: THE EFFECT OF SINGLE AND REPEATED ULTRAVIOLET RADIATION ON THE ANTERIOR SEGMENT OF THE RABBIT EYE
339. Svein Arne Aase: METHODS FOR IMPROVING QUALITY AND EFFICIENCY IN QUANTITATIVE ECHOCARDIOGRAPHY – ASPECTS OF USING HIGH FRAME RATE
340. Roger Almvik: ASSESSING THE RISK OF VIOLENCE: DEVELOPMENT AND VALIDATION OF THE BRØSET VIOLENCE CHECKLIST
341. Ottar Sundheim: STRUCTURE-FUNCTION ANALYSIS OF HUMAN ENZYMES INITIATING NUCLEOBASE REPAIR IN DNA AND RNA
342. Anne Mari Undheim: SHORT AND LONG-TERM OUTCOME OF EMOTIONAL AND BEHAVIOURAL PROBLEMS IN YOUNG ADOLESCENTS WITH AND WITHOUT READING DIFFICULTIES
343. Helge Garåsen: THE TRONDHEIM MODEL. IMPROVING THE PROFESSIONAL COMMUNICATION BETWEEN THE VARIOUS LEVELS OF HEALTH CARE SERVICES AND IMPLEMENTATION OF INTERMEDIATE CARE AT A COMMUNITY HOSPITAL COULD PROVIDE BETTER CARE FOR OLDER PATIENTS. SHORT AND LONG TERM EFFECTS
344. Olav A. Foss: "THE ROTATION RATIOS METHOD". A METHOD TO DESCRIBE ALTERED SPATIAL ORIENTATION IN SEQUENTIAL RADIOGRAPHS FROM ONE PELVIS
345. Bjørn Olav Åsvold: THYROID FUNCTION AND CARDIOVASCULAR HEALTH
346. Torun Margareta Melø: NEURONAL GLIAL INTERACTIONS IN EPILEPSY
347. Irina Poliakova Eide: FETAL GROWTH RESTRICTION AND PRE-ECLAMPSIA: SOME CHARACTERISTICS OF FETO-MATERNAL INTERACTIONS IN DECIDUA BASALIS
348. Torunn Askim: RECOVERY AFTER STROKE. ASSESSMENT AND TREATMENT; WITH FOCUS ON MOTOR FUNCTION
349. Ann Elisabeth Åsberg: NEUTROPHIL ACTIVATION IN A ROLLER PUMP MODEL OF CARDIOPULMONARY BYPASS. INFLUENCE ON BIOMATERIAL, PLATELETS AND COMPLEMENT

350. Lars Hagen: REGULATION OF DNA BASE EXCISION REPAIR BY PROTEIN INTERACTIONS AND POST TRANSLATIONAL MODIFICATIONS
351. Sigrun Beate Kjøtrød: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN ASSISTED REPRODUCTION
352. Steven Keita Nishiyama: PERSPECTIVES ON LIMB-VASCULAR HETEROGENEITY: IMPLICATIONS FOR HUMAN AGING, SEX, AND EXERCISE
353. Sven Peter Näsholm: ULTRASOUND BEAMS FOR ENHANCED IMAGE QUALITY
354. Jon Ståle Ritland: PRIMARY OPEN-ANGLE GLAUCOMA & EXFOLIATIVE GLAUCOMA. SURVIVAL, COMORBIDITY AND GENETICS
355. Sigrid Botne Sando: ALZHEIMER'S DISEASE IN CENTRAL NORWAY. GENETIC AND EDUCATIONAL ASPECTS
356. Parvinder Kaur: CELLULAR AND MOLECULAR MECHANISMS BEHIND METHYLMERCURY-INDUCED NEUROTOXICITY
357. Ismail Cüneyt Güzey: DOPAMINE AND SEROTONIN RECEPTOR AND TRANSPORTER GENE POLYMORPHISMS AND EXTRAPYRAMIDAL SYMPTOMS. STUDIES IN PARKINSON'S DISEASE AND IN PATIENTS TREATED WITH ANTIPSYCHOTIC OR ANTIDEPRESSANT DRUGS
358. Brit Dybdahl: EXTRA-CELLULAR INDUCIBLE HEAT-SHOCK PROTEIN 70 (Hsp70) – A ROLE IN THE INFLAMMATORY RESPONSE ?
359. Kristoffer Haugarvoll: IDENTIFYING GENETIC CAUSES OF PARKINSON'S DISEASE IN NORWAY
360. Nadra Nilsen: TOLL-LIKE RECEPTOR 2 –EXPRESSION, REGULATION AND SIGNALING
361. Johan Håkon Bjørngaard: PATIENT SATISFACTION WITH OUTPATIENT MENTAL HEALTH SERVICES – THE INFLUENCE OF ORGANIZATIONAL FACTORS.
362. Kjetil Høydal : EFFECTS OF HIGH INTENSITY AEROBIC TRAINING IN HEALTHY SUBJECTS AND CORONARY ARTERY DISEASE PATIENTS; THE IMPORTANCE OF INTENSITY,, DURATION AND FREQUENCY OF TRAINING.
363. Trine Karlsen: TRAINING IS MEDICINE: ENDURANCE AND STRENGTH TRAINING IN CORONARY ARTERY DISEASE AND HEALTH.
364. Marte Thuen: MANGANASE-ENHANCED AND DIFFUSION TENSOR MR IMAGING OF THE NORMAL, INJURED AND REGENERATING RAT VISUAL PATHWAY
365. Cathrine Broberg Vågbø: DIRECT REPAIR OF ALKYLATION DAMAGE IN DNA AND RNA BY 2-OXOGLUTARATE- AND IRON-DEPENDENT DIOXYGENASES
366. Arnt Erik Tjønna: AEROBIC EXERCISE AND CARDIOVASCULAR RISK FACTORS IN OVERWEIGHT AND OBESE ADOLESCENTS AND ADULTS
367. Marianne W. Furnes: FEEDING BEHAVIOR AND BODY WEIGHT DEVELOPMENT: LESSONS FROM RATS
368. Lene N. Johannessen: FUNGAL PRODUCTS AND INFLAMMATORY RESPONSES IN HUMAN MONOCYTES AND EPITHELIAL CELLS
369. Anja Bye: GENE EXPRESSION PROFILING OF *INHERITED* AND *ACQUIRED* MAXIMAL OXYGEN UPTAKE – RELATIONS TO THE METABOLIC SYNDROME.
370. Oluf Dimitri Røe: MALIGNANT MESOTHELIOMA: VIRUS, BIOMARKERS AND GENES. A TRANSLATIONAL APPROACH
371. Ane Cecilie Dale: DIABETES MELLITUS AND FATAL ISCHEMIC HEART DISEASE. ANALYSES FROM THE HUNT1 AND 2 STUDIES
372. Jacob Christian Hølen: PAIN ASSESSMENT IN PALLIATIVE CARE: VALIDATION OF METHODS FOR SELF-REPORT AND BEHAVIOURAL ASSESSMENT
373. Erming Tian: THE GENETIC IMPACTS IN THE ONCOGENESIS OF MULTIPLE MYELOMA
374. Ole Bosnes: KLINISK UTPRØVING AV NORSKE VERSJONER AV NOEN SENTRALE TESTER PÅ KOGNITIV FUNKSJON
375. Ola M. Rygh: 3D ULTRASOUND BASED NEURONAVIGATION IN NEUROSURGERY. A CLINICAL EVALUATION
376. Astrid Kamilla Stunes: ADIPOKINES, PEROXISOME PROFILERATOR ACTIVATED RECEPTOR (PPAR) AGONISTS AND SEROTONIN. COMMON REGULATORS OF BONE AND FAT METABOLISM
377. Silje Engdal: HERBAL REMEDIES USED BY NORWEGIAN CANCER PATIENTS AND THEIR ROLE IN HERB-DRUG INTERACTIONS
378. Kristin Offerdal: IMPROVED ULTRASOUND IMAGING OF THE FETUS AND ITS CONSEQUENCES FOR SEVERE AND LESS SEVERE ANOMALIES

379. Øivind Rognmo: HIGH-INTENSITY AEROBIC EXERCISE AND CARDIOVASCULAR HEALTH
380. Jo-Åsmund Lund: RADIOTHERAPY IN ANAL CARCINOMA AND PROSTATE CANCER

2009

381. Tore Grüner Bjåstad: HIGH FRAME RATE ULTRASOUND IMAGING USING PARALLEL BEAMFORMING
382. Erik Søndenaa: INTELLECTUAL DISABILITIES IN THE CRIMINAL JUSTICE SYSTEM
383. Berit Rostad: SOCIAL INEQUALITIES IN WOMEN'S HEALTH, HUNT 1984-86 AND 1995-97, THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
384. Jonas Crosby: ULTRASOUND-BASED QUANTIFICATION OF MYOCARDIAL DEFORMATION AND ROTATION
385. Erling Tronvik: MIGRAINE, BLOOD PRESSURE AND THE RENIN-ANGIOTENSIN SYSTEM
386. Tom Christensen: BRINGING THE GP TO THE FOREFRONT OF EPR DEVELOPMENT
387. Håkon Bergseng: ASPECTS OF GROUP B STREPTOCOCCUS (GBS) DISEASE IN THE NEWBORN. EPIDEMIOLOGY, CHARACTERISATION OF INVASIVE STRAINS AND EVALUATION OF INTRAPARTUM SCREENING
388. Ronny Myhre: GENETIC STUDIES OF CANDIDATE TENE3S IN PARKINSON'S DISEASE
389. Torbjørn Moe Eggebø: ULTRASOUND AND LABOUR
390. Eivind Wang: TRAINING IS MEDICINE FOR PATIENTS WITH PERIPHERAL ARTERIAL DISEASE
391. Thea Kristin Våtsveen: GENETIC ABERRATIONS IN MYELOMA CELLS
392. Thomas Jozefiak: QUALITY OF LIFE AND MENTAL HEALTH IN CHILDREN AND ADOLESCENTS: CHILD AND PARENT PERSPECTIVES
393. Jens Erik Slagsvold: N-3 POLYUNSATURATED FATTY ACIDS IN HEALTH AND DISEASE – CLINICAL AND MOLECULAR ASPECTS
394. Kristine Misund: A STUDY OF THE TRANSCRIPTIONAL REPRESSOR ICER. REGULATORY NETWORKS IN GASTRIN-INDUCED GENE EXPRESSION
395. Franco M. Impellizzeri: HIGH-INTENSITY TRAINING IN FOOTBALL PLAYERS. EFFECTS ON PHYSICAL AND TECHNICAL PERFORMANCE
396. Kari Hanne Gjeilo: HEALTH-RELATED QUALITY OF LIFE AND CHRONIC PAIN IN PATIENTS UNDERGOING CARDIAC SURGERY
397. Øyvind Hauso: NEUROENDOCRINE ASPECTS OF PHYSIOLOGY AND DISEASE
398. Ingvild Bjellmo Johnsen: INTRACELLULAR SIGNALING MECHANISMS IN THE INNATE IMMUNE RESPONSE TO VIRAL INFECTIONS
399. Linda Tømmerdal Roten: GENETIC PREDISPOSITION FOR DEVELOPMENT OF PREEMCLAMPSIA – CANDIDATE GENE STUDIES IN THE HUNT (NORD-TRØNDELAG HEALTH STUDY) POPULATION
400. Trude Teoline Nausthaug Rakvåg: PHARMACOGENETICS OF MORPHINE IN CANCER PAIN
401. Hanne Lehn: MEMORY FUNCTIONS OF THE HUMAN MEDIAL TEMPORAL LOBE STUDIED WITH fMRI
402. Randi Utne Holt: ADHESION AND MIGRATION OF MYELOMA CELLS – IN VITRO STUDIES –
403. Trygve Solstad: NEURAL REPRESENTATIONS OF EUCLIDEAN SPACE
404. Unn-Merete Fagerli: MULTIPLE MYELOMA CELLS AND CYTOKINES FROM THE BONE MARROW ENVIRONMENT; ASPECTS OF GROWTH REGULATION AND MIGRATION
405. Sigrid Bjørnelv: EATING– AND WEIGHT PROBLEMS IN ADOLESCENTS, THE YOUNG HUNT-STUDY
406. Mari Hoff: CORTICAL HAND BONE LOSS IN RHEUMATOID ARTHRITIS. EVALUATING DIGITAL X-RAY RADIOGRAMMETRY AS OUTCOME MEASURE OF DISEASE ACTIVITY, RESPONSE VARIABLE TO TREATMENT AND PREDICTOR OF BONE DAMAGE
407. Siri Bjørgen: AEROBIC HIGH INTENSITY INTERVAL TRAINING IS AN EFFECTIVE TREATMENT FOR PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE
408. Susanne Lindqvist: VISION AND BRAIN IN ADOLESCENTS WITH LOW BIRTH WEIGHT
409. Torbjørn Hergum: 3D ULTRASOUND FOR QUANTITATIVE ECHOCARDIOGRAPHY

410. Jørgen Urnes: PATIENT EDUCATION IN GASTRO-OESOPHAGEAL REFLUX DISEASE. VALIDATION OF A DIGESTIVE SYMPTOMS AND IMPACT QUESTIONNAIRE AND A RANDOMISED CONTROLLED TRIAL OF PATIENT EDUCATION
411. Elvar Eyjolfsson: 13C NMRS OF ANIMAL MODELS OF SCHIZOPHRENIA
412. Marius Steiro Fimland: CHRONIC AND ACUTE NEURAL ADAPTATIONS TO STRENGTH TRAINING
413. Øyvind Støren: RUNNING AND CYCLING ECONOMY IN ATHLETES; DETERMINING FACTORS, TRAINING INTERVENTIONS AND TESTING
414. Håkon Hov: HEPATOCYTE GROWTH FACTOR AND ITS RECEPTOR C-MET. AUTOCRINE GROWTH AND SIGNALING IN MULTIPLE MYELOMA CELLS
415. Maria Radtke: ROLE OF AUTOIMMUNITY AND OVERSTIMULATION FOR BETA-CELL DEFICIENCY. EPIDEMIOLOGICAL AND THERAPEUTIC PERSPECTIVES
416. Liv Bente Romundstad: ASSISTED FERTILIZATION IN NORWAY: SAFETY OF THE REPRODUCTIVE TECHNOLOGY
417. Erik Magnus Berntsen: PREOPERATIV PLANNING AND FUNCTIONAL NEURONAVIGATION – WITH FUNCTIONAL MRI AND DIFFUSION TENSOR TRACTOGRAPHY IN PATIENTS WITH BRAIN LESIONS
418. Tonje Strømmen Steigedal: MOLECULAR MECHANISMS OF THE PROLIFERATIVE RESPONSE TO THE HORMONE GASTRIN
419. Vidar Rao: EXTRACORPOREAL PHOTOCHEMOTHERAPY IN PATIENTS WITH CUTANEOUS T CELL LYMPHOMA OR GRAFT-vs-HOST DISEASE
420. Torkild Visnes: DNA EXCISION REPAIR OF URACIL AND 5-FLUOROURACIL IN HUMAN CANCER CELL LINES

2010

421. John Munkhaugen: BLOOD PRESSURE, BODY WEIGHT, AND KIDNEY FUNCTION IN THE NEAR-NORMAL RANGE: NORMALITY, RISK FACTOR OR MORBIDITY ?
422. Ingrid Castberg: PHARMACOKINETICS, DRUG INTERACTIONS AND ADHERENCE TO TREATMENT WITH ANTIPSYCHOTICS: STUDIES IN A NATURALISTIC SETTING
423. Jian Xu: BLOOD-OXYGEN-LEVEL-DEPENDENT-FUNCTIONAL MAGNETIC RESONANCE IMAGING AND DIFFUSION TENSOR IMAGING IN TRAUMATIC BRAIN INJURY RESEARCH
424. Sigmund Simonsen: ACCEPTABLE RISK AND THE REQUIREMENT OF PROPORTIONALITY IN EUROPEAN BIOMEDICAL RESEARCH LAW. WHAT DOES THE REQUIREMENT THAT BIOMEDICAL RESEARCH SHALL NOT INVOLVE RISKS AND BURDENS DISPROPORTIONATE TO ITS POTENTIAL BENEFITS MEAN?
425. Astrid Woodhouse: MOTOR CONTROL IN WHIPLASH AND CHRONIC NON-TRAUMATIC NECK PAIN
426. Line Rørstad Jensen: EVALUATION OF TREATMENT EFFECTS IN CANCER BY MR IMAGING AND SPECTROSCOPY
427. Trine Moholdt: AEROBIC EXERCISE IN CORONARY HEART DISEASE
428. Øystein Olsen: ANALYSIS OF MANGANESE ENHANCED MRI OF THE NORMAL AND INJURED RAT CENTRAL NERVOUS SYSTEM
429. Bjørn H. Grønberg: PEMETREXED IN THE TREATMENT OF ADVANCED LUNG CANCER
430. Vigdis Schnell Husby: REHABILITATION OF PATIENTS UNDERGOING TOTAL HIP ARTHROPLASTY WITH FOCUS ON MUSCLE STRENGTH, WALKING AND AEROBIC ENDURANCE PERFORMANCE
431. Torbjørn Øien: CHALLENGES IN PRIMARY PREVENTION OF ALLERGY. THE PREVENTION OF ALLERGY AMONG CHILDREN IN TRONDHEIM (PACT) STUDY.
432. Kari Anne Indredavik Evensen: BORN TOO SOON OR TOO SMALL: MOTOR PROBLEMS IN ADOLESCENCE
433. Lars Adde: PREDICTION OF CEREBRAL PALSY IN YOUNG INFANTS. COMPUTER BASED ASSESSMENT OF GENERAL MOVEMENTS
434. Magnus Fasting: PRE- AND POSTNATAL RISK FACTORS FOR CHILDHOOD ADIPOSITY
435. Vivi Talstad Monsen: MECHANISMS OF ALKYLATION DAMAGE REPAIR BY HUMAN AlkB HOMOLOGUES
436. Toril Skandsen: MODERATE AND SEVERE TRAUMATIC BRAIN INJURY. MAGNETIC RESONANCE IMAGING FINDINGS, COGNITION AND RISK FACTORS FOR DISABILITY

437. Ingeborg Smidesang: ALLERGY RELATED DISORDERS AMONG 2-YEAR OLDS AND ADOLESCENTS IN MID-NORWAY – PREVALENCE, SEVERITY AND IMPACT. THE PACT STUDY 2005, THE YOUNG HUNT STUDY 1995-97
438. Vidar Halsteinli: MEASURING EFFICIENCY IN MENTAL HEALTH SERVICE DELIVERY: A STUDY OF OUTPATIENT UNITS IN NORWAY
439. Karen Lehrmann Ægidius: THE PREVALENCE OF HEADACHE AND MIGRAINE IN RELATION TO SEX HORMONE STATUS IN WOMEN. THE HUNT 2 STUDY
440. Madelene Ericsson: EXERCISE TRAINING IN GENETIC MODELS OF HEART FAILURE
441. Marianne Klokk: THE ASSOCIATION BETWEEN SELF-REPORTED ECZEMA AND COMMON MENTAL DISORDERS IN THE GENERAL POPULATION. THE HORDALAND HEALTH STUDY (HUSK)
442. Tomas Ottemo Stølen: IMPAIRED CALCIUM HANDLING IN ANIMAL AND HUMAN CARDIOMYOCYTES REDUCE CONTRACTILITY AND INCREASE ARRHYTHMIA POTENTIAL – EFFECTS OF AEROBIC EXERCISE TRAINING
443. Bjarne Hansen: ENHANCING TREATMENT OUTCOME IN COGNITIVE BEHAVIOURAL THERAPY FOR OBSESSIVE COMPULSIVE DISORDER: THE IMPORTANCE OF COGNITIVE FACTORS
444. Mona Løvlien: WHEN EVERY MINUTE COUNTS. FROM SYMPTOMS TO ADMISSION FOR ACUTE MYOCARDIAL INFARCTION WITH SPECIAL EMPHASIS ON GENDER DIFFERECES
445. Karin Margaretha Gilljam: DNA REPAIR PROTEIN COMPLEXES, FUNCTIONALITY AND SIGNIFICANCE FOR REPAIR EFFICIENCY AND CELL SURVIVAL
446. Anne Byriel Walls: NEURONAL GLIAL INTERACTIONS IN CEREBRAL ENERGY – AND AMINO ACID HOMEOSTASIS – IMPLICATIONS OF GLUTAMATE AND GABA
447. Cathrine Fallang Knetter: MECHANISMS OF TOLL-LIKE RECEPTOR 9 ACTIVATION
448. Marit Følsvik Svindseth: A STUDY OF HUMILIATION, NARCISSISM AND TREATMENT OUTCOME IN PATIENTS ADMITTED TO PSYCHIATRIC EMERGENCY UNITS
449. Karin Elvenes Bakkelund: GASTRIC NEUROENDOCRINE CELLS – ROLE IN GASTRIC NEOPLASIA IN MAN AND RODENTS
450. Kirsten Brun Kjelstrup: DORSOVENTRAL DIFFERENCES IN THE SPATIAL REPRESENTATION AREAS OF THE RAT BRAIN
451. Roar Johansen: MR EVALUATION OF BREAST CANCER PATIENTS WITH POOR PROGNOSIS
452. Rigmor Myran: POST TRAUMATIC NECK PAIN. EPIDEMIOLOGICAL, NEURORADIOLOGICAL AND CLINICAL ASPECTS
453. Krisztina Kunszt Johansen: GENEALOGICAL, CLINICAL AND BIOCHEMICAL STUDIES IN *LRRK2* – ASSOCIATED PARKINSON'S DISEASE
454. Pål Gjerden: THE USE OF ANTICHOLINERGIC ANTIPARKINSON AGENTS IN NORWAY. EPIDEMIOLOGY, TOXICOLOGY AND CLINICAL IMPLICATIONS
455. Else Marie Huuse: ASSESSMENT OF TUMOR MICROENVIRONMENT AND TREATMENT EFFECTS IN HUMAN BREAST CANCER XENOGRAFTS USING MR IMAGING AND SPECTROSCOPY
456. Khalid S. Ibrahim: INTRAOPERATIVE ULTRASOUND ASSESSMENT IN CORONARY ARTERY BYPASS SURGERY – WITH SPECIAL REFERENCE TO CORONARY ANASTOMOSES AND THE ASCENDING AORTA
457. Bjørn Øglænd: ANTHROPOMETRY, BLOOD PRESSURE AND REPRODUCTIVE DEVELOPMENT IN ADOLESCENCE OF OFFSPRING OF MOTHERS WHO HAD PREECLAMPSIA IN PREGNANCY
458. John Olav Roaldset: RISK ASSESSMENT OF VIOLENT, SUICIDAL AND SELF-INJURIOUS BEHAVIOUR IN ACUTE PSYCHIATRY – A BIO-PSYCHO-SOCIAL APPROACH
459. Håvard Dalen: ECHOCARDIOGRAPHIC INDICES OF CARDIAC FUNCTION – NORMAL VALUES AND ASSOCIATIONS WITH CARDIAC RISK FACTORS IN A POPULATION FREE FROM CARDIOVASCULAR DISEASE, HYPERTENSION AND DIABETES: THE HUNT 3 STUDY
460. Beate André: CHANGE CAN BE CHALLENGING. INTRODUCTION TO CHANGES AND IMPLEMENTATION OF COMPUTERIZED TECHNOLOGY IN HEALTH CARE
461. Latha Nrugham: ASSOCIATES AND PREDICTORS OF ATTEMPTED SUICIDE AMONG DEPRESSED ADOLESCENTS – A 6-YEAR PROSPECTIVE STUDY

462. Håvard Bersås Nordgaard: TRANSIT-TIME FLOWMETRY AND WALL SHEAR STRESS ANALYSIS OF CORONARY ARTERY BYPASS GRAFTS – A CLINICAL AND EXPERIMENTAL STUDY

Cotutelle with University of Ghent: Abigail Emily Swillens: A MULTIPHYSICS MODEL FOR IMPROVING THE ULTRASONIC ASSESSMENT OF LARGE ARTERIES

2011

463. Marte Helene Bjørk: DO BRAIN RHYTHMS CHANGE BEFORE THE MIGRAINE ATTACK? A LONGITUDINAL CONTROLLED EEG STUDY
464. Carl-Jørgen Arum: A STUDY OF UROTHELIAL CARCINOMA: GENE EXPRESSION PROFILING, TUMORIGENESIS AND THERAPIES IN ORTHOTOPIC ANIMAL MODELS
465. Ingunn Harstad: TUBERCULOSIS INFECTION AND DISEASE AMONG ASYLUM SEEKERS IN NORWAY. SCREENING AND FOLLOW-UP IN PUBLIC HEALTH CARE
466. Leif Åge Strand: EPIDEMIOLOGICAL STUDIES AMONG ROYAL NORWEGIAN NAVY SERVICEMEN. COHORT ESTABLISHMENT, CANCER INCIDENCE AND CAUSE-SPECIFIC MORTALITY
467. Katrine Høyer Holgersen: SURVIVORS IN THEIR THIRD DECADE AFTER THE NORTH SEA OIL RIG DISASTER OF 1980. LONG-TERM PERSPECTIVES ON MENTAL HEALTH
468. MarianneWallenius: PREGNANCY RELATED ASPECTS OF CHRONIC INFLAMMATORY ARTHRITIDES: DISEASE ONSET POSTPARTUM, PREGNANCY OUTCOMES AND FERTILITY. DATA FROM A NORWEGIAN PATIENT REGISTRY LINKED TO THE MEDICAL BIRTH REGISTRY OF NORWAY
469. Ole Vegard Solberg: 3D ULTRASOUND AND NAVIGATION – APPLICATIONS IN LAPAROSCOPIC SURGERY
470. Inga Ekeberg Schjerve: EXERCISE-INDUCED IMPROVEMENT OF MAXIMAL OXYGEN UPTAKE AND ENDOTHELIAL FUNCTION IN OBESE AND OVERWEIGHT INDIVIDUALS ARE DEPENDENT ON EXERCISE-INTENSITY
471. Eva Veslemøy Tyldum: CARDIOVASCULAR FUNCTION IN PREECLAMPSIA – WITH REFERENCE TO ENDOTHELIAL FUNCTION, LEFT VENTRICULAR FUNCTION AND PRE-PREGNANCY PHYSICAL ACTIVITY
472. Benjamin Garzón Jiménez de Cisneros: CLINICAL APPLICATIONS OF MULTIMODAL MAGNETIC RESONANCE IMAGING
473. Halvard Knut Nilsen: ASSESSING CODEINE TREATMENT TO PATIENTS WITH CHRONIC NON-MALIGNANT PAIN: NEUROPSYCHOLOGICAL FUNCTIONING, DRIVING ABILITY AND WEANING
474. Eiliv Brenner: GLUTAMATE RELATED METABOLISM IN ANIMAL MODELS OF SCHIZOPHRENIA
475. Egil Jonsbu: CHEST PAIN AND PALPITATIONS IN A CARDIAC SETTING; PSYCHOLOGICAL FACTORS, OUTCOME AND TREATMENT
476. Mona Høysæter Fenstad: GENETIC SUSCEPTIBILITY TO PREECLAMPSIA : STUDIES ON THE NORD-TRØNDELAG HEALTH STUDY (HUNT) COHORT, AN AUSTRALIAN/NEW ZEALAND FAMILY COHORT AND DECIDUA BASALIS TISSUE
477. Svein Erik Gaustad: CARDIOVASCULAR CHANGES IN DIVING: FROM HUMAN RESPONSE TO CELL FUNCTION
478. Karin Torvik: PAIN AND QUALITY OF LIFE IN PATIENTS LIVING IN NURSING HOMES
479. Arne Solberg: OUTCOME ASSESSMENTS IN NON-METASTATIC PROSTATE CANCER
480. Henrik Sahlin Pettersen: CYTOTOXICITY AND REPAIR OF URACIL AND 5-FLUOROURACIL IN DNA
481. Pui-Lam Wong: PHYSICAL AND PHYSIOLOGICAL CAPACITY OF SOCCER PLAYERS: EFFECTS OF STRENGTH AND CONDITIONING
482. Ole Solheim: ULTRASOUND GUIDED SURGERY IN PATIENTS WITH INTRACRANIAL TUMOURS
483. Sten Roar Snare: QUANTITATIVE CARDIAC ANALYSIS ALGORITHMS FOR POCKET-SIZED ULTRASOUND DEVICES
484. Marit Skyrud Bratlie: LARGE-SCALE ANALYSIS OF ORTHOLOGS AND PARALOGS IN VIRUSES AND PROKARYOTES
485. Anne Elisabeth F. Isern: BREAST RECONSTRUCTION AFTER MASTECTOMY – RISK OF RECURRENCE AFTER DELAYED LARGE FLAP RECONSTRUCTION – AESTHETIC OUTCOME, PATIENT SATISFACTION, QUALITY OF LIFE AND SURGICAL RESULTS;

HISTOPATHOLOGICAL FINDINGS AND FOLLOW-UP AFTER PROPHYLACTIC MASTECTOMY IN HEREDITARY BREAST CANCER

486. Guro L. Andersen: CEREBRAL PALSY IN NORWAY – SUBTYPES, SEVERITY AND RISK FACTORS
487. Frode Kolstad: CERVICAL DISC DISEASE – BIOMECHANICAL ASPECTS
488. Bente Nordtug: CARING BURDEN OF COHABITANTS LIVING WITH PARTNERS SUFFERING FROM CHRONIC OBSTRUCTIVE PULMONARY DISEASE OR DEMENTIA
489. Mariann Gjervik Heldahl: EVALUATION OF NEOADJUVANT CHEMOTHERAPY IN LOCALLY ADVANCED BREAST CANCER BASED ON MR METHODOLOGY
490. Lise Tevik Løvseth: THE SUBJECTIVE BURDEN OF CONFIDENTIALITY
491. Marie Hjelmseth Aune: INFLAMMATORY RESPONSES AGAINST GRAM NEGATIVE BACTERIA INDUCED BY TLR4 AND NLRP12
492. Tina Strømdal Wik: EXPERIMENTAL EVALUATION OF NEW CONCEPTS IN HIP ARTHROPLASTY
493. Solveig Sigurdardottir: CLINICAL ASPECTS OF CEREBRAL PALSY IN ICELAND. A POPULATION-BASED STUDY OF PRESCHOOL CHILDREN
494. Arne Reimers: CLINICAL PHARMACOKINETICS OF LAMOTRIGINE
495. Monica Wegling: KULTURMENNESKETS BYRDE OG SYKDOMMENS VELSIGNELSE. KAN MEDISINSK UTREDNING OG INTERVENSJON HA EN SELVSTENDIG FUNKSJON UAVHENGIG AV DET KURATIVE?
496. Silje Alvestad: ASTROCYTE-NEURON INTERACTIONS IN EXPERIMENTAL MESIAL TEMPORAL LOBE EPILEPSY – A STUDY OF UNDERLYING MECHANISMS AND POSSIBLE BIOMARKERS OF EPILEPTOGENESIS
497. Javaid Nauman: RESTING HEART RATE: A MATTER OF LIFE OR DEATH – PROSPECTIVE STUDIES OF RESTING HEART RATE AND CARDIOVASCULAR RISK (THE HUNT STUDY, NORWAY)
498. Thuy Nguyen: THE ROLE OF C-SRC TYROSINE KINASE IN ANTIVIRAL IMMUNE RESPONSES
499. Trine Naalsund Andreassen: PHARMACOKINETIC, PHARMACODYNAMIC AND PHARMACOGENETIC ASPECTS OF OXYCODONE TREATMENT IN CANCER PAIN
500. Eivor Alette Laugsand: SYMPTOMS IN PATIENTS RECEIVING OPIOIDS FOR CANCER PAIN – CLINICAL AND PHARMACOGENETIC ASPECTS
501. Dorthe Stensvold: PHYSICAL ACTIVITY, CARDIOVASCULAR HEALTH AND LONGEVITY IN PATIENTS WITH METABOLIC SYNDROME
502. Stian Thoresen Aspenes: PEAK OXYGEN UPTAKE AMONG HEALTHY ADULTS – CROSS-SECTIONAL DESCRIPTIONS AND PROSPECTIVE ANALYSES OF PEAK OXYGEN UPTAKE, PHYSICAL ACTIVITY AND CARDIOVASCULAR RISK FACTORS IN HEALTHY ADULTS (20-90 YEARS)
503. Reidar Alexander Vigen: PATHOBIOLOGY OF GASTRIC CARCINOIDS AND ADENOCARCINOMAS IN RODENT MODELS AND PATIENTS. STUDIES OF GASTROCYSTOPLASTY, GENDER-RELATED FACTORS, AND AUTOPHAGY
504. Halvard Høilund-Kaupang: MODELS AND METHODS FOR INVESTIGATION OF REVERBERATIONS IN NONLINEAR ULTRASOUND IMAGING
505. Audhild Løhre: WELLBEING AMONG SCHOOL CHILDREN IN GRADES 1-10: PROMOTING AND ADVERSE FACTORS
506. Torgrim Tandstad: VOX POPULI. POPULATION-BASED OUTCOME STUDIES IN TESTICULAR CANCER
507. Anna Brenne Grønskag: THE EPIDEMIOLOGY OF HIP FRACTURES AMONG ELDERLY WOMEN IN NORD-TRØNDELAG. HUNT 1995-97, THE NORD-TRØNDELAG HEALTH STUDY
508. Kari Ravndal Risnes: BIRTH SIZE AND ADULT MORTALITY: A SYSTEMATIC REVIEW AND A LONG-TERM FOLLOW-UP OF NEARLY 40 000 INDIVIDUALS BORN AT ST. OLAV UNIVERSITY HOSPITAL IN TRONDHEIM 1920-1960
509. Hans Jakob Bøe: LONG-TERM POSTTRAUMATIC STRESS AFTER DISASTER – A CONTROLLED STUDY OF SURVIVORS' HEALTH 27 YEARS AFTER THE CAPSIZED NORTH SEA OIL RIG
510. Cathrin Barbara Canto, Cotutelle with University of Amsterdam: LAYER SPECIFIC INTEGRATIVE PROPERTIES OF ENTORHINAL PRINCIPAL NEURONS
511. Ioanna Sandvig: THE ROLE OF OLFACTORY ENSHEATHING CELLS, MRI, AND BIOMATERIALS IN TRANSPLANT-MEDIATED CNS REPAIR

512. Karin Fahl Wader: HEPATOCYTE GROWTH FACTOR, C-MET AND SYNDECAN-1 IN MULTIPLE MYELOMA
513. Gerd Tranø: FAMILIAL COLORECTAL CANCER
514. Bjarte Bergstrøm: INNATE ANTIVIRAL IMMUNITY – MECHANISMS OF THE RIG-I-MEDIATED RESPONSE
515. Marie Søfteland Sandvei: INCIDENCE, MORTALITY, AND RISK FACTORS FOR ANEURYSMAL SUBARACHNOID HEMORRHAGE. PROSPECTIVE ANALYZES OF THE HUNT AND TROMSØ STUDIES
516. Mary-Elizabeth Bradley Eilertsen: CHILDREN AND ADOLESCENTS SURVIVING CANCER: PSYCHOSOCIAL HEALTH, QUALITY OF LIFE AND SOCIAL SUPPORT
517. Takaya Saito: COMPUTATIONAL ANALYSIS OF REGULATORY MECHANISM AND INTERACTIONS OF MICRORNAS

Godkjent for disputas, publisert post mortem: Eivind Jullumstrø: COLORECTAL CANCER AT LEVANGER HOSPITAL 1980-2004

518. Christian Gutvik: A PHYSIOLOGICAL APPROACH TO A NEW DECOMPRESSION ALGORITHM USING NONLINEAR MODEL PREDICTIVE CONTROL
519. Ola Storrø: MODIFICATION OF ADJUVANT RISK FACTOR BEHAVIOURS FOR ALLERGIC DISEASE AND ASSOCIATION BETWEEN EARLY GUT MICROBIOTA AND ATOPIC SENSITIZATION AND ECZEMA. EARLY LIFE EVENTS DEFINING THE FUTURE HEALTH OF OUR CHILDREN
520. Guro Fanneløb Giskeødegård: IDENTIFICATION AND CHARACTERIZATION OF PROGNOSTIC FACTORS IN BREAST CANCER USING MR METABOLOMICS
521. Gro Christine Christensen Løhaugen: BORN PRETERM WITH VERY LOW BIRTH WEIGHT – NEVER ENDING COGNITIVE CONSEQUENCES?
522. Sigrid Nakrem: MEASURING QUALITY OF CARE IN NURSING HOMES – WHAT MATTERS?
523. Brita Pukstad: CHARACTERIZATION OF INNATE INFLAMMATORY RESPONSES IN ACUTE AND CHRONIC WOUNDS

2012

524. Hans H. Wasmuth: ILEAL POUCHES
525. Inger Økland: BIASES IN SECOND-TRIMESTER ULTRASOUND DATING RELATED TO PREDICTION MODELS AND FETAL MEASUREMENTS
526. Bjørn Mørkedal: BLOOD PRESSURE, OBESITY, SERUM IRON AND LIPIDS AS RISK FACTORS OF ISCHAEMIC HEART DISEASE
527. Siver Andreas Moestue: MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF BREAST CANCER THROUGH A COMBINATION OF MR IMAGING, TRANSCRIPTOMICS AND METABOLOMICS
528. Guro Aune: CLINICAL, PATHOLOGICAL, AND MOLECULAR CLASSIFICATION OF OVARIAN CARCINOMA
529. Ingrid Alsos Lian: MECHANISMS INVOLVED IN THE PATHOGENESIS OF PRE-ECLAMPSIA AND FETAL GROWTH RESTRICTION. TRANSCRIPTIONAL ANALYSES OF PLACENTAL AND DECIDUAL TISSUE
530. Karin Solvang-Garten: X-RAY REPAIR CROSS-COMPLEMENTING PROTEIN 1 – THE ROLE AS A SCAFFOLD PROTEIN IN BASE EXCISION REPAIR AND SINGLE STRAND BREAK REPAIR
531. Toril Holien: BONE MORPHOGENETIC PROTEINS AND MYC IN MULTIPLE MYELOMA
532. Rooyen Mavenyengwa: *STREPTOCOCCUS AGALACTIAE* IN PREGNANT WOMEN IN ZIMBABWE: EPIDEMIOLOGY AND SEROTYPE MARKER CHARACTERISTICS
533. Tormod Rimehaug: EMOTIONAL DISTRESS AND PARENTING AMONG COMMUNITY AND CLINIC PARENTS
534. Maria Dung Cao: MR METABOLIC CHARACTERIZATION OF LOCALLY ADVANCED BREAST CANCER – TREATMENT EFFECTS AND PROGNOSIS
535. Mirta Mittelstedt Leal de Sousa: PROTEOMICS ANALYSIS OF PROTEINS INVOLVED IN DNA BASE REPAIR AND CANCER THERAPY
536. Halfdan Petursson: THE VALIDITY AND RELEVANCE OF INTERNATIONAL CARDIOVASCULAR DISEASE PREVENTION GUIDELINES FOR GENERAL PRACTICE
537. Marit By Rise: LIFTING THE VEIL FROM USER PARTICIPATION IN CLINICAL WORK – WHAT IS IT AND DOES IT WORK?

538. Lene Thoresen: NUTRITION CARE IN CANCER PATIENTS. NUTRITION ASSESSMENT: DIAGNOSTIC CRITERIA AND THE ASSOCIATION TO SURVIVAL AND HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH ADVANCED COLORECTAL CARCINOMA
539. Berit Doseth: PROCESSING OF GENOMIC URACIL IN MAN AND MOUSE
540. Gro Falkenér Bertheussen: PHYSICAL ACTIVITY AND HEALTH IN A GENERAL POPULATION AND IN CANCER SURVIVORS – METHODOLOGICAL, OBSERVATIONAL AND CLINICAL ASPECTS
541. Anne Kari Knudsen: CANCER PAIN CLASSIFICATION
542. Sjur Urdson Gjerald: A FAST ULTRASOUND SIMULATOR
543. Harald Edvard Mølmen Hansen: CARDIOVASCULAR EFFECTS OF HIGH INTENSITY AEROBIC INTERVAL TRAINING IN HYPERTENSITIVE PATIENTS, HEALTHY AGED AND YOUNG PERSONS
544. Sasha Gulati: SURGICAL RESECTION OF HIGH-GRADE GLIOMAS
545. John Chr. Fløvig: FREQUENCY AND EFFECT OF SUBSTANCES AND PSYCHOACTIVE MEDICATIONS THE WEEK BEFORE ADMISSION TO AN ACUTE PSYCHIATRIC DEPARTMENT
546. Kristin Moksnes Husby: OPTIMIZING OPIOID TREATMENT FOR CANCER PAIN – CLINICAL AND PHARMACOLOGICAL ASPECTS
547. Audun Hanssen-Bauer: X-RAY REPAIR CROSS-COMPLEMENTING PROTEIN 1 ASSOCIATED MULTIPROTEIN COMPLEXES IN BASE EXCISION REPAIR
548. Marit Saunes: ECZEMA IN CHILDREN AND ADOLESCENTS – EPIDEMIOLOGY, COURSE AND IMPACT. THE PREVENTION OF ALLERGY AMONG CHILDREN IN TRONDHEIM (PACT) STUDY, YOUNG-HUNT 1995-97
549. Guri Kaurstad: CARDIOMYOCYTE FUNCTION AND CALCIUM HANDLING IN ANIMAL MODELS OF INBORN AND ACQUIRED MAXIMAL OXYGEN UPTAKE
550. Kristian Svendsen: METHODOLOGICAL CHALLENGES IN PHARMACOEPIDEMIOLOGICAL STUDIES OF OPIOID CONSUMPTION
551. Signe Nilssen Stafne: EXERCISE DURING PREGNANCY
552. Marius Widerøe: MAGNETIC RESONANCE IMAGING OF HYPOXIC-ISCHEMIC BRAIN INJURY DEVELOPMENT IN THE NEWBORN RAT – MANGANESE AND DIFFUSION CONTRASTS
553. Andreas Radtke: MOLECULAR METHODS FOR TYPING *STREPTOCOCCUS AGALACTIAE* WITH SPECIAL EMPHASIS ON THE DEVELOPMENT AND VALIDATION OF A MULTI-LOCUS VARIABLE NUMBER OF TANDEM REPEATS ASSAY (MLVA)
554. Thor Wilhelm Bjelland: PHARMACOLOGICAL ASPECTS OF THERAPEUTIC HYPOTHERMIA
555. Caroline Hild Hakvåg Pettersen: THE EFFECT OF OMEGA-3 POLYUNSATURATED FATTY ACIDS ON HUMAN CANCER CELLS – MOLECULAR MECHANISMS INVOLVED
556. Inga Thorsen Vengen: INFLAMMATION AND ATHEROSCLEROSIS – RISK ASSOCIATIONS IN THE HUNT SURVEYS
557. Elisabeth Balstad Magnussen: PREECLAMPSIA, PRETERM BIRTH AND MATERNAL CARDIOVASCULAR RISK FACTORS
558. Monica Unsgaard-Tøndel: MOTOR CONTROL EXERCISES FOR PATIENTS WITH LOW BACK PAIN
559. Lars Erik Sande Laugsand: INSOMNIA AND RISK FOR CARDIOVASCULAR DISEASE
560. Kjersti Grønning: PATIENT EDUCATION AND CHRONIC INFLAMMATORY POLYARTHRITIS – COPING AND EFFECT
561. Hanne Gro Wenzel: PRE AND POST-INJURY HEALTH IN PERSONS WITH WHIPLASH: THE HUNT STUDY. EXPLORATION OF THE FUNCTIONAL SOMATIC MODEL FOR CHRONIC WHIPLASH
562. Øystein Grimstad: TOLL-LIKE RECEPTOR-MEDIATED INFLAMMATORY RESPONSES IN KERATINOCYTES
563. Håkon Olav Leira: DEVELOPMENT OF AN IMAGE GUIDANCE RESEARCH SYSTEM FOR BRONCHOSCOPY
564. Michael A. Lang: DIVING IN EXTREME ENVIRONMENTS: THE SCIENTIFIC DIVING EXPERIENCE

565. Helena Bertilsson: PROSTATE CANCER-TRANSLATIONAL RESEARCH. OPTIMIZING TISSUE SAMPLING SUITABLE FOR HISTOPATHOLOGIC, TRANSCRIPTOMIC AND METABOLIC PROFILING
566. Kirsten M. Selnæs: MR IMAGING AND SPECTROSCOPY IN PROSTATE AND COLON CANCER DIAGNOSTICS
567. Gunvor Steine Fosnes: CONSTIPATION AND DIARRHOEA. EFFECTIVENESS AND ADVERSE EFFECTS OF DRUGS
568. Areej Elkamil: SPASTIC CEREBRAL PALSY: RISK FACTORS, BOTULINUM TOXIN USE AND PREVENTION OF HIP DISLOCATION
569. Ruth Derdikman Eiron: SYMPTOMS OF ANXIETY AND DEPRESSION AND PSYCHOSOCIAL FUNCTION IN MALES AND FEMALES FROM ADOLESCENCE TO ADULTHOOD: LONGITUDINAL FINDINGS FROM THE NORD-TRØNDELAG HEALTH STUDY
570. Constantin Sergiu Jianu: PROTON PUMP INHIBITORS AND GASTRIC NEOPLASIA IN MAN
571. Øystein Finset Sørdal: THE ROLE OF GASTRIN AND THE ECL CELL IN GASTRIC CARCINOGENESIS
572. Lisbeth Østgaard Rygg: GROUP EDUCATION FOR PATIENTS WITH TYPE 2 DIABETES – NEEDS, EXPERIENCES AND EFFECTS
573. Viola Lobert: IDENTIFICATION OF NOVEL REGULATORS OF EPITHELIAL POLARITY AND CELL MIGRATION
574. Maria Tunset Grinde: CHARACTERIZATION OF BREAST CANCER USING MR METABOLOMICS AND GENE EXPRESSION ANALYSIS
575. Grete Kjelvik: HUMAN ODOR IDENTIFICATION STUDIES IN HEALTHY INDIVIDUALS, MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE
576. Tor Eivind Bernstein: RECTAL CANCER SURGERY. PROGNOSTIC FACTORS RELATED TO TREATMENT
577. Kari Sand: INFORMED CONSENT DOCUMENTS FOR CANCER RESEARCH: TEXTUAL AND CONTEXTUAL FACTORS OF RELEVANCE FOR UNDERSTANDING
578. Laurent Francois Thomas: EFFECTS OF SINGLE-NUCLEOTIDE POLYMORPHISMS ON microRNA-BASED GENE REGULATION AND THEIR ASSOCIATION WITH DISEASE
579. Øystein Sandanger: THE INNATE IMMUNE SYSTEM: A PARADOXICAL MEDIATOR OF HOST DEFENSE, TISSUE REPAIR AND COLLATERAL DAMAGE
580. Line Knutsen Lund: MENTAL HEALTH IN LOW BIRTH WEIGHT INDIVIDUALS APPROACHING ADULTHOOD
581. Nils Kristian Skjærvold: AUTOMATED BLOOD GLUCOSE CONTROL – DEVELOPMENT AND TESTING OF AN ARTIFICIAL ENDOCRINE PANCREAS USING A NOVEL INTRAVASCULAR GLUCOSE MONITOR AND A NEW APPROACH TO INSULIN PHARMACOLOGY