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PERIOPERATIVE GLUCOSE CONTROL

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ABSTRACT

Trauma or surgery elicit a physiological stress response, which among others induces insulin resistance. It could be described as a state where the biological response to a given dose of insulin is reduced, and is associated with an altered glucose metabolism, as well as a disturbed lipid and protein metabolism and dysfunctional immune, inflammatory and coagulation systems. The endogenous glucose production (EGP) is enhanced, whereas the whole-body glucose disposal (WGD) is reduced with the result of hyperglycemia.

Hyperglycemia, > 11.1 mmol/l glucose, in surgical patients is associated with increased rates of infection and mortality. On the other hand, hypoglycemia, <3.9 mmol/l glucose, is associated with adverse events and increased mortality. Still, attempts to improve clinical outcome in critically ill patients, by maintaining normoglycemia, have resulted in diverging results for morbidity and mortality. The latter could be the result of increasing glucose variability with the treatment. Reduced glucose variability, independent the glucose range, seems beneficial and could possibly be accomplished by continuous glucose measurement. One suggested method for monitoring mainly tissue metabolism, such as glucose, is microdialysis (MD). The overall aims of this thesis are to better understand the metabolic consequences of insulin treatment on glucose metabolism in relation to major stress and to test a new approach for continuous glucose monitoring.

The first half of this thesis investigated the accuracy of intravenous glucose MD measurements using various perfusion rates and length of peripheral catheters in volunteers (paper I), and continuous on-line MD measurements via a central venous catheter performed in surgical patients (paper II). In the second half of the thesis, the effect of glucose control on postoperative insulin resistance and glucose kinetics in liver surgery was investigated by the hyperinsulinemic normoglycemic clamp technique (HNC) (paper III-IV) and the isotopic tracer dilution method (paper IV).

In paper I, reduced rate of perfusion fluid and increased length of semipermeable membrane improved the accuracy of microdialysis readings and plasma reference values. In paper II, the use of a continuous on-line real time MD systems proceeded over 20 hours, with measurements every minute, demonstrated good correlation to plasma reference values. All values were found in zone A and B in a Clark Error Grid, indicating safe usage. In paper III and IV, insulin resistance was assessed pre- and postoperatively in patients subjected to liver surgery. During surgery, intravenous insulin was administered to maintain glucose at 6-8 mmol/l in the treatment group, whereas the control group was allowed glucose > 11 mmol/l before intervention. Intraoperative mean glucose was significantly different between groups. Postoperative insulin resistance was significantly higher in the control group (paper III-IV) and glucose kinetics (paper IV) were altered after the surgical trauma, with increased EGP and substantially reduced WGD, without any statistical difference between the groups. Intraoperative kinetic alterations revealed a reduced EGP and an unaltered WGD, despite

evolving hyperglycemia, possibly due to undetected rapid changes in glucose kinetics earlier during the surgical procedure.

In conclusion, microdialysis is a feasible technique for intravenous continuous on-line glucose measurements monitoring. Intraoperative glucose control during liver surgery maintains insulin sensitivity assessed by HNC. In all patients, reduced WGD is a major contributor to early postoperative insulin resistance. Intraoperative glucose kinetics indicate reduced EGP and stable WGD despite evolving hyperglycemia.

LIST OF SCIENTIFIC PAPERS

I. **Continuous glucose monitoring by intravenous microdialysis: influence of membrane length and dialysis flow rate**
O. Rooyackers, C. Blixt, P. Mattsson and J. Wernerman
Acta Anaesthesiol Scand 2013; 57**:** 214–219

II. **Continuous on-line glucose measurement by microdialysis in a central vein. A pilot study**
C. Blixt, O. Rooyackers, B. Isaksson, J. Wernerman
Critical Care 2013, 17:R87

III. **The effect of perioperative glucose control on postoperative insulin resistance**
C. Blixt, C. Ahlstedt, O. Ljungqvist, B. Isaksson, S. Kalman, O. Rooyackers
Clinical Nutrition 21 (2012) 676-681

IV. **The effect of glucose control in liver surgery on glucose kinetics and insulin resistance**
C. Blixt, M. Larsson, B. Isaksson, O. Ljungqvist, O. Rooyackers
(In manuscript)

CONTENTS

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
CGM	Continuous glucose measurement
CRP	C-reactive protein
CVC	Central venous catheter
DM	Diabetes mellitus
EDA	Epidural anesthesia
EGP	Endogenous glucose production
FSIVGTT	Frequently sampled intravenous glucose tolerance test
GCMS	Gas-chromatography mass-spectrometer
GDH	Glucose-1-dehydrogenase
GLUT	Glucose transporters
GOD	Glucose oxidase
GH	Growth hormone
HNC	Hyperinsulinemic normoglycemic clamp
HOMA-IR	Homeostatic Model Assessment-Insulin Resistance
IL	Interleukin
IR	Insulin receptor
ICU	Intensive Care Unit
MARD	Mean absolute relative difference
MD	Microdialysis
MPE	Molar percent excess
NF-KB	Nuclear factor-KB
OGTT	Oral glucose tolerance test
PAI-1	Plasminogen activator inhibitor-1
POC	Point of care
POD	Peroxidase
PVC	Peripheral venous catheter
RBC	Packed red blood cells

TNF-α	Tissue necrosis factor-α
WGD	Whole-body glucose disposal

1 INTRODUCTION

In Sweden, about 900 patients are subjected to liver surgery every year[1]. A physiological response to surgical injury, trauma or critical illness is the transient insulin resistance and subsequent metabolic disturbances, similar to the alterations observed in diabetes type 2.

In 1942 Cuthbertson described the systemic metabolic changes as a response to surgery in the lower extremities. The terms of ebb and flow in metabolism were introduced to explain the initial period of depressed metabolism that precedes the later hypermetabolism[2]. This stress response has been regarded as a beneficial adaptation to trauma, to increase the chance of survival of an injured animal. The net effect is an increased tissue catabolism of carbohydrates, fat and protein, which results in hyperglycemia, thereby securing the cellular energy supply to vital organs[3]. Insulin resistance swiftly alters glucose kinetics by increasing the hepatic endogenous glucose production and by reducing the glucose disposal in peripheral tissues, resulting in elevated glucose levels.

The belief of hyperglycemia as a possible survival benefit has later been revised, since several studies have shown potential positive effects on surgical outcome by modulating the subsequent hyperglycemia[4-6]. The development of insulin resistance can be attenuated by minimizing the size of surgery, an adequate pain control and by maintaining perioperative normoglycemia, either accomplished by reducing the preoperative fasting time, with preoperative beverage or glucose infusion, or by insulin treatment.

Though hyperglycemia has repeatedly been proven to aggravate postoperative adverse events, the attempts to improve outcome by introducing tight glucose control in severely ill patients, have dramatically increased the risk for hypoglycemic events[7,8]. The downside effect of tight glucose control has been demonstrated in several multicenter studies[9-11], which has bred the important question of the necessity of the concept. A less rigid glucose target and avoidance of high variability, regardless of dysglycemia, may have the same positive impact on the stress response. Hence, this notion has put the light on how, where and when to monitor glucose levels, and has introduced the need for a feasible, accurate and continuous measurement technique to detect swift alterations in glucose levels. Several different technologies, applying intravascular or subcutaneous sampling, have been suggested in the field of continuous glucose monitoring, microdialysis being one promising alternative. This technique, by using passive diffusion over a semipermeable membrane, creates a dialysate containing the extracellular concentration of a certain compound, which can be immediately analyzed. Microdialysis can be applicable in most tissues, and the intravascular approach is of special interest for continuous glucose measurement systems in critically ill patients, which are at risk of having compromised tissue perfusion.

In summary, the optimal glucose range for tight glucose control during major surgery and critical illness is not settled. The use of accurate continuous glucose measurement devices has been suggested beneficial to reduce hypoglycemic events and the intravascular microdialysis

technique is one suggested method. The postoperative insulin resistance and hyperglycemia may be attenuated by glucose control, which effects the endogenous glucose production and the whole-body glucose disposal, though the intraoperative alterations are less investigated.

2 GLUCOSE HOMEOSTASIS AT NORMAL CONDITIONS

Glucose concentration is normally kept at a stable level, regulated by several complex interactions. The principal organs involved in glucose homeostasis include the central nervous system, pancreas, muscles, adipose tissue, liver and kidney[12].

2.1 CELLULAR TRANSPORT AND REGULATION

Glucose enters the cell by two ways; either by sodium dependent active transport or by facilitated diffusion, the first requires glucose transporters (GLUTs). The different types of GLUTs have various affinities for their substrates, and tissue-specific expression and regulation [12]. As insulin binds to the insulin receptor (IR) in the cell membrane, a cellular cascade is initiated, which results in the GLUTs being released and fusing with the plasma membrane, thereby permitting an increased glucose uptake. Several types of GLUTs have been identified; having different features, allowing for glucose transport under a wide glucose range. GLUT-1 is expressed in erythrocytes and neurons, transporting glucose under normoglycemic conditions. GLUT-4 is predominantly expressed in skeletal muscle, cardiac muscle, and adipose tissue, being the main contributor of insulin-dependent peripheral glucose disposal. The hepatocytes, renal cells and pancreatic β-cells have predominantly GLUT-2 on the cell surface, a transporter with high capacity and low sensitivity to insulin, which enables the cells to absorb large quantities of glucose after ingestion of food[3].

In adipose and muscular tissues, insulin mediates the cellular glucose uptake. However, in most other tissues, the major part of glucose disposal is non-insulin mediated, driven by a facilitated diffusion. In the post-absorptive state, uptake in these tissues represents >70% of whole body glucose disposal[13]. Pancreatic cells, erythrocytes, neurons, immune and endothelial cells have all insulin-independent glucose uptake. In a hyperglycemic state, glucose uptake in the central nervous system is saturated[14], resulting in glucose overload in other tissues[15].

2.2 INSULIN SECRETION AND REGULATION

Insulin is the main anabolic hormone, a polypeptide produced and pulsatile secreted by the β-cells in the pancreas. The secretion is mainly regulated by the blood glucose concentration, but also by amino acids and ketone bodies[3]. The basal insulin concentration is 10-15mU/l, which can be rapidly increased by 4-5 times as a response to elevated blood glucose level after ingesting a meal[16].

The hormone reduces the glucose concentration by promoting glucose uptake in peripheral tissue, such as muscle and adipose cells, and by inhibiting glucose production within hepatocytes. Insulin is the most important hormone involved in glucose homeostasis, yet not the only one. Its actions are counter-regulated by other hormones. Glucagon, produced and secreted from the pancreatic α-cells, has the opposite effect of insulin. Hormones secreted by the adrenal gland, such as catecholamines (adrenaline and noradrenaline) and cortisol, also increase glucose concentration[17]. Incretins are a collective name for hormones released by the

intestines and that also modulate insulin secretion, hence influence glucose homeostasis[18] when nutrients are absorbed by the intestines.

2.3 METABOLIC AND NON-METABOLIC EFFECTS OF INSULIN

Apart from its action on carbohydrate metabolism, insulin also affects lipid and protein metabolism by decreasing lipolysis and stimulating protein anabolism.

In addition, the hormone has effects in other areas beyond regulating the energy supply for the cell. It has anti-inflammatory effects, by regulating the accumulation of macrophages and neutrophils in areas of inflammation. Supressed expression of pro-inflammatory transcription factors (NF-KB)[19] and the concentration of inflammatory mediators, IL-1β, IL-6 and TNF-α as well as CRP, adds to the anti-inflammatory qualities of insulin. Furthermore, it augments the production of nitric oxide[20], which results in depressed platelet aggregation and vasodilation, thereby demonstrating a combined anti-inflammatory and anti-thrombotic action[21,22].

Insulin suppress the generation of tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1), and reduces the levels of reactive oxygen species[21], which together yields anti-thrombotic, pro-fibrinolytic as well as anti-oxidant effects.

2.4 GLUCOSE TURNOVER

Glucose molecules are always present in the blood, and the blood glucose level is kept constant with a continuous turn-over rate. The inflow derives from either intestinal absorption after a meal or endogenous glucose production. The hepatocytes are the main producer of glucose, with an additional contribution of roughly 25% from the kidney[23]. The hepatocyte creates glucose either by glycogenolysis or gluconeogenesis.

Glucose is the main cellular energy source for various tissues. The muscles dominates the body glucose disposal after a meal, accounting for 70-90% of the total glucose uptake, together with adipose tissue[24]. However, even if many cells can utilise other energy sources, for example lactate and ketone bodies, some cells like neurons, erythrocytes and the renal medulla, are totally dependent on glucose as an energy source and a stable glucose level.

2.5 GLUCOSE METABOLISM IN THE POSTABSORPTIVE STATE

The normal human glucose level in a fasted state is <7 mmol/l[25]. The turn-over rate of glucose in the post-absorptive state is about 2 mg/kg/min, or 200 g per day for a 70 kg person[26]. In this state, glucose level is kept steady by a mixture of breakdown of glycogen and new production of glucose, to counter-act declining levels resulting from glucose disposal.

Glycogen is stored in the liver after a meal, as a long-term glucose supply. Liver glycogen stores can keep the blood glucose level constant for 24 hours, if no food is ingested. As a comparison, the amounts of free glucose molecules in the body can only supply energy for about 30 min[3]. Glycogen is also produced and stored in muscular tissue. However, here it is

used for internal cellular energy requirement only, as the muscle is unable to convert the molecule back into glucose. Instead, by further metabolizing the glucose-phosphate to pyruvate and lactate, the molecule can re-enter the gluconeogenesis in the liver in the form of lactate, better known as the Cori cycle.

The predominant precursor for gluconeogenesis is lactate, recycled from various tissues using anaerobic glycolysis, as described above. However, also the substrates can be used, such as amino acids, mostly alanine[27], from muscle protein breakdown as well as glycerol from lipocytes in adipose tissue. These are all transported via the blood to the liver, the main place for gluconeogenesis.

2.6 GLUCOSE METABOLISM AFTER A MEAL

Ingested glucose is absorbed by the intestines and transported via the portal vein to the liver. The molecules are taken up by the hepatocytes, via GLUT-2, and transformed to glucose-6-phosphate. Inside the cell, the phosphorylated glucose can then be stored as glycogen or degraded for energy supply, depending on the insulin/glucagon ratio. In a healthy person, ingestion of food will make the blood glucose level rise and this increase will induce the liver to shift metabolism from catabolism to anabolism.

Insulin is secreted from the pancreas in response to a raised glucose level, and delivered to the liver via the portal vein to coordinate glucose metabolism[28]. A combined high glucose and insulin level activate glycogen storage and glycolysis, in addition to inhibition of glycogen breakdown and gluconeogenesis. This effect is counter-regulated, mostly by glucagon but also by catecholamines and cortisol[3]. However, catechoamines and cortisol have been demonstrated to depress only insulin-mediated uptake, with no reduction of non-insulin mediated uptake[29,30].

The effect of eating on glucose turnover is well established. The blood glucose level, as well as the insulin concentration, are clearly elevated, while the hepatic endogenous glucose production rate is almost totally suppressed in response to the supply of exogenous glucose[3]. In addition, the glucose clearance from plasma is faster since the insulin stimulates peripheral glucose uptake.

This describes the normal pattern in a healthy individual, whilst metabolism dramatically alters in conditions of stress.

3 STRESS RESPONSE TO TRAUMA

Transient insulin resistance is a common physiological phenomenon developing in response to stress, trauma or critical illness. The modifications observed in the endocrine and metabolic responses to a potentially fatal trauma, may have been adapted to improve chances for survival, ensuring an energy supply and a hemodynamic balance. The metabolic alterations resemble the changes observed in type 2 diabetes, presenting a stress induced hyperglycemia in addition to increased lipolysis and proteolysis[31].

3.1 ENDOCRINE RESPONSE

Immediately following trauma there is a local tissue reaction, where afferent neural impulses and inflammatory mediators, cytokines, from the damaged area activate the neuroendocrine system[32]. The hypothalamus acts on the sympathetic autonomous system by affecting nerve terminals to release noradrenaline[17]. In addition, the hypothalamus stimulates the pituitary-adrenal axis, via increased pituitary secretion of ACTH and GH, to increase the release of adrenal hormones, catecholamines and cortisol[17]. The net result is a patient presenting with clinical signs of tachycardia and hypertension. In addition, increased secretion of vasopressin from the pituitary gland retains salt and water, which enables the body to maintain hemodynamic stability.

3.2 INSULIN RESISTANCE AND PERIOPERATIVE GLUCOSE TURNOVER

Insulin resistance can be defined as a state where a normal concentration of insulin has a reduced biologic effect. It is rapidly developed in response to surgical trauma or critical illness, and is mainly characterized by a decreased peripheral glucose uptake and an increased endogenous hepatic glucose production[33], see Figure 1. However, in addition insulin resistance renders several non-metabolic effects in other tissues, resulting in a pro-inflammatory, pro-thrombotic and high oxidative stress state.

The complex teamwork between released cytokines and stress hormones adds up to a reduced action of insulin and stress hyperglycemia. An increased cytokine level resulting from tissue damage has been suggested to be one potential mechanism for inducing hepatic and peripheral insulin resistance[34,35]. TNF-α, in addition to IL-1 and IL-6, has been shown to interfere with the insulin signal transduction[36]. In addition, hyperglycemia per se exacerbates a flood of proinflammatory cytokines in combination with markers for oxidative stress[37], thus having the potential to set up a vicious circle, where the mounting hyperglycemia could contribute to further hyperglycemia.

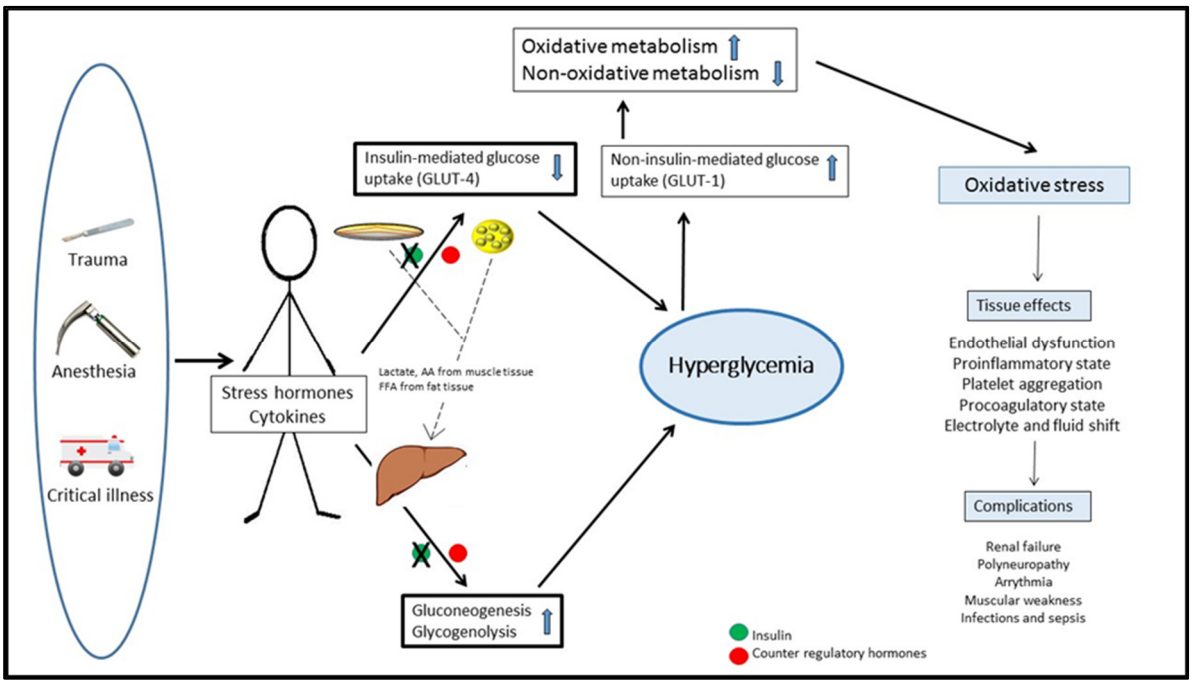

Figure 1: Schematic overview of insulin resistance. After an acute insult, critical illness or trauma, stress hormones and cytokines are released, thereby affecting the endogenous glucose production, which leads to an increased gluconeogenesis and glycogenolysis, and reducing the insulin-mediated glucose uptake via GLUT-4. The net hyperglycemia and glucose overload in insulin-independent tissues increases the oxidative metabolism and results in an oxidative stress, which is suggested to cause tissue pathology and clinical complications.

3.2.1 Glucose kinetics during stress hyperglycemia

Stress hyperglycemia is characterized by a disturbed glucose turn-over, indicated by a markedly elevated endogenous glucose production, since the net effect of stress hormones on the liver renders the hepatocytes unresponsive to the action of insulin. Moreover, in the periphery insulin dependent glucose uptake is reduced because of down-regulation of GLUT-4, an impaired insulin receptor signaling and a decreased muscle glycogen storage[38]. Even though glucose uptake may be impaired in these cells; glucose oxidation within the cell seems to be unaffected[39]. In contrast, the non-insulin mediated glucose uptake via GLUT-1 is enhanced, resulting in an increased whole-body glucose uptake[36].

3.3 ALTERATIONS IN TISSUE METABOLISM IN RESPONSE TO HYPERGLYCEMIA

An elevated blood glucose level is associated with a dysfunctional immune system[34] and endothelium[40], in addition to an increased inflammatory and procoagulant state. This disturbed

balance generates delayed wound healing, increased infection rates and injuries to myocardial, renal and cerebral tissues[32].

In tissues with non-insulin dependent glucose uptake, cells are exposed to excessive levels of glucose. Certain cell types, such as renal glomeruli, endothelial and neural cells, which are unable to reduce this glucose- uptake, are vulnerable to hyperglycemia[41]. This burst of energy supply induces an intracellular oxidative stress, where mitochondria produce reactive oxygen species instead of oxidizing glucose. Brownlee suggested that this superoxide production damages pathways, leading to disrupted immune and inflammatory systems, thereby creating a possible explanation for the damages noted in different organs and tissues in response to acute stress hyperglycemia[42], see Figure 1.

This tissue damage has been observed in kidney and liver, where changes in mitochondrial enzymes, or increased transaminases postoperatively have been demonstrated [43-45]. Moreover, after short-term exposure to hyperglycemia, in vivo and in vitro studies have shown increased platelet activation[46], and significantly impaired endothelial vasodilation[40]. The detrimental organ failure due to hyperglycemia and critical illness may be a result of cellular toxicity and impaired cellular autophagy, as suggested by Vanhorebeek et al[47].

However, it has also been argued that hyperglycemia should be left untreated, as human metabolism is adapted for survival during long term starvation in critical illness[48,49]. As previously discussed, during shock, hyperglycemia initially promotes circulatory stability and the non-insulin mediated glucose uptake is enhanced. As insulin resistance develops, energy is provided from oxidation of fat and ketone bodies, instead of glucose, which spares protein. In addition, intracellular glucose metabolism is turned into production of NADPH, an energy form needed by macrophages and neutrophils in host defense, and a substance needed in cell proliferation.

4 HYPERGLYCEMIA AND OUTCOME IN ICU AND SURGERY

Diabetes mellitus is a well-known cause to renal, cardiac and neurological complications. Though these patients per se have a significantly raised risk for developing postoperative complications[50] and death[51], it has been demonstrated in numerous studies that acute hyperglycemia and insulin resistance induce the risk for postoperative complications irrespective the diabetic status, in surgical[52-54] as well as medical/ICU settings[55-58]. Pathological alterations on a cellular level may account for the unfavorable findings in multiple patient populations. However, it can be discussed whether the glycemic alterations per se causes harm, or is mere a marker of severe illness.

Non-DM patients do not seem to be protected from adverse events, rather the opposite[59]. As Egi et al. demonstrated in a ICU setting, non-DM patients with a glucose range of 8-10 mmol/l or 10-11 mmol/l had an increased mortality risk by 1.7 and 3.3 times respectively, compared to DM patients in the same glucose range[60]. Moreover, non-DM patients displaying hyperglycemia at admittance to the hospital, demonstrated a raised risk for ICU-need and an even higher in-hospital mortality - 16% vs. 3% in diagnosed DM patients[61]. Hence, the premorbid glycemic status seems to matters for the clinical outcome, as demonstrated by that an elevated glycosylated hemoglobin (HbA1c) correlates to postoperative adverse events[53,62]. This was also reported for prior non-DM patients in non-cardiac surgery presenting with glucose values > 12 mmol/l, thereby indicating an acute disturbed glucose status, which had a higher one-year mortality compared to hyperglycemic DM patients[63]. These results are confirmed in different patient groups; in non-DM patients admitted with myocardial infarction and simultaneous hyperglycemia, in-hospital mortality is four times higher compared to normoglycemic patients[55], an additional poor clinical outcome has also been shown in hyperglycemic non-DM patients suffering from stroke[56].

Over the past years, the negative impact of intraoperative hyperglycemia during cardiovascular surgery has been repeatedly correlated to higher mortality and total postoperative complications, irrespective diabetic status[4,64-67]. Duncan et al found that a perioperative glucose level > 11 mmol/l, regardless diabetic status, augments total postoperative morbidity, showing an increased risk for adverse respiratory and renal events as well as infections after cardiac surgery[64]. Though in this study, correcting intraoperative glucose levels were beneficial on outcome only up to a point, as the risk for adverse events increased at normoglycemic levels.

The unfavorable outcome of perioperative hyperglycemia was also found in patients subjected to noncardiac surgery. Outtara et al correlated intraoperative glucose level > 8.2 mmol/l in diabetics to high total in-hospital morbidity, with significantly increased cardiac, renal, neurological and respiratory morbidity[65]. In a large study on general surgical patients by Kotagal et al., DM patients had higher risk for postoperative complications, independent of the glucose level[68]. However, non-DM with perioperative hyperglycemia showed a dose-response relationship between adverse events and glucose level, with an OR of 1.6 if blood glucose > 10 mmol/l. The results were repeated in a retrospective study on over 3000 non-cardiac surgical

patients, though DM patients displayed higher morbidity and mortality, the strongest association between the degree of perioperative hyperglycemia and the risk of mortality was found in non-DM patients[69].

Similar results were found in transplant surgery, where perioperative hyperglycemia is associated with delayed renal[70] or liver graft[71] function. Perioperative hyperglycemia has been repeatedly associated with increased risk for surgical site infections, not only in cardiac surgery[72], but also in liver[73], colorectal[74], transplant[75] and orthopedic[76] procedures.

4.1 LIVER SURGERY

The liver is pivotal in the regulation of glucose metabolism, being the major contributor of glucose production[23]. Theoretically, major liver resection would raise the risk for hypoglycemia due to a reduced remnant parenchyma. Fortunately, a functioning liver has a substantial residual capacity, and even major resections may not result in hypoglycemia[77]. However, the extent of resection matters, a large resection volume predisposes for postoperative morbidity[78], and is possibly associated with a suboptimal remnant hepatic immune system[79].

Preoperative liver dysfunction may correlate with perioperative hyperglycemia[80]. However, liver surgery per se induces insulin resistance, even in non-DM patients without liver failure, as reported by Durzynski et al[81]. In response to the surgical stress during hepatic resection, cytokine levels are increased[82]. The level of cytokines correlated to the incidence of liver dysfunction after hepatic resections, defined as hyperbilirubinemia, and postoperative infections. In addition, the degree of liver necrosis after injury has been associated to the increase in liver transaminases[83].

Perioperative hyperglycemia during liver surgery for colorectal metastases, was associated with postoperative complications[84]. In major resections, DM patients have a raised risk for fatal early postoperative liver failure, whereas non-DM patients are not spared from adverse events[85]. Postoperative complications per se have a major impact on long term survival after hepatic resection[86]. Han et al. concluded that the development of perioperative hyperglycemia is associated to the extent of hepatocyte injury, as demonstrated in a trial, including patients with liver cirrhosis[45]. The study recommended moderate perioperative glucose control, since increased levels of liver transaminases were associated with hyperglycemia >10 mmol/l, whereas lower glucose levels failed to show the same effect.

4.1.1 Ischemia and Reperfusions injury - Pringle maneuver

Occasionally in liver surgery, the Pringle maneuver (which implies temporary vascular occlusion of the hepatic artery and portal vein in the hepatoduodenal ligament) is used to reduce hemorrhage during complicated partial liver resections. This maneuver may cause ischemic reperfusion injury to the hepatocytes. Repeated Pringle maneuvers have been shown to induce rapidly increasing hyperglycemia when the ligament is unclamped, a phenomenon that may be

associated to glycogen breakdown[87] and studies have suggested that the cells are more vulnerable to damage if liver glycogen storage is depleted[88].

Experimental data demonstrated overt hyperglycemia during ischemia/reperfusion in surgery aggravate the injury in a liver[89], as well as a renal[90] animal model. Furthermore, other trials indicated that intraoperative hyperglycemia per se aggravate the ischemic reperfusion damage, rendering a vicious circle with negative impact on postoperative outcome[91,92]. The same phenomenon was demonstrated in liver and renal transplantation, where perioperative hyperglycemia was associated with postoperative infections[75], delayed graft function or rejection[70,71,93] as well as with a raised risk for mortality[94].

However, contradictive data was presented in a trial on renal transplant patients, where in-hospital hyperglycemia had no adverse effect on outcome one year later[95]. The discrepancy may depend on the timing of acute hyperglycemia, as demonstrated in experiments by Hirose et al[90]. By targeting intraoperative glucose levels <9 mmol/l, Parekh et al. showed improved renal function 30 days after renal transplantation[96].

Even though hyperglycemia has been acknowledged to contribute to substantial morbidity and mortality, in surgical as well as ICU-patients, the question is whether a reduction of the glucose level accomplish clinical benefits? Accordingly, the optimal glucose target and intervention period remain frequently and vividly debated[97,98].

5 MODULATION OF INSULIN RESISTANCE

As previously mentioned, stress induced hyperglycemia is linked to the development of insulin resistance. The stress response is also associated to clinical outcome, as the degree of postoperative resistance is significantly correlated to length of stay[99]. Indeed, in cardiac surgery, Sato et al. made a clear association between postoperative adverse events and the degree of insulin resistance[53]. For each 1mg/ml of reduced insulin sensitivity, the risk for major complications was doubled and for severe infections was five-fold higher.

The degree of whole-body insulin resistance was most pronounced on the day after surgery, where over 50% of preoperative values may be lost, and may persist for weeks[100]. In addition, on a cellular level, glycogen synthase was impaired up till a month after surgery[101], and protein catabolism may cause considerable nitrogen loss. These findings may partly explain the delayed recovery in postoperative as well as ICU patients. Consequently, several strategies have been developed to prevent or modulate the stress response, in an attempt to improve clinical outcome. Some of the major factors will be discussed below.

5.1 MAGNITUDE OF TRAUMA

The extent of surgery is one factor influencing the degree of insulin resistance and hyperglycemia[102], where abdominal surgery seems to provoke the largest response[103]. Thorell et al. demonstrated the relative reduction of insulin sensitivity correlated to the size of surgery[102]. Other investigators have made the same associations between the magnitude of surgery and the degree of insulin resistance[104], or the degree of increased endogenous glucose production[105]. By reducing the perioperative trauma, for example by performing minimal invasive surgery, the development of postoperative insulin resistance can be moderated and has been associated to increased glucose disposal after abdominal surgery compared to open surgery[106-109].

An important aspect of trauma and surgery, is perioperative hemorrhage, which is suggested to correlate with the perioperative glucose level[110]. Moreover, blood transfusion may affect perioperative glycemic levels, since RBCs are stored in SAG-MAN solutions (Saline-Adenosine-Glucose-Mannitol), which contains about 15-25 mmol glucose/unit[111].

5.2 FASTING AND NUTRITION

Normoglycemia

Patients presenting for surgery, may unexpectedly have abnormal glucose homeostasis. In one prospective study 26% of the patients had prior undiagnosed impaired fasting glucose[112]. As high preoperative glucose levels are suggested a predictor of perioperative adverse events and mortality[63], patients with preoperative dysglycemia may be regarded as having the same risks for complications as a previously diagnosed DM patient.

Insulin treatment

By maintaining normoglycemia with concurrent insulin and glucose infusion during surgery, the early development of insulin resistance can be reduced, and the decreased peripheral glucose disposal attenuated[113]. In the later phase of trauma, insulin treatment may have its major action on reducing the endogenous glucose production[114].

As described, insulin per se possesses non-metabolic properties, with potential benefits for surgical patients. Levels of IL-6, IL-8 and TNF-α, as well as troponin, decrease with insulin dosage[115,116]. Moreover, insulin exhibits a positive effect on the inflammatory response to trauma, in addition to suppressing platelet activation[15,36]. As demonstrated, patients subjected to an hyperinsulinemic normoglycemic clamp during cardiac surgery displayed an attenuated inflammatory response[117] and a decreased and delayed stress response, indicated by a reduced ACTH and cortisol release[118]. Nygren et al. could present similar reduction in cortisol response during insulin infusion[113]. The insulin effect on the stress response is the rationale for glucose control protocols, where revealed cardioprotective properties, augmented myocardial perfusion as well as inotropic effects, indicate positive clinical implications[6,116,119]. Studies have used administration of a fixed rate of insulin, often in context of a hyperinsulinemic clamp technique[120] or a strict protocol, sometimes administered as a GIK-infusion, glucose-insulin-potassium (KCl)[6]. The use of variable insulin dosage in trial protocols, is less common.

Fasting, pre- and intraoperative nutrition

Historically, elective surgery has been performed after an overnight fast. The rationale has been to avoid pulmonary aspiration of gastric content. A prolonged preoperative fasting time depletes liver glycogen content, and has been proven to negatively influence insulin sensitivity[121].

Glucose administration decreases the endogenous glucose production, though intraoperatively the suppression is less due to the emerging insulin resistance[122]. However, as demonstrated in several studies, preoperative carbohydrate load, given as a glucose infusion or an oral beverage, preserves postoperative insulin sensitivity[113,123-125]. Preoperative beverage seems to primarily attenuate whole body glucose uptake in the immediate postoperative phase[123], while a later attenuating effect on glucose production has been demonstrated up till three days after surgery[126]. In an animal study, Gjessing et al. suggested the beneficial effect on postoperative glucose uptake, is due to an improved insulin signaling in the muscle tissues[127].

In addition, the insulin resistant state negatively affects protein and lipid metabolism, turning the patient into a catabolic state. Even though exogenous nutrients may be available, the patient is unable to use substrates for anabolism[128]. By maintaining normoglycemia during surgery[129,130] while providing nutrients, or after surgery[131], postoperative protein catabolism has been blunted. Similar results were reported by Svanfeldt et al., where administration of preoperative carbohydrates reduced postoperative endogenous glucose production and maintained postoperative protein balance[132]. Whether fat metabolism is affected by concurrent

glucose infusion is unclear. Glucose infused postoperatively had no effect on lipolysis in colorectal surgery[133]. Though, in another trial by Kambe et al., intraoperative administration of glucose, lowered the concentration of stress hormones and free fatty acids in blood, without causing hyperglycemia[134].

5.3 THE EFFECT OF ANESTHESIA

Notably, the choice of anesthetic technique during surgery matters for the development of insulin resistance. In addition, mere bedrest and immobilization have been shown to induce a reduction in peripheral glucose uptake[135].

Local anesthesia

The addition of epidural anesthesia has been repeatedly shown to mitigate the early neuroendocrine stress response and insulin resistance after major surgery, by reducing levels of stress hormones[136], the inflammatory response[137], endogenous glucose production[138] and protein breakdown[139,140]. As Lattermann et al. demonstrated, by applying an epidural anesthesia, a better suppression of the endogenous glucose production was observed during exogenous glucose administration[138]. However, later in the postoperative phase, it is suggested that an epidural has no further beneficial effect on glucose kinetics[141].

Agents used in general anesthesia

Opioids used during general anesthesia may blunt the metabolic response during surgery[142]. A similar effect was demonstrated for Propofol at single use. Moreover, the combination of Propofol and opioids, commonly used in general anesthesia, reduces the stress response during surgery, compared to inhaled anesthesia[143]. However, the effect seems to be limited to the intraoperative period only. In contrast to both neuraxial block and intravenous anesthesia, some inhaled anesthetics (isoflurane and sevoflurane) seem to cause intraoperative hyperglycemia, by decreasing the peripheral glucose uptake and increasing the endogenous glucose production[144], where an impaired insulin secretion has been suggested as the explanation for the glycemic effects[145].

The α_2-agonist clonidine seems to suppress the neuroendocrine stress response in a dose-dependent way. Glucose metabolism is also affected, possibly caused by a direct inhibitory effect on the pancreatic β-cells[146]. Regarding glucocorticoids, which is often used intraoperatively for prophylaxis of postoperative nausea, it is known to exacerbate short-term hyperglycemia[147].

5.4 CORRELATION TO BMI, GENDER AND AGE

Increasing body weight and expanding adipose tissue have been suggested to be closely linked to the development of insulin resistance[148]. Insulin resistance has also been demonstrated to increase with age, possibly associated to decreased insulin secretion and action. Premenopausal woman are suggested to have a better insulin sensitivity[149]. However, when considering BMI,

age or gender, there was no correlation to insulin resistance for either, when insulin sensitivity was assessed by HNC in over 70 subjects[113].

6 GLUCOSE CONTROL IN ICU AND SURGERY

6.1 GLUCOSE CONTROL IN ICU

Hyperglycemia was previously regarded as a normal adaptive reaction to stress, not being a cause for medical intervention. In response to accumulating evidence from several trials that the condition might be harmful[55-57,150,151], the attitude to glucose control in postoperative or in critically ill patients changed.

The landmark Leuven study, presented in 2001 by van den Berghe et al., had a pronounced impact on the medical society. This prospective randomized non-blinded trial on 1548 adult ICU patients, investigated the effects of tight glucose control, i.e. blood glucose of 4.4-6.1 mmol/l, compared to blood glucose in the range of 10-11.1 mmol/l[152]. By accomplishing normoglycemia, during concurrent parenteral nutrition, in-hospital mortality was reduced by 34%. In addition, the observed survival benefit was reflected by a decreased morbidity. The ICU patients receiving insulin therapy demonstrated a reduced risk for renal failure, blood stream infections, liver dysfunction, peripheral neuropathy and muscle weakness, as well as less need for mechanical ventilation.

The remarkable results from this and subsequent trials[4,153] had a tremendous effect on the glycemic management in ICU-settings. Nevertheless, the debate on whether these results could be applied to other ICU populations, not only a subset of surgical patients, or on patients subjected to other nutritional, i.e. enteral, regimens, not only high doses of intravenous glucose, continued.

Subsequently, a new major prospective study was published by the same Leuven-group in 2006, though this time with the focus on medical-ICU patients. 1200 patients were included, and the identical protocol as in the previous trial was applied. In contrast, this subsequent trial could not reproduce the overall beneficial outcome for mortality. Though, the investigators could demonstrate positive effects of glucose control in a subgroup analysis of patients requiring 3 days of ICU-care, or more. Among this subset of patients, in-hospital mortality was reduced by 18%. Furthermore, a decreased rate of acute kidney injury, requirement of mechanical ventilation and a reduced ICU- and hospital-length of stay were observed. However, the trial reported a concerning backside in that over 18% of the patients had, at some point, a glucose value <2.2 mmol/l.

The investigators presented pooled data from the two studies, confirming the positive effects of intensive glucose control. By maintaining glucose level < 8 mmol/l mortality was reduced and additional benefits on morbidity were demonstrated at levels <6 mmol/l[154].

Consequently, several studies were launched to confirm the positive outcome results in mixed ICU-populations. However, two major multicenter-trials were prematurely interrupted partly due to high incidence of hypoglycemic events[9,11], a concerning finding which was repeatedly reported from other investigators[7,8,155]. The largest multicenter trial to this date, could not

disclose any beneficial effect of intensive insulin treatment. In contrast, it demonstrated a substantially raised risk for hypoglycemia[10], which dampened the enthusiasm for tight glucose control in the ICU.

6.2 GLUCOSE CONTROL IN SURGERY

6.2.1 Cardiovascular and general surgery

The proposed tight regimen for glucose control was subsequently adapted in the perioperative period with cardiac surgery, especially in DM patients, since these are per se at risk for developing postoperative complications[156]. Gandhi et al. demonstrated a linear relationship between intraoperative glucose level and the risk for adverse events[157]. Notably, in a population of prior non-DM patients subjected to cardiac surgery, about 70% was found to be pre-diabetics[158], thus implying that a majority of these patients have a disturb glycemic control and are at risk for complications. In this type of surgery, acute hyperglycemia has repeatedly been associated with unfavorable outcome. Accordingly, a substantial body of literature has focused on perioperative glucose control in patients subjected to cardiovascular intervention, suggesting beneficial effects on wound infections[159,160], renal function[161] and reduced incidence and degree of postoperative myocardial infarction[162]. Tight glucose control in cardiac surgery has been suggested cardioprotective, indicated by an improved myocardial function and reduced postoperative troponin levels[116,120].

Results are difficult to compare, as many studies do not have the same timing of intraoperative hyperglycemia. Some investigators have initiated glucose control during[65] and others after surgery[4]. Some trials mix DM-patients and non-DM patients, others do not. Some trials were retrospective[54,157,161], others observational[69] or sometimes randomized[163]. Studies have prospectively investigated glucose control in the intraoperative period, though had different glucose target ranges in the treatment group[6,163]. Hence, the possible optimal glucose range is not yet established, and different targets have been suggested over the years[164]. In addition, the certain positive effect of glucose control during the surgical intervention is not quite clear[165].

6.2.2 Liver surgery

As previously mentioned, the liver plays a key role in glucose metabolism and maintaining its metabolic properties during critical illness or surgery could be essential. In animal studies, insulin treatment has been associated to improved hepatocyte morphology and suggested to play an anti-apoptopic role. Accordingly, keeping a tight glucose control in ICU patients, has been suggested by Vanhorebeek et al, to improve hepatocyte mitochondrial structure and function[44].

Several studies have reported beneficial effects of glucose control in liver surgery. Okabayashi et al. reported favorable outcomes in a mixed population (DM and non-DM), by using an artificial pancreas to maintain blood glucose level between 4.1-6 mmol/l postoperatively, which exhibited a reduced number of surgical site infections, and shortened total hospital length

of stay[166]. A subsequent study, using the same protocol, suggested a beneficial effect of insulin treatment on liver regeneration, assessed by higher levels of liver transaminases in the control group[167]. More resent, Mita et al. reproduced this protective effect, in this case on postoperative kidney failure, which was presented by lower creatinine values[168].

Moreover, Fisette et al. performed a HNC to maintain normoglycemic glucose levels in the perioperative period, in combination with preoperative carbohydrate loading[169]. They indicated that insulin treatment improved energy storage and reduced liver cell apoptosis and necrosis, as presented by reduced markers associated with liver cell damage and dysfunction (liver transaminases, IL-6 and TNFα). In addition, the trial suggested an association to improved postoperative outcome, since data correlated to reduced postoperative liver dysfunction scoring. These results are in line with the findings presented by Hassanain et al., using a similar protocol, where improved liver glycogen content and postoperative dysfunction scores in hepatic surgery were demonstrated[170].

In conclusion, glucose control has been suggested to have positive effects on perioperative outcome in liver surgery. To our knowledge, no prior trial has studied the effect of intraoperative glucose control in liver surgery, by using a target range of 6-8 mmol/l, without a fixed insulin rate. Two of the major aims in this thesis are to investigate the effect of this insulin regimen on the development of postoperative insulin resistance, and on the alterations of glucose turnover.

7 GLUCOSE CONTROL - A RISKY BUSINESS?

7.1 HYPOGLYCEMIA IN ICU AND SURGERY

As previously discussed, the impressive favorable results presented in the Leuven study have been debated since they were published[171,172]. Apart from the limitations that it is single center design, with a population of patients predominantly admitted to ICU after cardiac procedures, the high amount of administered intravenous glucose in the study has been an item for discussion[173], since the amount may cause a relative hyperglycemia in need of insulin treatment. The questions are how to predict which patients would extend their stay in the ICU, and if the results are generalizable to other patient populations? However, the randomized clinical trials consequently launched in attempt to reproduce the brilliant results from Leuven, were unable to confirm the beneficial effects of glucose control[7,8]. Instead an emerging concern for hypoglycemic events became evident. A normal physiological response to hypoglycemia is first a reduced insulin secretion and secondly an increased glucagon secretion, which induce hepatic glucose production, all reactions in attempt to restore glucose levels[174]. Symptomatic hypoglycemia may produce behavioral changes, progressive focal neurological deficits, seizures and ultimately death[175]. However, in certain patient populations, such as critically ill or sedated patients subjected to surgery, warning signs of hypoglycemia may go undetected.

Hypoglycemic events were indeed noted in the Leuven trials as well. In the first trial, these were only briefly mentioned by the investigators[152], whereas in the second study somewhat more analyzed[176]. In the medical ICU, the treatment group experienced over five times more hypoglycemic events. The adverse events were found more commonly in this medical population, indicating that patients in prolonged need for ICU-care and/or vasoactive drugs or on dialysis, having liver failure or sepsis, had an especially raised risk for hypoglycemia[155,177].

However, subsequent trials, using different nutrition algorithms with predominantly enteral administration, could not easily establish and preserve normoglycemia[7,8,155]. In contrast, two major multicenter trials were prematurely stopped, partly due to high incidence of hypoglycemic events, i.e. the VISEP-study and the Glucontrol-trial. In the first study from 2008, the investigators compared tight glucose control, as well as the effects of circulatory resuscitation using different fluid regiments, in ICU-patients admitted for sepsis[9]. The second, Glucontrol, applied a lower glucose range in the control group than in the Leuven protocol (8-10 mmol/l), on a mixed-ICU population[11]. The VISEP-study reported an increased rate of severe hypoglycemia in the treatment group, 17% vs 4.1% in the control group. In the latter trial, researchers demonstrated a rate of hypoglycemic events of 8.7% in the treatment group vs. 2.7% in the control group, as well as a high number of protocol violations. In addition, none of the studies could demonstrated any mortality benefit.

In a meta-analysis, Wiener et al. concluded that tight glucose control in ICU patients was associated with a decreased risk for septicemia without showing any significant difference in mortality, however increasing the incidence of hypoglycemic events five-folded[178].

Finally, the largest multi-center study to this date, the NICE-SUGAR-study, was presented in 2009[10]. The investigators compared the effects on ICU mortality of intensive insulin treatment (4.5-6 mmol/l) to conventional treatment (8-10 mmol/l) in 6104 ICU-patients. The glucose range in the control group was similar the one in the Glucontrol trial. NICE-SUGAR reported episodes of severe hypoglycemia in 6.8 % of the patients in the treatment group compared to 0.5% in the control group. The overall conclusion was that tight glucose control increased the risk for hypoglycemia, which in turn was associated with a significantly increased mortality, 27.5% compared to 24.9% (p=0,02). In addition, no beneficial effect on outcome could be demonstrated.

Griesdale et al. concluded in a meta-analysis, that tight glucose control increased the risk for hypoglycemia without any improved clinical outcome, apart from surgical ICU-patients which could benefit from the treatment[179]. In another later meta-analysis, Marik et al. compared data from seven studies, unable to find any support for intensive insulin treatment reducing the mortality, the incidence of infections or the need for dialysis[173]. Instead a 7-fold raised risk for hypoglycemic events was demonstrated, which was influenced by the administration of parenteral nutrition.

These larger randomized trials reported an incidence of severe hypoglycemic events in the treatment groups, ranging from 5.1-28.6%[7,8,10,152,176]. Notably, the trials reporting the highest incidence of hypoglycemia do not always employ the tightest glucose range, indicating glucose control may not be sole causality to hypoglycemia.[180]. However, this is concerning data, since several studies have demonstrated an association between the degree of hypoglycemia and mortality. Even milder degrees of hypoglycemia have been indicated to increase the risk of death in the ICU[181,182], independent of the diabetic status[183,184], though contradictive findings have been presented[185]. Although, the causality of death is difficult to demonstrate, except in a trial by Hermanides et al., where severe hypoglycemia (<2.2 mmol/l) was suggested a marker for the severity of illness[186].

Nevertheless, the impact of hypoglycemia on ICU patients is difficult to settle, due to concurrent severe illness and neurological dysfunctions, which may mask the hypoglycemic signs. Moreover, the patient´s glycemic control prior hospitalization, HbA1c, may significantly influence the risk for hypoglycemic events in the ICU, thereby also being associated with mortality[187]. Perhaps, this may explain why spontaneous hypoglycemic events are associated more with poorer outcome than hypoglycemia caused by insulin treatment[188].

After all, there is still an interest in the subject, recently, a study in a pediatric ICU was prematurely stopped due to hypoglycemic events, while showing no beneficial effect on clinical outcome[189]. Though, to our knowledge no RCTs are presently being performed.

However, changing the focus to the perioperative period, subsequent studies on tight glucose control, predominantly in cardiac surgery patients, have had various conclusions[190-192]. Subramaniam et al., could demonstrate that glucose control (5.6-8.3 mmol/l) in cardiovascular surgery patients, was superior to minimize major adverse events, possibly due to reduced

glycemic variability[162]. However, this trial reported increased hypoglycemic events in the treatment group, 8.8% vs 4.1% in the control group, an adverse risk, which was also noted in a study by Lazar et al[191]. Similarly, another randomized trial on cardiac surgery patients, which compared intraoperative glucose control (4.4-5.6 mmol/l) to standard treatment (<11.1 mmol/l), could not present positive clinical effects, in contrary reported an increased incidence of stroke and mortality[193]. A later meta-analysis, including 6 RCTs, suggested some benefits of tight glucose control on cardiac events and mortality, though with the allowance that the included studies displayed various glucose levels, different periods of glucose control and reported different outcomes[190]. Moreover, in a meta-analysis, Hua et al. concluded that intraoperative glucose control only decreased the rate of infection, whilst having no beneficial effect on hypoglycemia or mortality[159].

7.2 GLUCOSE VARIABILITY

Marked fluctuation in blood glucose levels, i.e. high glycemic variability, may be harmful, independent of the absolute mean glucose level[194]. Avoiding large variations may be metabolically important, since acute changes in glucose concentration are associated to higher oxidative stress on the cellular level[195], which has been a suggested explanation for development of diabetic complications[42]. A larger retrospective study of >7000 patients could demonstrate a strong association between glucose variation (SD) and in-hospital mortality[196]. These findings were confirmed by Ali et al, where ICU patients had a five-folded increase of hospital mortality, despite normoglycemia[197]. In addition, Hermanides et al. demonstrated decreased mortality in patients with low variability, though presented with higher mean glucose level[186]. Glucose variability may also be considered a warning sign, since it seemed to increase prior to a hypoglycemic event[198]. A recent retrospective trial confirmed the findings that ICU and all-hospital mortality are strongly associated to the degree of dysfunctional glycemic control (dysglycemia), hyper- and hypoglycemia in combination with glucose variability, predominantly in non-DM patients[199]. In addition, the investigators suggested lowering the target in non-DM patients and pointed to the importance of maintaining glucose control during the entire hospital stay. Prevailing in a stable glucose range is associated with improved outcome for ICU-[200], as well as non-critical ill patients[201]. However, to our knowledge, prospective randomized studies are lacking.

The definition of glucose variability is somewhat unclear. It has been reported as the standard deviation, the variation coefficient, mean absolute change over time or glucose variability index for example. In addition, there are different definitions of hyper- and hypoglycemia. In a meta-analysis, the association between glucose variability and mortality was difficult to settle, due to different glucose metrics and definitions[202].

Another important variable of glucose control is the variable time in range/band, meaning the amount of time during which glucose values are within a preset range. The variable combine the variability and the average value of glycemia, and has been associated with improved outcome in both the ICU[200,203] and the surgical settings[204]. Since time in range was higher in

the Leuven trial, compared to the more recent major studies, this has also been proposed as an explanation for the diverging results.

7.3 WHY THE EFFECT? GLUCOSE CONTROL OR INSULIN TREATMENT VS DIABETIC STATUS?

It has been discussed whether glucose control or the administration of insulin itself improves clinical outcome. Insulin has, as previous mentioned, metabolic and non-metabolic effects, which possibly are beneficial to different patient groups. In studies performing high dose HNC (5 mU/kg/min) during cardiac surgery, beneficial effects have been demonstrated on myocardial injury markers, troponin[120], and postoperative myocardial function[205]. However, a post-hoc analysis by the Leuven group demonstrated that normoglycemia, rather than the insulin dosage, correlated with improved outcome[206]. This conclusion was later confirmed in a randomized animal trial, where mortality was significantly reduced in the normoglycemic groups, independent of insulin dosage[207].

Another possible explanation for the diverging outcomes in several large trials on glucose control could be the diagnosis, or not, of diabetes[60,163,184,199,208]. Interestingly, DM patients have an altered, higher, threshold for counter-acting hormone secretion[209], indicating an adapted reaction to rapidly normalized glucose value. Moreover, as previously discussed, the preoperative glycemic control, measured as HbA1c, matters, irrespective of diabetes diagnosis, as an elevated HbA1c has been found to predict postoperative hyperglycemia, glucose variability and adverse outcome in abdominal[62], as well as cardiac surgery[204,210,211].In addition, specific patient populations, surgical or medical, seem to have various risk for hypoglycemia, thereby influencing the result[212]. However, patients undergoing cardiac surgery, are considered to benefit from perioperative glucose control[190,213]. Thus, it seems important to critically evaluate the patient population, since diabetics may not benefit from tight glucose control[199], whereas the regimen in certain surgical patient populations, even for DM-patients, is suggested to improve outcome[214].

Altogether, hyperglycemia and hypoglycemia are both negatively associated to adverse events [215], as patients admitted for acute myocardial infarction which presented a glucose level >6.7 or <3.9 mmol/l, had an increased the risk for mortality. Patients presenting with high variability have an additional poor clinical outcome[194]. MacKenzie et al. demonstrated in a single center study that glucose control was associated with outcome in the ICU, in which three different independent glucose metrics of importance were identified, central tendency (mean values of different ways of presentation), dispersion (variability) and hypoglycemia[216].

In conclusion, the ideal glucose range remains to be settled, it is still uncertain and could vary according to specific patient populations.

8 ASSESSMENT OF GLUCOSE AND INSULIN RESISTANCE

Glucose target recommendations have altered over time, depending on the results from various studies, as discussed above. One suggested explanation for diverging results between the first Leuven study and later trials, have been the use of different devices for blood glucose measurement. Glucose control, as previous discussed, has been suggested more beneficial in a surgical setting and in non-DM patients, by mainly decreasing the rate of postoperative complications, though having less effect on mortality[208]. Recently, some recommended to initiate insulin treatment in intraoperative settings, at glucose level <10 mmol/l[97]. In severely ill patients, a more aggressive approach has been suggested, by starting treatment at glucose level >7.7-8.3 mmol/l. Moreover, several different insulin protocols have been suggested to minimize the risk for hypoglycemia[4,217]. Since critically ill patients, or patients subjected to longer surgical procedures, may have a disturbed tissue perfusion, intravenous insulin administration is preferred. Aside from a suitable insulin treatment, a proper assessment of glucose concentration is of the greatest importance to reduce the risk for dysglycemia, which is strongly associated with poor clinical outcome.

A more frequent measurement may improve glucose control by reducing hyper- or hypoglycemia and glucose variability, and is suggested to increase the time in glucose range[218]. In hope of improved clinical outcome and to avoid intense labor for ICU personnel, there is a call for reliable continuous glucose measurement (CGM) devices[219]. Central laboratory testing is considered the reference standard method for glucose measurement, whilst blood gas analysis is almost as good[220]. In contrast, point-of care analyzers (POC) are considered less accurate[221]. Though having several limitations, they provide swift bedside testing, thereby enabling important and quick decisions to treat hypo- or hyperglycemic events. Nevertheless, all methods mentioned are time- and labor consuming[222], therefore different continuous measurements systems have been developed and tested in the ICU settings. Some various methods for blood glucose assessment will be mentioned in this chapter.

8.1 BLOOD GLUCOSE

Glucose can be measured in whole blood, serum or plasma. Glucose concentration is higher in arterial blood, than in the capillary, which in turn is higher than in venous whole blood[223]. Since glucose is dissolved only in the aqueous part of the sample and not in the solid part, for example blood cells or proteins, glucose concentration is also about 11% higher in plasma than in whole blood. For that reason, abnormal hematocrit levels can produce inaccurate glucose values. In addition, glucose can be assessed from arterial, venous or capillary samples. Capillary sampling is advised against in the ICU, since hypoperfusion is suggested to interference with readings. The glucose measurements are performed by using different enzymatic reactions, glucose-1-dehydrogenase (GDH), hexokinase or glucose oxidase (GOD). The two latter are most frequently used in laboratory glucose meters.

GOD is an enzymatic reaction oxidizing glucose in presence of water and oxygen, with the net production of gluconic acid and hydrogen peroxide[224]. The reaction is detected amperometrically or colorimetrically. In the first variant, peroxide reacts with the subsequent loss of electrons, sensed by a platinum electrode, thereby evoking an electric signal. In colorimetric detection, peroxide reacts with hydrogen together with a chromogene, producing a color change measured by a photometer. The stronger signal produced, the higher glucose concentration. These reactions can be used on arterial, venous and interstitial fluid samples, and is the predominant method in POC glucose meters.

GDH resembles GOD, being specific for D-glucose, but has less risk of interference than the GOD reaction. This enzymatic reaction, in combination with a colorimetrical detection on lysed whole blood, is used by the POC meter HemoCue[225]. Hexokinase converts glucose to glucose-6-phosphate, where under reduction NADH is produced and detected.

Certain patient conditions, or compounds, may interact with the GOD-reaction. This applies to critically ill patients with abnormal pH or hyperoxia, which provide inaccurate values or anemia, which overestimate the glucose level. In addition, different drugs (paracetamol) or metabolic disorders (hyperlipidemia or hyperbilirubinemia) may interfere with readings[226].

8.1.1 Blood gas analyzers and point of care glucose meters

Blood gas analyzers used in the ICU or operation settings, provide data with close accuracy to central laboratory values, and is regarded a reliable substitute to laboratory measurements. The device uses the glucose oxidase method. An alternative to blood gas analyzers are POC-meters, which could be considered more user-friendly and are used for in- as well as out-of-hospital settings. POC glucose meters often analyze whole blood by a GOD-reaction, and an internal correction factor eliminates the difference in glucose concentration between plasma and whole blood. However, there are diverging results concerning the agreement between samples analyzed by a POC-device and reference samples measured by blood gas analyzers or the laboratory. One trial demonstrated acceptable results in stable patients, except in conditions of hypoperfusion[227], whereas other investigators report substantially lower accuracy, where the glucometers often provide falsely higher readings [228,229]. Moreover, one should bear in mind that not only tissue perfusion may have an impact on the measurements, but additional confounders are the source of blood and the amount of blood on the glucometer strip. Though, Kanji et al. reported POC-results being 20% off reference value regardless of venous, arterial or capillary site of sampling[230].In contrast, Cortjens et al. presented reliable accuracy by using arterial sampling only[231]. One explanation for this discrepancy could be that different devices have been evaluated, and newer POCs may have altered the analyzing techniques, as Karon et al. indicated improved accuracy even by capillary sampling[232].

8.1.2 Continuous glucose measurement

CGM systems have been proven efficient in out-of-hospital monitoring of DM patients, by reducing the risk for hypoglycemic events and improving glycemic control[233]. The hope is that they could provide the same benefits for the ICU population, since the systems have the ability of reporting not only mean glucose values, but also the direction and rate of glucose changes[219]. In addition, the systems can be integrated with a closed-loop insulin administration, potentially thereby reducing hypoglycemic events. However, many studies have focused on accuracy and feasibility, few on clinical outcome in critical ill patients. The conclusions have been diverging, some stated that the rate of severe hypoglycemic events could be reduced[234-236], whereas others demonstrated no positive effects on hypoglycemic events or glycemic variability by using subcutaneous CGMS compared to regular POC[235,237]. Since glucose metrics have not been standardized, investigators report different parameters, thus making comparison of trials difficult.

8.1.2.1 Principles:

Most of the available CGM systems sample glucose from interstitial fluid in subcutaneous tissues. Newer devices also use intravascular sampling. The CGMS sensors and catheters are placed, either together inside the tissue space or the catheter draw intermittent samples to a sensor placed outside the tissue. Glucose is frequently sampled, commonly at an interval of 5 minutes or less, and measured by glucose oxidase or fluorescence method, mid-infrared spectroscopy or hydrogel methods. However, there are limitations, apart from the analysis method, where intravenous sampling can be affected by thrombosis, catheter occlusion or infections, and the interfering factors associated with subcutaneous devises; sensor drifting and the need for (repeated) calibrations. In addition, an invasive device may initially cause tissue damage and an inflammatory reaction, in need of a stabilizing period.

The CGM technique has been evaluated with various accuracy in ICU patients, possibly due to differences in interstitial and intravenous glucose concentrations. The impact of tissue hypoperfusion in critically ill patients, has been a concern for inaccurate readings in subcutaneous CGMS. There is a reduced agreement between the intravascular glucose readings and the interstitial concentration in critically ill patients[238]. Normally, glucose is transported by passive diffusion over the capillary membrane, along a concentration gradient. The interstitial glucose level is determined by the rate of diffusion and the uptake in dermal or subcutaneous tissue cells. Hence, factors influencing the interstitial glucose level are, aside from the metabolic rate in adjacent cells, the blood flow and the capillary permeability. Basu et al. observed, by using microdialysis and tracer technique, a physiological time lag of 5- 10 minutes, between glucose in plasma and in the interstitial fluid[239,240], which agrees with the calculated lag time reported by Schiavon et al., using a mathematical model incorporating tracer and microdialysis data[241]. Kulku et al., demonstrated a 5-minute time lag in DM patients using a subcutaneous MD device, though the investigators reported longer delays in interstitial glucose levels during rapid changes in blood glucose[242].

8.1.2.2 Accuracy of measurements

Critically ill patients often have altered microcirculation[243], though there is a disagreement whether the tissue perfusion influences measurement accuracy or not. Siegelaar et al. presented no effect on accuracy by reduced microcirculation, but instead stated that the peripheral temperature could interfere[244], nor could Holzinger et al. report a reduced accuracy in subcutaneous readings during administration of noradrenaline[245]. In contrast, others reported a reduced accuracy and increased glucose variability with the use of vasopressors[246], and Rabiee et al. concluded that the subcutaneous CGMS is unsafe and underestimates hypoglycemia[247]. Thus, the idea of intravascular sampling was introduced and several papers have been published on the subject.

The intravenous devices for continuous measurements, with rapid development over the recent years, use various approaches for glucose sampling and analysis. Some have the sensor inserted inside an intravenous catheter, using mid-infrared spectroscopy[248,249], or a central venous catheter (CVC) with the use of the fluorescence technique[250], whereas others have used an external analyzer in combination with a CVC with an integrated microdialysis (MD) membrane[251-254]. Direct comparisons between intravenous and subcutaneous CGMS show diverging results. One study demonstrated superior accuracy for the intravascular devise[255], and the other reported no difference in accuracy[256], though increased frequency of calibration was suggested to produce higher accuracy in subcutaneous CGMS readings[257]. Recently, Bocchichio et al. demonstrated high accuracy for an intravenous CGM system compared to reference values, in addition presented data in both the hyper- and hypoglycemic range[248], the latter is often lacking in trials.

Over time, the microdialysis technique has been refined, thereby becoming a useful tool for continuous glucose monitoring. In 2010, being one of the first trials on the subject, Rooyackers et al. presented data for the feasibility of a intravascular microdialysis catheter in ICU patients over 5 days and in healthy volunteers[258]. Therefore, our aim in paper I and II, was to further investigate the factors influencing the accuracy of glucose measurements via intravenous microdialysis.

8.2 MICRODIALYSIS

This technique, first described in research in the 60 and 70´s and further developed over the past decades, makes it possible to achieve continuous in vivo measurements in virtually every tissue (including blood) or organs in the body, without any biopsies[259]. The method has mostly been used in research settings, both in vivo and in vitro. Almost every tissue is the body, even those normally not possible to access, have been studied in microdialysis experiments. This often in search of substances related to energy metabolism or in study of drug delivery, since the technique allows monitoring of both endogenous and exogenous compounds[260]. However, one must consider, that the analysis mirrors only the tissue metabolism in close proximity of the catheter.

The technique has been evaluated in patients after neurological insults, liver transplantation[261] and major abdominal surgery[262], as a tool to detect acute ischemic events.

The technique, by mimicking the characteristics of a capillary, analyses the extracellular concentration of the molecules derived by passive diffusion from the interstitial fluid in the tissue of interest, whilst no fluid is removed. However, some conditions must be fulfilled. The substance must be dialyzable, i.e., it must be small enough to pass the membrane pores. In addition, the analyzing method must be simple and fast and achievable for the small volumes derived, and the recovery ratio easily determinable. In summary, the microdialysis technique implies no impact on the physiological systems and thereby makes long term monitoring feasible.

8.2.1 Principles

The microdialysis catheter consists of an outer and an inner tube, with a semi-permeable membrane at the tip, over which an exchange of substances takes place based on passive diffusion. The ingoing fluid, the perfusate, is pumped slowly through the outer tube, see figure 2. The perfusate is similarly composed as the surrounding tissue fluid, but free of the substance of interest. Small molecules from the interstitial fluid freely diffuse over the membrane following the concentration gradient. After exchange over the semipermeable membrane, the fluid, now called dialysate, flows through the inner tube. The dialysate contains a sample separated from proteins, not further degraded by enzymes, and is collected in small, 10-50 µL samples volumes, microvials[224].

8.2.2 Recovery

If there is a difference between the concentration of the analyzed substance in the dialysate and the concentration in the tissue, it implies that complete equilibrium has not been reached. The ratio between the two is referred to as recovery, and can be described as absolute or relative[224]. Absolute recovery is the total amount of substance recovered over a defined period. 100% recovery can be impractical and time-consuming, as it often demands low velocity of the perfusate. Mostly, the relative recovery is used during higher fluid velocity, which is the proportion of the substance in the dialysate compared to the content in the surrounding tissue.

The relative recovery is influenced by the membrane area, the membrane pore size, the rate and composition of the perfusion fluid, as well as the polarization of the compound[263]. In addition, the physiological properties of the surrounding tissues, for example changes in blood flow may impact recovery[258]. Therefore, before interpreting any in vivo microdialysis data, the system should be calibrated to establish the relative recovery of the substance of interest, given that the incomplete equilibrium remains constant. Various methods can be applied; some will be mentioned.

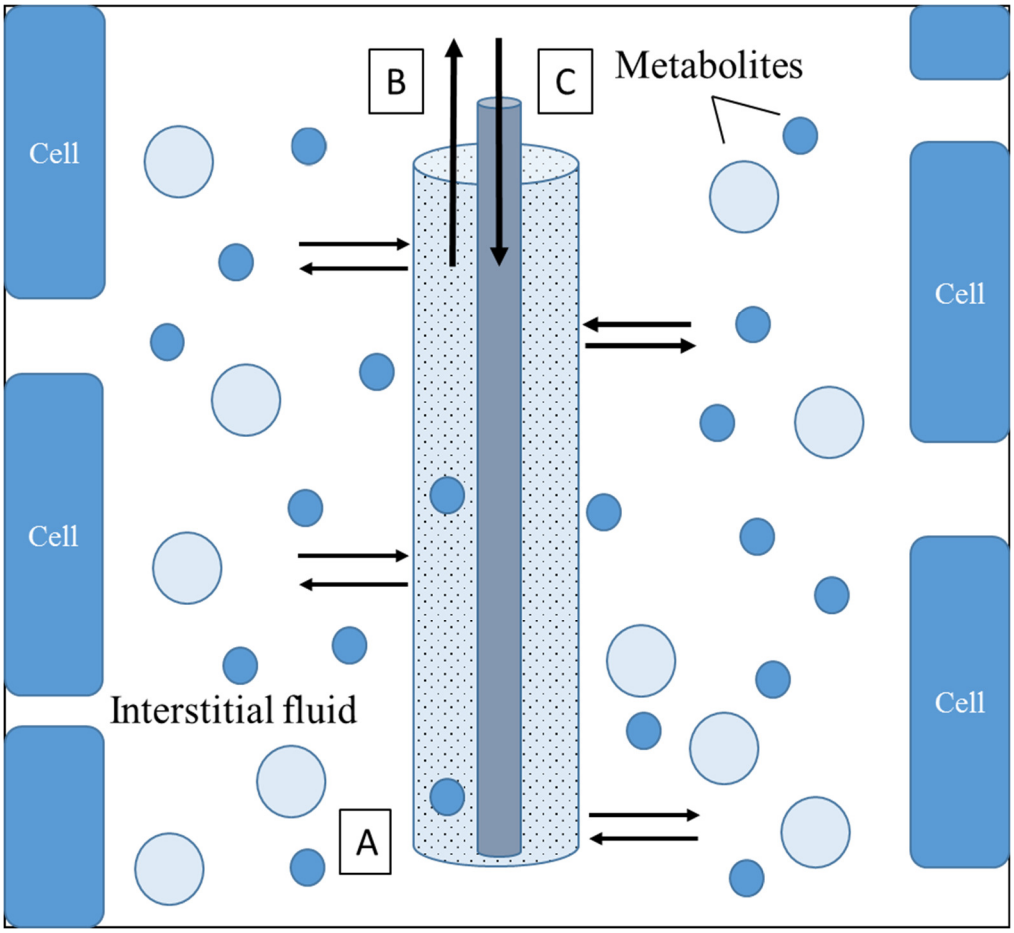

Figure 2: Principles of microdialysis: A microdialysis catheter, inserted in the tissue of interest (inclusive blood), consists of two tubes of which the outer distal part is made of a semi-permeable membrane (A). A perfusion fluid (perfusate) is run through the inner tube (C)and the liquid is equilibrated over time with the metabolites passing over the semipermeable membrane. The fluid then returns through the outer tube (B), now containing a dialysate of the metabolites from the surrounding tissues and can be sampled for analyses or analysed on-line.

In the no-net-flux method, the catheter is perfused with fluids of different concentrations. At the point of no difference between the perfusate and dialysate concentrations, it is assumed that this equals the concentration in the surrounding tissue and that no-net exchange is occurring[263].

The low-flow-rate method involves perfusion with different fluid rates, under the assumption that at no velocity the recovery in the dialysate is total, and equals the concentration of the surrounding tissues. This point can be found by plotting and comparing the concentration at different rates.

Another reliable technique uses a labelled compound added to the perfusion fluid, with similar biochemical qualities as the substance of interest[260]. It is assumed that the net loss into the surrounding tissues of this compound, is equal to the relative recovery.

8.2.3 Fluid rate and membrane properties

The recovery, as previously mentioned, is positively correlated with the area and the pore-size of the semipermeable membrane[224]. In addition, the composition and the velocity of the perfusate, impacts the recovery ratio.

The perfusion velocity can differ from 0.3-10 µL/min[224], but often range within 1-5 µL/min. A lower velocity permits longer diffusion time over the membrane, resulting in a higher recovery. The perfusate can be pumped through the system in different modes, the push-mode, the pull-mode or combined push-pull-mode. The push-mode may result in a net loss of fluid due to elevated hydrostatic and osmotic pressure across the membrane. The pull-mode results in a negative pressure and hence fluid might be sucked over the membrane. The last mode with combined push and pull, allows a low pressure over the membrane, though it entails a more expensive and complicated system[224].

The semi-permeable membranes have different pore sizes, allowing for collection or exclusion of different molecules, defined as the molecular weight cut-off size. Glucose is a small compound with a molecular weight of 180 Da, and a common pore size used in analyses is 20 kDa. A larger size may be used, but then often in combination with osmotic perfusion solution, to avoid sample dilution[260].

8.2.4 Tissue factors

Recovery is affected by conditions in the surrounding tissue. For example, substances may undergo metabolism prior to reaching the membrane and the tissues may react to the insertion of the catheter. Hence, a run-in-time of 1-2 h is recommended for avoiding disturbances for cell damage in rate of recovery after insertion[224]. In addition, it is suggested that the core temperature and changes in blood flow influence the recovery. The temperature positively affects the recovery with an increase of 1-2% for every raise in ºC[224], and the recovery in blood is higher than in interstitial fluid.

8.2.5 Sample analysis

By using microdialysis technique, only the substances of interest are collected from the surrounding tissue, and it needs no further separation before analysis. The technique is especially useful for sampling small water-soluble molecules such as glucose, lactate, pyruvate and glycerol. The time elapsing between sampling and on-line sensor analysis is linked to the fluid rate, often referred to as physical lag-time. A condition for reliable continuous on-line measurement in real time is a swift analyzing method, and the relationship between fluid rate and recovery is important and must be considered when developing CGM systems.

Several types of analyzing methods have been developed. There are biosensors that react in response to biological changes by an electrical signal; an enzyme-based or electrochemical with optical detection[264]. For glucose measurement, an enzyme-based method is used in all current

systems, i.e. the GOD analyzer, where glucose oxidase initiates the reaction with a subsequent electrochemical detection of H_2O_2.For later off-line analyses, there are immuno-assay methods, preferably used for measuring peptides or drugs, and mass spectrometry that identify low-concentrated samples with low molecular weight[264]. If separation of substances in the dialysate is needed, other methods may be used, for example liquid chromatography or electrophoresis[264].

8.3 INSULIN RESISTANCE

Insulin resistance and glucose metabolism may be assessed by a variety of methods. Depending on the purpose, ranging from large epidemiological studies to limited trials on physiological events, the available methods can be divided into quantitative or qualitative/dynamic function assessments, of which some will be mentioned.

Indirect assessment:

- Fasting plasma insulin
- Homeostasis model assessment, Insulin Resistance test (HOMA-IR)
- Quantitative insulin sensitivity check index (QUICKI)

Dynamic assessment:

- Short insulin tolerance test
- Continuous infusion of glucose with model assessment (CIGMA)
- Oral glucose tolerance test (OGTT)
- The frequently sampled intravenous glucose tolerance test (FSIVGTT)
- Hyperinsulinemic normoglycemic clamp (HNC) – gold standard, and hyperglycemic clamp

8.3.1 Fasting plasma insulin

Sampling of fasting insulin value, is performed in the morning, after an overnight fast. A high value derives from increased β-cell secretion and reflects the presence of insulin resistance.

8.3.2 Homeostatic model assessment - insulin resistance (HOMA-IR)

This method, first described in 1985, uses fasting insulin and fasting glucose values in a mathematical model to estimate insulin resistance[265]. By the using the formula, the product of the insulin value, which represent the pancreatic β-cell function, and the glucose value, which is dependent on the endogenous glucose production, give an approximative value of the basal insulin sensitivity. In this model, insulin sensitivity is assumed to be equivalent in the liver and in peripheral tissues, which is not always correct. HOMA-IR reflects insulin sensitivity best in normoglycemic or mild hyperglycemic persons. However, in severe hyperglycemia, it may be inaccurate[266]. It correlates well with the HNC, is simple to use, and is therefore mostly used in larger population studies.

8.3.3 Quantitative insulin sensitivity check index (QUICKI)

QUICKI is another mathematical model for estimating insulin sensitivity. It corresponds well to HOMA, by using log-transformed basal insulin and glucose values[266]. The log-transformation of values accounts for non-normally distributed insulin concentrations. The advantage is it gives a more correct value of insulin sensitivity in hyperglycemic patients, it is simple, inexpensive and it correlates well with the HNC values[267]. However, in individuals with low insulin concentration and β-cell dysfunction, the correlation is weaker[268]. The use for QUICKI is mainly in large population trials.

8.3.4 Short insulin tolerance test

In this method, a single intravenous bolus of insulin is injected and by the rate of decline in glucose concentration, an estimate of insulin sensitivity is obtained[265]. Glucose samples are collected during 15 minutes, and the test is then terminated. The test can also be performed over a 60-minute period, with the disadvantage of an increased risk for hypoglycemia and the release of counter-acting hormones, which may interfere with the interpretation of the results. The data can give an estimate of the glucose clearance, but it cannot discriminate the site of insulin resistance[266]. The test correlates acceptably well with HNC, and can be used in large trials.

8.3.5 Continuous infusion of glucose with model assessment (CIGMA)

This method was developed for assessing insulin sensitivity and β-cell function. A continuous glucose infusion is administered over 60 minutes. Samples for insulin and glucose are collected the last 10 min, and values are compared to reference values in a mathematical model[268]. The CIGMA reflects insulin sensitivity better than HOMA, since the glucose infusion stimulates an insulin response[266]. The insulin enhances the peripheral glucose uptake, thereby producing a glucose steady-state. CIGMA correlates well with the HNC, but is also limited by the need for a mathematical model like HOMA and QUICKI.

8.3.6 Oral glucose tolerance test (OGTT)

By administering 75 g glucose orally and collecting blood glucose samples over the following two hours, a value representing the insulin sensitivity is obtained. By giving glucose orally, OGGT reflects the physiological response to carbohydrate loading well, which is not the case with HNC and FSIVGTT[267]. The data in combination with the fasting glucose value represent the individual diabetic status. Data derived from OGTT can also be converted into an insulin sensitivity index (ISI), reflecting peripheral insulin sensitivity, and correlates well with the HNC[268]. The estimate is more accurate than HOMA-IR and QUICKI, since not only fasting values are considered. Though, it is limited by the fact that gastrointestinal function and incretins influence the results. OGTT represents a more clinically applicable alternative to HNC and FSIVGTT in large population studies, and in the diagnose of diabetes mellitus[266].

8.3.7 Frequently sampled intravenous glucose tolerance test (FSIVGTT)

This technique is a less labor intense alternative to the HNC, thereby advantageous in larger studies. By injecting a single bolus of glucose and subsequent sampling of plasma insulin and glucose over the next 180 minutes, the dynamic test assesses both the insulin sensitivity and the β-cell function. The dynamic data for insulin and glucose are fitted in two separate mathematical models, where insulin sensitivity and the ability of glucose to mediate its own uptake are estimated[268]. FSIVGTT can be modified by administering concurrent insulin infusion to account for a decreased insulin response in patients with reduced β-cell function, which improves the correlation to HNC. However, since this method obtains data in a non-steady state, and insulin and glucose kinetics used in calculations are based on various assumptions, the correlation to the HNC may differ considerably[266]. Still, the FSIVGTT method is considered the "second" gold standard for insulin sensitivity assessment, and can be used in population trials[266].

8.3.8 Hyperinsulinemic normoglycemic clamp (HNC) and hyperglycemic clamp:

The hyperinsulinemic normoglycemic clamp technique, first described in 1979, is considered the gold-standard in research setting for quantification of insulin sensitivity, and is regarded highly reproducible[269]. Together with the minimal model method of FSIVGTT, HNC represents the only technique to adequately assess peripheral insulin resistance. However, the procedure is time- and labor consuming, and is mainly applicable in research settings for a limited number of patients.

The technique is based on a constant insulin infusion combined with a variable glucose infusion to maintain normoglycemia. Plasma glucose is frequently assessed, every 5 minutes, to avoid hypoglycemia. Due to the arterio-venous difference of glucose in plasma, glucose sampling is preferred in an arterial, or an arterialized venous access. At steady state, the rate of exogenous glucose infusion, is equal to the amount of overall glucose disposal, the metabolic clearance rate (M-value, mg/kg/min), with the assumption that exogenous insulin infusion accomplishes total suppression of the endogenous glucose production (EGP). The higher M-value, the more insulin sensitive the patient is, see figure 3.

Some suggest M- values > 7.5 as a normal value, whereas an insulin resistant person has values < 4 mg/kg/min[268]. However, the absolute M-value is dependent on the dosage of insulin given[270]. Different insulin infusion rates can be used, in research settings common rates are 40-120 mU/m^2/min[266]. In obese subjects a higher rate is necessary to suppress the hepatic glucose production, whereas a lower rate is sufficient in normal-weight persons. If the clamp is prolonged, the non-oxidative disposal has been demonstrated to increase, which was most pronounced in low-sensitive persons[271].

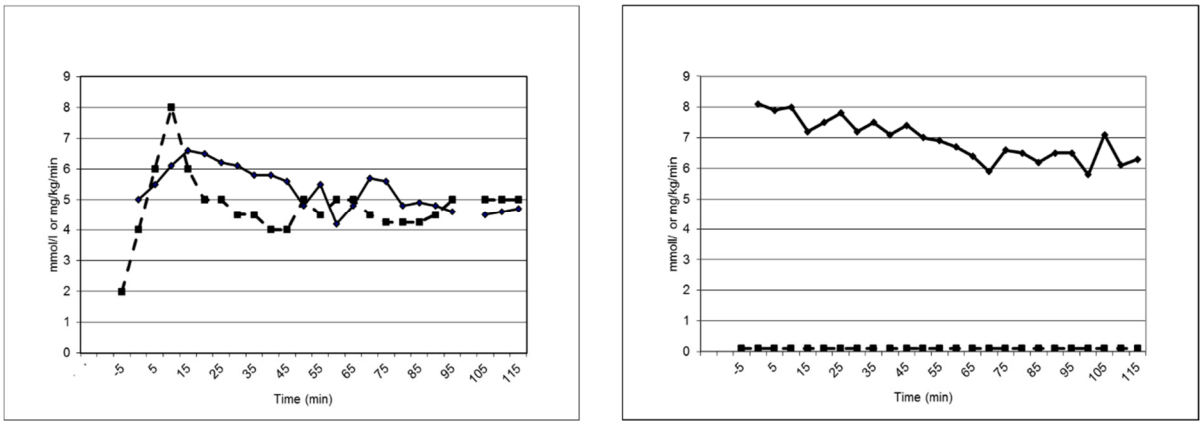

Figure 3: Examples of a preoperative HNC (to the left) and a postoperative HNC (to the right), in the same patient. M-values, the variable glucose infusion rate (the dotted line) are given as mg/kg/min and the blood glucose values (the bold line) are given as mmol/l.

The HNC is limited by the fact that the method fail to reflect physiological dynamics, since glucose is administered intravenously, without passing the effect of the gastrointestinal tract. To further elucidate and discriminate glucose metabolism, the HNC can be combined with other techniques; isotope dilution, local tissue sampling, indirect calorimetry, or nuclear magnetic resonance scans. Though, the HNC is still unable to distinguish insulin- and non-insulin dependent glucose disposal.

Hyperglycemic clamp:

In this method, used for assessing the β-cell function, glucose is intermittently infused at a variable rate to achieve a predetermined hyperglycemic value, often about 12 mmol/l, and is maintained for 2 hours[268]. The mean glucose infusion rate over the last 30 minutes represents the glucose metabolism and gives an estimate of the endogenous insulin secretion. The hyper- and normoglycemic clamps share the same limitations.

8.4 ISOTOPIC TRACER DILUTION METHODOLOGY

The estimation of insulin sensitivity by HNC, assumes a fully suppressed endogenous glucose production. Otherwise, the clearance rate reflects the disposal of both exogenous and endogenous glucose. To discriminate the endogenous contribution, the arteriovenous difference technique, labelled nuclear magnetic resonance spectroscopy or isotope dilution technique can be used[272]. The latter methodology, intravenous or oral, can be used in combination with FSIVGTT, OGTT or HNC to assess the endogenous glucose production[267].

By adding a labelled isotope, it enables assessment of different parts of the human metabolism. The technique determines differences in the metabolic substrates kinetics, on a whole-body

level, as well as on a tissue level. In this case, the method is recommended for distinguishing glucose production from peripheral glucose disposal.

8.4.1 Tracer and tracee

The isotope dilution method uses a labelled form of a molecule, tracer, where one or several given atoms are replaced by its isotopic form. Ideally, the tracer molecule is metabolically and structurally identical with the molecule that is being studied, the tracee. The isotope form merely makes it distinguishable from its natural form, thereby being possible to detect and measure. The glucose molecule used for this method may have either a radioactive or a stable isotope of a hydrogen or a carbon atom. For labelled glucose, the position of the isotope is important, being a determinant of the degree of recycling. If glucose is metabolized, and the isotope label is lost in degradation, the molecule may be regarded as a new molecule, thereby leading to overestimating the glucose turn-over. The isotope variant 6,6-$^{2}H_{2}$-glucose, is considered the best, since the two labelled carbon atoms from the sixth carbon are lost late in the glycolysis, thereby giving the best estimate of de novo glucose production[272]. Stable isotope tracers are measured by mass spectrometry technique and the main disadvantage is the cost for the tracers and the analyses.

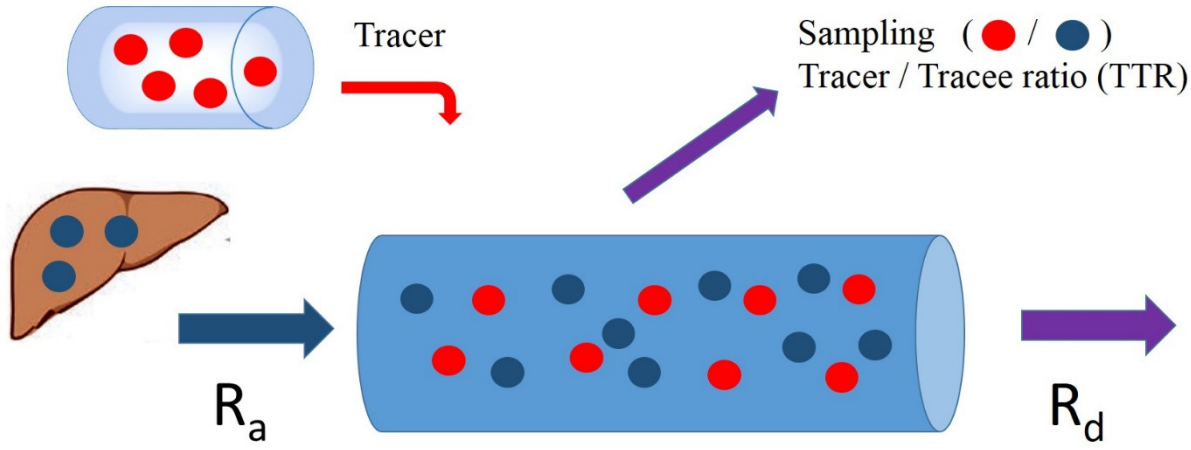

Figure 4: Principles for the isotope dilution technique. The endogenous glucose production, expressed as the rate of appearance (R_a), which at steady-state is equal to the whole-body glucose uptake, the rate of disappearance (R_d). Under equilibrium, these rates are equal to the infusion rate of the tracer divided by the ratio of plasma tracer/tracee (enrichment).

8.4.2 Calculation

For calculation, the terminology “pool” is used to represent the volume in which the tracer/tracee is evenly distributed. In steady-state, the concentration of the substance in the pool

is stable, therefore the rate of appearance of the substance is assumed to be equal to the rate of disappearance. In a non-steady-state, in which an exogenous infusion is administered, a modified Steele´s equation is used to account for the disturbance[273]. A prime constant infusion allowed for 60 min is commonly used to accomplish a steady state- condition[272].

When a stable isotope is used, tracer and tracee are sampled and analyzed using a mass spectrometer. Under steady-state condition and a constant volume, tracer enrichment in plasma (ratio of tracer/tracee) is assumed to be equal to the rate of appearance (R_a), to the rate of disappearance (R_d) and to the ratio of tracer infusion rate/R_a, see figure 4. The rate of appearance corresponds to the endogenous glucose production in the post-absorptive state, and a high rate in the presence of insulin is indices of hepatic insulin resistance. However, R_a reflects not only the hepatic glucose production since glucose is also synthesized in the kidney.

9 AIMS

The overall aims of the thesis were:

- To investigate factors influencing the accuracy of glucose measurements by intravenous microdialysis.

I. To identify the optimal rate of perfusion fluid and membrane length, which present the best agreement to plasma glucose reference values, in a catheter for intravenous microdialysis measurement.

II. To investigate the agreement of an on-line intravenous continuous glucose measurement system via microdialysis, to plasma reference values.

- To investigate the effect of intraoperative glucose control in liver surgery.

III. To determine the effect of glucose control on postoperative insulin resistance, assessed by a hyperinsulinemic normoglycemic clamp technique.

IV. To characterize the effect of glucose control on intra- and postoperative glucose kinetics, by discriminating alterations in endogenous glucose production and whole-body glucose disposal, assessed by stable isotopic tracers and a hyperinsulinemic normoglycemic clamp technique.

10 METHODOLOGICAL CONSIDERATIONS

The study designs for paper I-IV were all reviewed and approved by the regional ethics committee in Stockholm. The patients and volunteers were informed about the purpose and the nature of the study and the risks involved, before written informed consent was obtained.

10.1 PAPER I

The aim for paper I was to investigate the effects of various semi-permeable membrane lengths and perfusion fluid velocities, on the accuracy of intravenous microdialysis glucose measurements.

Study I was divided in two parts. In the first part, the effect of the membrane length and in the second part the effect of, the perfusion fluid rate, on the agreement of microdialysis (MD) glucose reading to plasma reference value were evaluated.

Method: The volunteers were allowed a light breakfast before arrival to the research facility. All veins were measured with an ultrasound devise (Site Rite® 5, Bard, Salt Lake City, UT, USA), and only veins with a diameter of 3 mm or more in a non-stasis state, were used. This was 5 times the diameter of the catheter, thereby assumed sufficient to ensure enough blood flow around the catheter. The diameter was assessed before and after catheter insertion, and before and after the sampling period. A period of 60 minutes was allowed after insertion, for stabilization of the microdialysis readings. The MD-catheters were perfused with a solution of Ringer-Acetate and 25 U/ml of low molecular heparin (Fragmin®, Pfizer, New York, NY, USA), driven by a low-voltage pump (CMA107). Samples for MD-glucose were collected in microvials over 10 minutes, during a 70-min period. Blood samples for reference glucose were collected in the middle of each 10-min period.

Protocol I: 30 volunteers were included and randomized to one of three groups. In group 1 (the control group), the subjects received a peripheral venous catheter (PVC) in an antecubital vein in both arms for blood sampling. In group 2, a PVC was inserted in one arm and a MD catheter (CMA, Microdialysis AB, Solna Sweden) with a membrane length of 10 mm was inserted in the other. Group 3 was handled as group 2, with the difference that a membrane length of 20 mm was used.

Protocol II: 15 volunteers were included. All subjects received a MD catheter, with membrane length of 30 mm, in an antecubital vein one arm, and a PVC in the other. Three measurement periods with different perfusion rates, 0.5, 1 and 2 µl/min, were performed in all subjects. The subjects were randomized to one of three groups. In group one; subsequent fluid rates of 0.5, 1 and 2 µl/min, in group two; 1, 2 and 0.5 µl/min, in group three; 2, 0.5 and 1 µl/min, were infused. Between every change of fluid rate, a 60-min period was allowed for stabilization of MD-readings.

10.2 PAPER II

The aim for paper II was to evaluate the feasibility and accuracy of an intravenous on-line continuous glucose measurement system inserted in a central vein, applying the microdialysis technique.

10 patients scheduled for major upper abdominal surgery were included in this prospective observational study. Exclusion criteria were; any coagulopathy, CVC planned in other vein than in the right internal jugular vein, a deviating CVC or under 18 years of age.

Method: The patients were studied during and after surgery, in the postoperative ward, for a total of 20 hours. After induction of anesthesia and before surgery, two CVCs were inserted in the right internal jugular vein, one standard 2- or 3-lumen catheter (5F, BD CareFlow™, Becton Dickinson Medical Surgical Systems, Franklin Lakes, NJ, USA, or 12F Mahurkar™, Covidien, Mansfield, MA, USA) and one additional one-lumen catheter with a MD-membrane (4 Fr Eirus SLC, Dipylon Medical AB, Solna Sweden). As a standard clinical routine, 25 mg/ml intravenous glucose infusion was administered during surgery. The MD-catheter was placed proximal to the CVC tip, to minimize the risk for falsely high glucose levels due to this local glucose infusion in comparison with arterial reference measurements. The catheters had a minimal distance between the tips of 3.9 cm, see figure 5. The placement of the CVCs was documented postoperatively by a chest X-ray. The MD-catheter had a membrane length of 40 mm, and was perfused with saline.

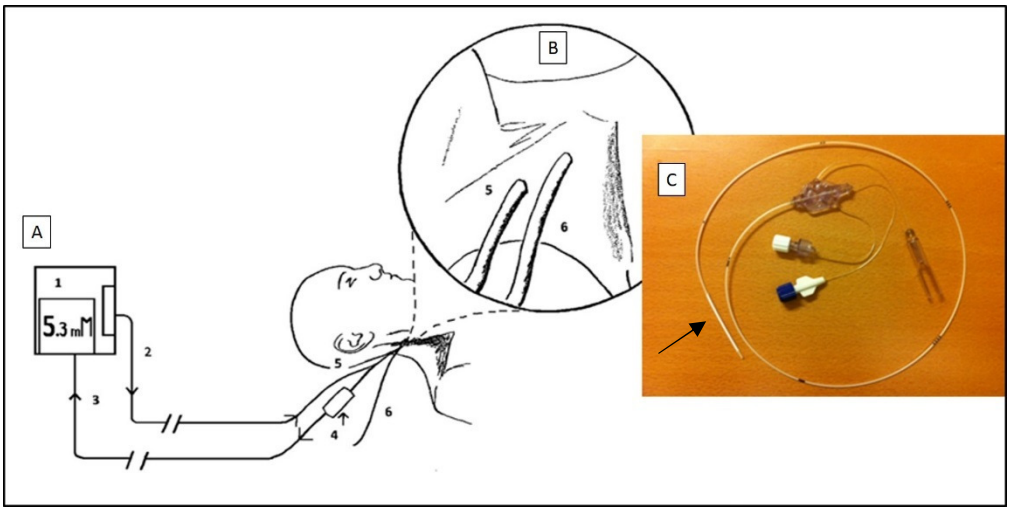

Figure 5: (Paper II) Schematic overview of the central vein microdialysis(MD) system. Picture A: 1. The MD-system with the sensor holder and display. 2. The perfusate line (NaCl). 3. The dialysate line. 4. The MD sensor with integrated vials. 5. MD catheter 6. The adjacent 2-lumen CVC. Picture B. Zoomed view of the insertion area: 5. Proximally inserted MD-catheter, 6. Distally inserted CVC. Picture C. MD-catheter with a semi-permeable membrane (arrow). Reproduced with permission from Critical Care (Blixt et al., 2013).

Microdialysis started after insertion, but monitoring started after a run-in time of at least 30 minutes. Continuous glucose measurements proceeded for 20 hours, online values were recorded every minute. The device analyzed glucose levels every second, while presenting a rolling minute average. Two reference arterial samples were collected every hour, two minutes apart, during the whole study period.

10.3 PAPER III+IV

The aim for paper III and IV was to investigate the effects of glucose control on postoperative insulin resistance and glucose kinetics in liver surgery (paper IV).

Patients scheduled for elective open hepatectomy were enrolled in these randomized prospective studies. Twenty patients per protocol and study were planned. Patients scheduled for open laparotomy, over 18 years of age, with no contraindications for EDA were included. Patients with BMI>30, known history of diabetes mellitus or medication with systemic corticosteroids were excluded. Paper IV was registered at ANZCTR (Trial id number 12614000278639).

Method: Insulin sensitivity was assessed by a hyperinsulinemic normoglycemic clamp, HNC, as described below, on the day before surgery and immediately after surgery. In paper IV, a stable isotope tracer infusion with 6,6-$^{2}H_{2}$-D-glucose was added, during the HNC and intraoperatively. Patients were randomized by using a sealed opaque envelop, on the day of surgery before induction, to intraoperative insulin treatment (glucose target of 6-8 mmol/l) or to a control group. The control group was treated accordingly to current clinical practice, receiving intermittent intravenous insulin if blood glucose exceeded 11 mmol/l.

At the day of surgery, the patient arrived to the operating theatre at 7.30 am, fasting since midnight. A 6,6-$^{2}H_{2}$-D-glucose-infusion was started after arterial baseline sampling. The patients received oxycodone (Oxycontin®, Mundipharma, Sweden) as premedication, intra- and postoperative analgesia was managed with a thoracic epidural catheter, inserted at level Th 7-10. After a test dose with Bupivacaine adrenaline 5 mg/ml (3-5 ml, Marcain adrenalin®, Astra Zeneca, Sweden) and an epidural bolus dose of fentanyl (50 µg) (Fentanyl, B Braun, Melsungen AG, Germany), an epidural infusion, containing Bupivacaine (1 mg/ml), adrenaline (2 µg/ml), and fentanyl (2 µg/ml) (15ml/h), was started. General anaesthesia was induced with Propofol-Lipuro® (B. Braun, Melsungen AG, Germany) and fentanyl and maintained with Sevoflurane (Sevorane®, Abbott, Solna, Sweden). Atracurium-Hameln (Biocodex, Kista, Sweden) was used for muscle relaxation. A central venous catheter(CVC) was inserted after induction of anaesthesia in all patients. Continuous infusion of glucose 25 mg/ml, 1 ml/kg/h, was routinely started as soon as the CVC was inserted. Arterial plasma glucose was measured every 10 minutes using a POC glucose monitor (Hemocue Glucose 201+®, Hemocue AB, Ängelholm, Sweden). In addition, hourly reference plasma samples were obtained.

Plasma samples for insulin and C-peptide were obtained at the start and the end of the HNC, as well as at three times during surgery; at the start of anaesthesia, at the start and at the end of the liver resection phase. In paper IV, plasma samples for tracer enrichment were obtained at identical surgery phases as for the hormones, apart from an additional sampling at the start of operation, see the study protocol, figure 6. Data for baseline characteristics; hemodynamic parameters, saturation, the amount of haemorrhage and blood transfusion, the size of resection and times of the anaesthetic, surgical and resection phases were collected. In addition, the rates of the glucose-, noradrenaline- and insulin-infusions were recorded. Crystalloid infusion (Ringer-Acetate®, Baxter International Inc., Ill, USA) 0-4ml/kg was given as intraoperative fluid replacement. Intraoperative haemorrhage was replaced with crystalloids, colloids and/or blood products, depending on the staff in charge.

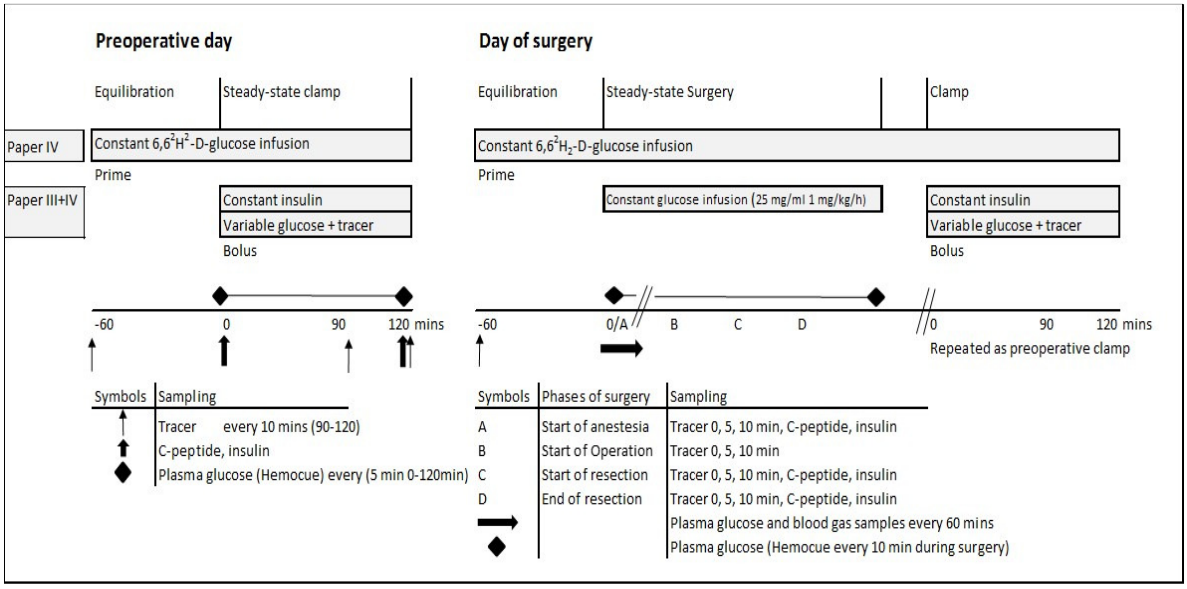

Figure 6: Study protocol for paper III and IV. Insulin sensitivity was assessed by a pre- and postoperative hyperinsulinemic normoglycemic clamp, HNC. In paper IV, a stable isotope tracer infusion with 6,6-$^2H_2$-D-glucose was added, during the HNC and intraoperatively.

10.3.1 Hyperinsulinemic normoglycemic clamp (HNC)

The patients arrived at the hospital at the day before surgery and measurements were performed after an overnight fast, or after a minimum of 6 hours. A peripheral vein catheter and an arterial catheter, though for the preoperative HNC in paper III two peripheral vein catheters, were inserted in separate arms, for infusions and blood sampling.

Two separate plasma glucose values, 10 and 5 minutes before the start of the HNC, analysed on a blood-gas analyser (ABL 800 Flex, Radiometer, Denmark), were used as a baseline mean

value. The HNC was initiated with a bolus of insulin (1.1U/m^2, Humulin® Regular, Lilly Ltd, Indianapolis, USA) followed by a constant insulin infusion (80 mU/m^2/min) with added albumin (400 mg, CSL Bering, PA, USA) and potassium (32 mmol, Addex®-Kaliumklorid, Fresenius-Kabi, Fresenius AG Bad Homburg, Germany). Normoglycemia was maintained by a variable infusion of glucose (Glucose 200mg/ml, B. Braun, Melsungen AG, Germany). During HNC, plasma glucose was analysed every 5 minutes on the POC device. For safety reason, plasma potassium was measured before and after the HNC, analysed on a blood-gas analyser. The target level for glucose steady-state was set to ±0.5 mmol/l of the baseline mean value. The HNC was continued for 120 minutes, and steady-state condition was assumed to be obtained after 60 minutes.

The HNC was repeated at the postoperative ward, approximately one hour after arrival, and after assessment of the EDA. The preoperative glucose target level was also used for the postoperative HNC. Arterial sampling was used in the postoperative HNC in paper III and IV. After 120 min, the insulin infusion was interrupted, whereas the glucose infusion continued as a security measure for an additional 30 minutes, and was terminated when a control sample showed normoglycemia. To avoid hypoglycemia after the preoperative HNC, patients were requested to eat before returning to the surgical ward.

Steady states for the glucose infusion and the glucose concentrations during the final 60 minutes of the HNC were later evaluated blinded, by a person not involved in performing the HNC, and unaware of the randomization. A steady state period, coinciding for glucose infusion and glucose levels, of minimum 30 minutes was identified and used for calculations. HNCs with no steady state for at least 30 minutes were excluded. The HNC validation was performed prior to any calculation. The amount of glucose given during the steady state period was used for calculation of the M-value (mg/kg/min).

In paper IV, after baseline isotopic enrichment sampling, a primed continuous (3 mg/kg + 2.4 mg/kg/min) infusion of 6,6-$^2H_2$-D-glucose was started, 60 minutes before start of the HNC. The steady-state conditions of 6,6-$^2H_2$-D-glucose and the HNC was considered to coincide. 6,6-$^2H_2$-D-glucose was added to the variable glucose infusion, to compensate for changes in plasma enrichment during HNC, due to changes in the glucose infusion rate. The mean glucose isotope enrichment (MPE) was estimated to be 1.2% and 0.7%, in the pre- and the postoperative HNC respectively.

At the day of operation, sampling for baseline enrichment was performed via an arterial catheter. A primed continuous infusion (3 mg/kg + 2.8 mg/kg/h) was started 60 min before tracer sampling, and continued uninterrupted during surgery and the postoperative HNC. Sampling for 6,6-$^2H_2$-D-glucose enrichment in plasma was made every 10 minutes during the last 30 minutes of the HNC period (90 to 120 min), and every 5 minutes for 10 minutes during surgery, at the start of anaesthesia, the start of surgery, the start of resection and the end of resection. The standard continuous intraoperative glucose infusion (glucose 25 mg/ml,

1ml/kg/h) was unlabelled. This infusion and any ongoing insulin infusion were terminated at the postoperative ward, and the HNC was performed as previously described.

10.3.2 Isotopic tracer dilution technique

The enrichment of 6,6-$^{2}H_2$-D-glucose and glucose concentrations were measured in all infused solutions. Plasma enrichment values, molar percent excess (MPE), used in further calculation, represent the mean MPE value of the four 10-minute samples collected during the final 30 min period of HNC, or the three 5-minute samples collected during the four surgical phases. Glucose kinetics were calculated by using a modified Steele´s equation. For WGD, the glucose pool volume (V) was assumed to be 250 ml/kg and a pool correction factor (P) of 65% was assumed. The rate of change in plasma glucose concentration (ΔG, mmol/l/min) as well as the total rate of tracer infusion (TI, continuous and added labelled glucose, mmol/kg/min) were calculated.

Under steady-state, it is assumed that the tracer infusion rate divided by the tracer enrichment (MPE), equals the rate of appearance, Ra, which in the post-absorptive state represents the EGP, see figure 3. However, with concurrent glucose infusion, endogenous glucose R_a was calculated by subtracting the rates of exogenous glucose infusion (GIR) from total glucose R_a. At steady state rate of appearance is also assumed to be equal to rate of disappearance, R_d.

10.3.2.1 Glucose kinetics equations:

EGP (mmol/kg/min) = (TIR / MPE x100) - GIR - TIR

WGD (mmol/kg/min) = GIR + EGP – (P x V x ΔG)

EGP = endogenous glucose production (µmol/kg/min)

WGD = Whole body glucose disposal (µmol/kg/min)

TIR = Tracer infusion rate (µmol/kg/min)

GIR = Labelled glucose infusion rate (µmol/kg/min)

MPE = Molar percent excess, enrichment of labelled glucose in plasma (%)

ΔG = Rate of change in plasma glucose concentration (µmol/l/min)

P = Correction factor of glucose pool (0.65)

V = Distribution volume of glucose (250 ml/kg)

11 SAMPLING AND ANALYSES

Paper II-IV: Reference glucose sampling was collected using an arterial line. In paper I, a PVC was used for collection, as in paper III, during the preoperative HNC.

Paper I-IV: Reference plasma glucose were taken in pre-chilled Sodium fluoride/potassium oxalate tubes, kept on ice, centrifuged within 60-90 min (1200G, 10 minutes in 4° C, Universal 32 R Hettich Zentrifugen®, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany) and stored at –80 ºC until later analysis. Samples were analysed on an automatic analyser (Konelab 20, Thermo Scientific, Jönköping, Sweden) using a GOD-POD analysis (Thermo Fisher Scientific, Vantaa, Finland).

Paper I: Microdialysis vials were collected and analyzed within 5 hours on an automated analyzer (CMA600, CMA Microdialysis, Solna, Sweden).

Paper II: The microdialysate was analysed in an online analyser module, using a GOD reaction. The results were sent to and displayed on the Eirus monitor. Retrospectively, MD-measurements were calibrated to plasma glucose values by two different ways of calibration, by using the first plasma value only (MD1) or by recalibration every eight hour (MD8). A mean value of the two reference values was calculated and compared to the MD-value. Due to the transport of fluid in the MD catheter, there was a lag-time of 10 minutes between measurement and analysis. Consequently, MD-values were corrected for these 10 minutes. Only measurements coinciding with hourly plasma reference samples were used for calculations.

Paper III+IV: Repeated plasma glucose measurements were performed using a bedside glucose analyser (Hemocue Glucose 201+ ®, Hemocue AB, Ängelholm, Sweden) both during surgery and clamping. In addition, safety measurements of plasma glucose and potassium were done hourly during surgery as well as before and after HNC using a blood gas analyser (ABL 800 Flex, Radiometer, Denmark).

Paper III+IV: Samples for Insulin, C-peptide and Cortisol were taken in pre-chilled EDTA tubes, centrifuged and stored as described above. Plasma cortisol (Paper III), insulin and C-peptide (Paper III+IV) were analysed using an ELISA-based standard analysing kit (IMMULITE® 1000 Immunoassay System, Siemens®, Ill, USA). In the hormone analysis, some insulin values were reported as <2 or >300 µIU/ml. No apparent explanation for outliners, for example hemolysis, was found. For calculation, numbers 2 and 300 were used.

Paper IV: Samples for 6,6-$^{2}H_{2}$-D-glucose were collected in pre-chilled EDTA tubes, centrifuged and stored as described above. Plasma glucose samples was deproteinized and purified using ion exchange chromatography, glucose was derivatized to its trimethylsilyl-O-methyloxime form. 6,6-$^{2}H_{2}$-D-glucose enrichment was analysed on gas-chromatography mass-spectrometer (GCMS) (Agilent 6890 and 5975C with Triple-Axis Detector, Agilent Technologies Inc., CA, USA).

12 STATISTICS

Paper I-IV: Values are presented as median and lower and upper quartile or mean (standard deviation) for non-normally and normally distributed data, respectively. Shapiro-Wilks test was used for testing normal distribution of data. Student's t-test and repeated measures-ANOVA were used to analyse normally distributed data, Mann-Whitney test and Kruskal-Wallis ANOVA, was used for analysing continuous non-parametric data and Fischer´s exact test was used for analysing categorical data.

A p-value<0,05 was considered statistical significant. Statistical analyses were performed using Statistica 10 (Paper I-III) or 13 (Paper IV and combined data) (StatSoft® Inc, OK, USA).

Paper I: The percentage difference between measurements from both arms was calculated for each of the seven sampling timepoints in each subject.

Paper I+II: Mean difference between reference sampling and MD-glucose is presented in Bland-Altman plots, (paper II) regression analysis and a Clark-Error Grid.

Paper III+IV: Twenty plus twenty patients were analysed per protocol. In paper III one patient in the treatment and two in the control group were excluded, and in paper IV one patient in each group were excluded, due to suboptimal insulin clamping, assessed blinded.

In paper III Kruskal-Wallis ANOVA was used for analysing the hormone levels. M-values were presented as mean±SD, and the difference between pre- and postoperative HNC values was reported as percentage. In paper IV and the combined data, M-values were reported as median (lower-upper quartile), and the difference between pre- and postoperative values was reported as M-ratio.

In the analysis of the combined data, a correlation and forward stepwise logistic regression analysis were performed. The sample size of 35 patients, had a power of 80% to find a correlation of 0.46, in continuous data, at α 0.05. Non-normally distributed data for ANOVA analyses was log transformed for analysis (paper III and IV).

13 RESULTS

13.1 PAPER I

Protocol I: The difference between glucose measurements in two peripheral veins (control), was up to 10%, with an average difference of 3±3% (mean±SD). For the 10 and 20 mm membrane, the average difference was 30±21% and 14±13%, respectively.

Protocol II: At the lowest perfusion rate, 0.5 µl/min, 12 of 15 subject had a difference of less than 10% between plasma and microdialysis measurements, with an average of 8±7%. For the perfusion rates of 1 and 2 µl/min, the average differences were 25±19% and 39±28%, respectively.

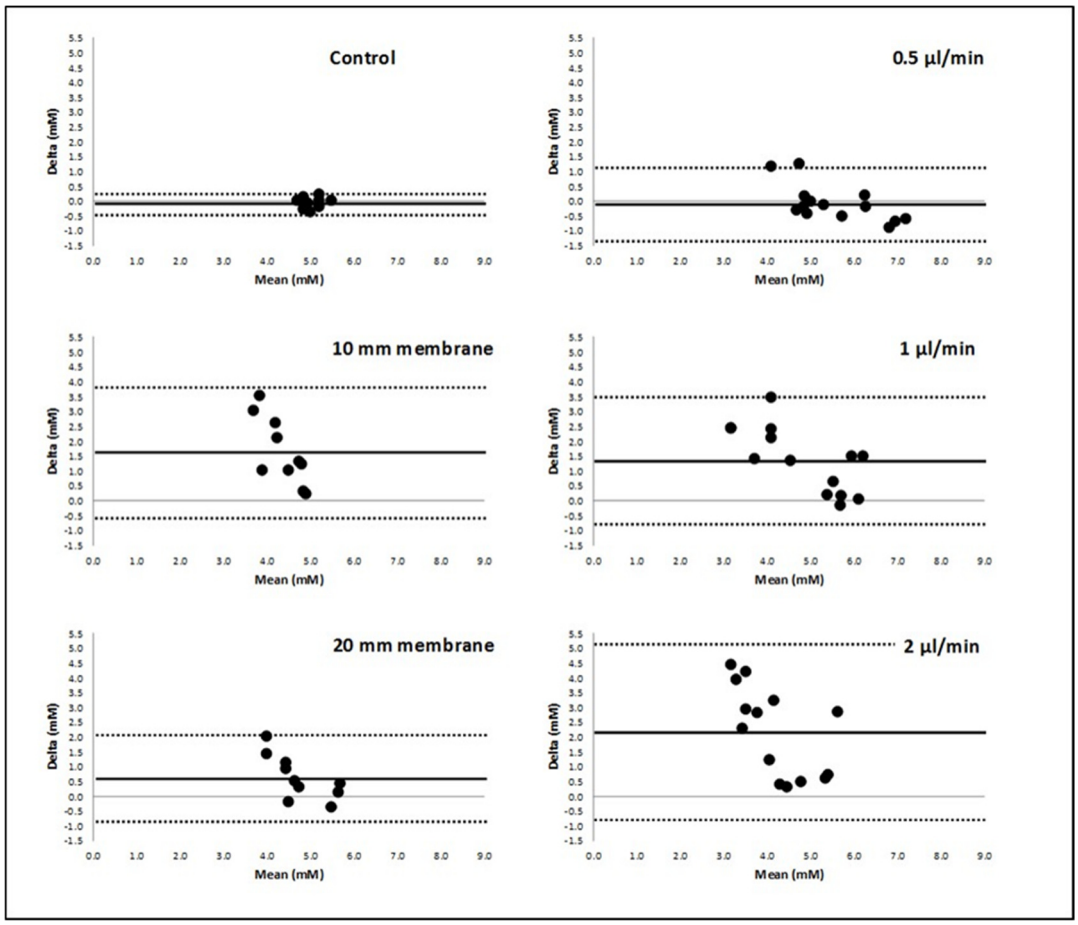

Figure 7: (Paper I) Bland-Altman plots of the comparison of the two plasma measurements from different veins in the same subject (top left), and the glucose measurements by microdialysis using different settings (variable membrane length and perfusion fluid rates), as described in chapter 11.1. For the left panels, the perfusion rate was 0.5 µl/min and for the right panels the membrane length was 30 mm. Values are given as the median values of the seven consecutive measurements over 70 min. The bold lines represent the line of equality, and the dotted lines the limit of agreement (SD x 1.96 of the difference). Reproduced with permission from Acta Anaest Scand (Rooyackers et al., 2012).

To compare the two different measurement methods, which had large interindividual variations, the median values were presented in a Bland Altman plot. For the two plasma measurements, i.e. the control, the line of equality was -0.09 mmol/l, and the limits of agreement 0.27 and -0.45 mmol/l. For the two microdialysis membrane, 10 and 20 mm, the limits of agreement were larger than for the control group. In addition, the lines of equality were different from zero, 1,62 and 0.61 mmol/l respectively. The setup used in protocol II, using a 30-mm membrane with a perfusion rate of 0.5 µl/min, demonstrated the best line of equality, -0.11 mmol/l, and limits of agreement,1.13 and -1.35 mmol/l, see figure 7.

Consequently, the conclusion was that the lowest perfusion rate combined with the longest membrane, demonstrated the best agreement, 8±7%, to plasma reference values. An interesting observation was the average difference between two plasma measurements, 3±3%.

13.2 PAPER II

In all patients included in the study, continuous measurements proceeded for 20 hours. Eight women and two men were included, with a mean age of 60 years (range 27-81). Of the 10 patients, 3 patients had pancreatic, 6 liver and 1 gastric surgery. Four patients received a large bore (12F) CVC, six patients received a standard double lumen (5F) CVC, along with the microdialysis catheter. To avoid falsely high values due to interference from the glucose infusions through the venous catheters, the mean distance between the tips of the microdialysis and venous catheter was 59 mm (39-82mm). This placement was confirmed by postoperative X-ray.

The recorded 195 individual glucose values ranged from 4.2 to 17.1 mmol/l. Mean single calibration glucose values (MD1) were 9.6±2.5 mmol/l, and for eight-hour calibration (MD8) 9.8±2.4 mmol/l. Both calibration methods showed a close agreement between the continuous readings and reference values. They showed a mean absolute glucose difference of 0.85±0.82 mmol/l or a mean absolute relative difference (MARD) of 8.8±8.4% and 0.61±0.76 mmol/l or 6.8±9.3% to the reference value, in MD1 and MD8 respectively.

In addition, both calibrations showed a high correlation to plasma readings, $r=0.89$ ($p<0.001$) and $r=0.92$ ($p<0.001$; t-test) in MD1 and MD 8 respectively. The agreement of the microdialysis glucose measurements were also presented in a Bland Altman plot. Both calibration methods showed lines of equality close to zero. The limit of agreement (±1.96 SD: CI 95%) was 24.2% (2.34 mmol/l) and 23.0% (1.94 mmol/l) for the MD1 and MD 8 calibration respectively, see figure 8. There was no statistical difference between MD1 and MD 8 calibrations ($p=0.09$, t-test). When the values were presented in a Clarke Error Grid, 100 % of all values were found in the A or B areas (benign). In MD1, 92.7%, and in MD8 93.3%, of the values were in the A area, see figure 8.

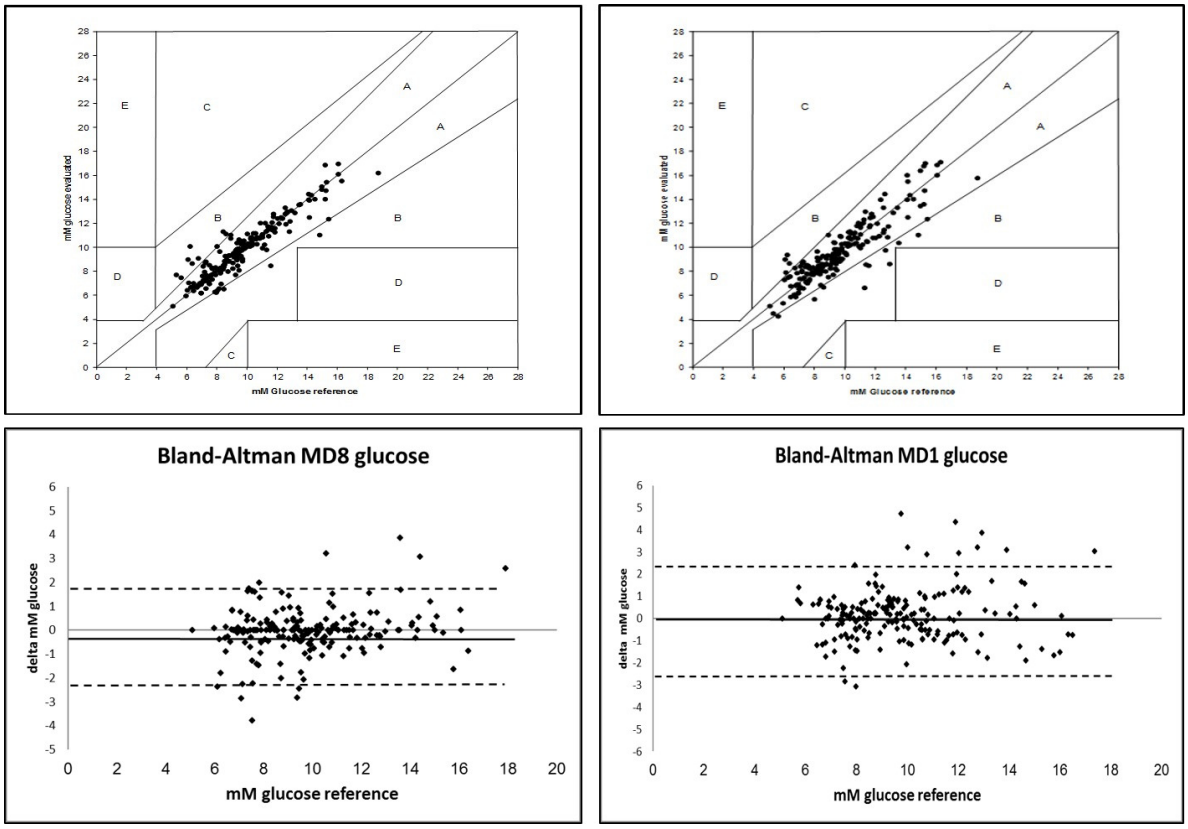

Figure 8: (Paper II) Upper row: Clark Error Grid of the reference vs the microdialysis glucose values, the eight-hour calibration (MD8) to the left, and the single calibration (MD1) to the right. Lower row: Bland-Altman plots of the mean absolute reference vs the mean absolute microdialysis glucose values (mmol/l), to the left MD8-calibration, to the right MD1-calibration. Bold lines represent the lines of equality, and dotted lines the limit of agreement (SD x 1.96 of the difference). Reproduced with permission from Critical Care (Blixt et al., 2013).

The paper concluded that 20 hours on-line continuous microdialysis glucose measurements, via a central vein catheter, showed a close agreement to plasma reference values. 100% of values were within A or B area in a Clark Error Grid, and the device demonstrated a MARD of 6.8±9.3%.

13.3 PAPER III

In paper III, 22 patients were included per protocol. Two patients were excluded in the intraoperative period due to unexpected corticosteroid treatment. Ten patients were planned in each group. Due to suboptimal quality of the hyperinsulinemic normoglycemic clamping (i.e. unable to reach steady state) another three patients were excluded (assess blinded), one patient in the treatment group and two patients in the control group. The groups were comparable for BMI, age, gender, operation and resection time, as well as blood loss and blood transfusions.

In the treatment group, all patients had surgery due to metastasized liver cancer, in the control group 6 out of 8 had the same indication for surgery, one had hepatocellular cancer, and one was diagnosed with benign tumor after microscopic evaluation of the resected parenchyma.

At start, mean glucose was 6.7±0.7 and 6.7±0.9 mmol/l in the treatment group and control group respectively (p=0.69, t-test). Total mean intraoperative blood glucose was 6.9±0.4 and 8.8±1.5 mmol/l in the treatment and control group respectively, and the difference between the groups during surgery were significant statistically (p=0.003, t-test). The mean M-value decreased from preoperative 6.9±2.3 to 3.3±1.7 mg/kg/min and from 7.0±2.5 to 1.6±1.7 mg/kg/min, in the treatment and control group respectively (p=0.056, ANOVA). In paper III, the relative difference in postoperative insulin sensitivity is expressed as M%. It corresponds to the ratio between postoperative and preoperative M-value, M-ratio, given in percentage. Though the decrease was pronounced in both groups, it differed significantly between the groups, the M% was 46.8±15.5 and 21.9±16.2 in the treatment and control group respectively (p<0.005, t-test), see figure 9.

Consequently, postoperative insulin resistance after liver surgery, though pronounced in both groups, was significantly attenuated by intraoperative glucose control.

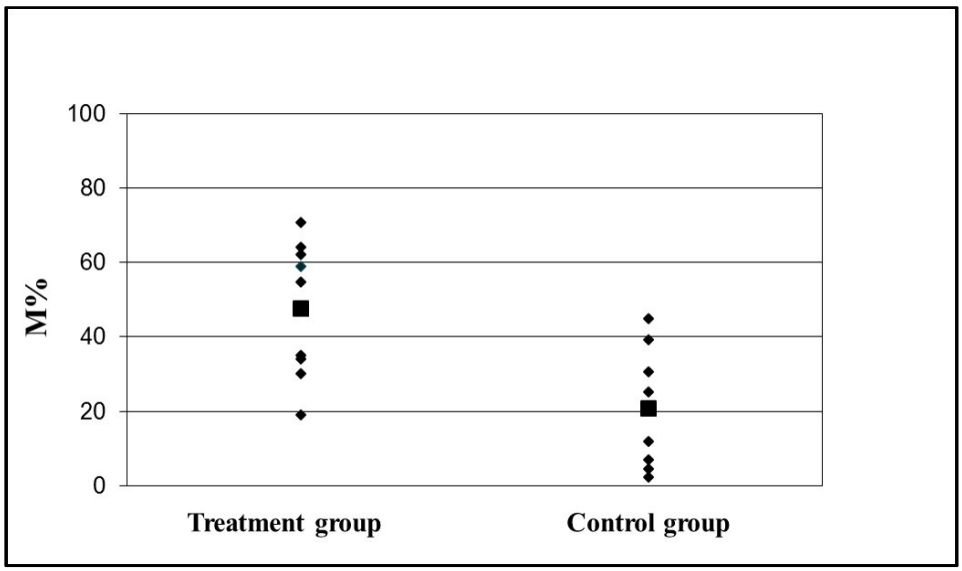

Figure 9: (Paper III) The difference between pre- and postoperative M-values presented as the percentage of the retained insulin sensitivity after surgery (M%). Individual and mean±SD values are presented. The groups preserved 46.8±15.5% vs 21.9±16.2%, in the treatment and control group respectively (p< 0.005, t-test). Reproduced with permission from Clinical Nutrition (Blixt et al., 2012).

13.4 PAPER IV

In paper IV the protocol from paper III was repeated, except for the addition of isotope labelled glucose. The aim was to evaluate the alterations in glucose kinetics during intraoperative glucose control and the development of insulin resistance.

In paper IV, another 22 patients were included. Two patients were excluded, one due to undiagnosed diabetes and in one the surgery was unexpectedly interrupted. As in paper III, another two patients, one patient in each group, were excluded due to suboptimal quality of the hyperinsulinemic normoglycemic clamping (assess blinded). As in paper III, the groups were comparable for BMI, age, gender, operation and resection time as well as blood loss and blood transfusions. In the treatment group, seven patients had surgery due to metastasized liver cancer, one had cholangiocarcinoma and one was diagnosed with a benign tumor after surgery. In the control group 5 patients had metastasized liver cancer, one was later diagnosed with neuroendocrine tumor (without overt symptoms or laboratory findings from the tumor), and three was diagnosed with benign tumor after microscopic evaluation of the resected parenchyma.

Mean glucose at start was 6.7±0.9 and 6.3±0.7 mmol/l in the treatment and control group respectively (p=0.35, t-test). Mean intraoperative glucose for the first 220 mins used for calculations, were 7.0±0.8 and 7.7±1.1 mmol/l, in the treatment and control group respectively (p<0.001; ANOVA). Insulin resistance decreased in both groups, from preoperative M-value of 4.6(4.4-6.8) to 2.1(1.2-2.6) and from 4.6(4.1-5.0) to 0.6(0.1-1.8) mg/kg/min in the treatment and control group (p=0.03; ANOVA, log-transformed data), see figure 10. However, the relative reduction in insulin sensitivity, in paper IV reported as the ratio between postoperative and preoperative M-value, failed to reach significance between groups, 0.35(0.26-0.51) vs 0.11(0.02-0.41) in treatment and control group respectively (p=0.11; Mann-Whitney).

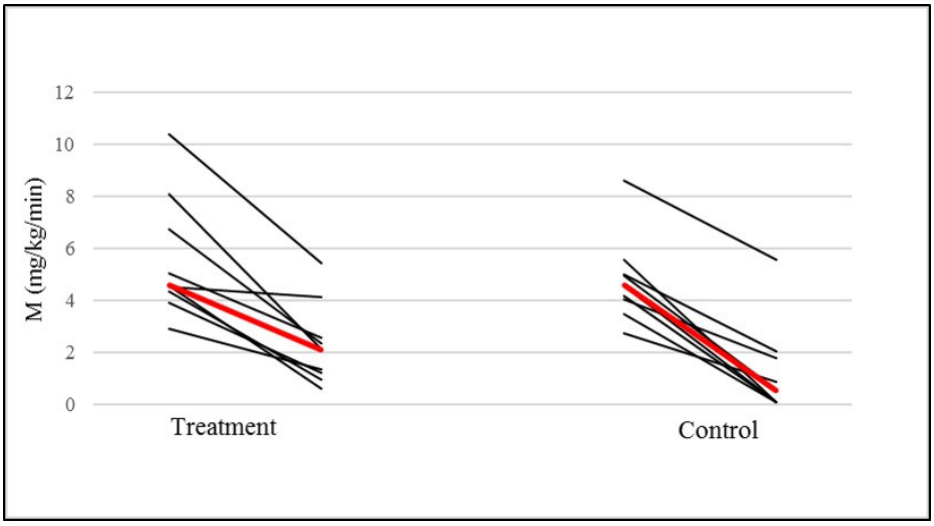

Figure 10: (Paper IV) Individual M-values (mg/kg/min) and median M-values (red), at the preoperative (left) to the postoperative (right) HNC, in the treatment and the control group respectively (p=0.03, ANOVA).

The preoperative endogenous glucose production (EGP) was comparable between the groups, 1.8(-0.9-4.2) vs 2.9(1.9-3.8) µmol/kg/min. Postoperatively these increased significantly to 3.4(2.6-6.5) vs 6.4(3.2-10.8) µmol/kg/min, in the treatment and control group respectively. Though a time effect was observed (p=0.05, ANOVA), the interaction did not reach significant difference between the groups (p=0.85, ANOVA). Similarly, whole-body glucose disposal (WGD) decreased significantly in both groups, from 31.6(25.6-43.0) vs 32.7(31.6-34.5) µmol/kg/min to 15.4(12.0-22.3) vs 14.0(11.6-16.3) µmol/kg/min, in the treatment and control group respectively. Whereas a significant time effect was demonstrated (p<0.0001, ANOVA), no statistical difference in interactions between the groups was observed (p=0.60, ANOVA).

Intraoperative glucose kinetics revealed a decreasing EGP, with a significant difference between groups, from 17.2 to 11.6 µmol/kg/min in the treatment group vs 15.3 to 10.1 µmol/kg/min in the control group (p=0.02, ANOVA). In contrast, no difference in WGD for either group, could be detected during surgery (p=0.67, ANOVA), see figure 11 in chapter 14.8.

The findings were in line with the results from paper III, which also demonstrated that intraoperative glucose control significantly improved postoperative insulin resistance. In paper IV, only the absolute reduction in insulin sensitivity reached significance (p=0.03, ANOVA). Postoperative glucose kinetics suggested that WGD is the major contributor to postoperative insulin resistance. During surgery, no clear explanation to the increased hyperglycemia was found, though a reduction of the EGP was revealed, possibly due to insulin treatment, while the WGD remained unchanged.

13.5 COMBINED RESULTS FROM PAPER III AND IV

Both studies, paper III and IV, were limited by the number of included subjects, 17 and 18 respectively. The subjects were treated according to the same protocol, apart from the addition of the isotopic tracer dilution technique in paper IV. Consequently, the results from the papers were combined in the thesis, to 1) improve statistic power, and 2) to increase the possibility to detect significant correlations explaining the level of postoperative insulin resistance. In the results for the combined data, values for M-PRE, M-POP and M%/M-ratio reported in paper III and IV are here given as median (lower-upper quartile).

The two groups, 18 and 17 patients in the treatment and control group respectively, were comparable for age, gender, BMI, blood loss, operation and resection time. Moreover, the groups did not differ in amount of transfused blood products or mean preoperative glucose values. The amount of noradrenaline administered was slightly higher in the treatment group, without reaching significance, 0.05±0.03 µg/kg/min and 0.04±0.02 µg/kg/min, in the treatment and control group respectively (p=0.06; t-test). The treatment group received insulin at a dosage of 21.8±11.9 mU/m^2/min or 0.55±0.27 mU/kg/min. The control group only received single intermittent boluses, insulin was administered to 4 individual patients, at a total of 1-4 U/patient. Notably, both groups in paper IV have lower median M-PRE values than the

corresponding group in paper III. Consequently, the control groups in paper III and IV are not comparable for that parameter (p=0.03, Mann-Whitney), while the difference between the treatment groups was non-significant. The baseline characteristics are presented in table 1.

Blood glucose levels at the start of anesthesia were 6.7±0.8 and 6.4±0.8 mmol/l in the treatment and control group respectively (p=0.27, t-test). During surgery, the groups diverged to have 7.0±0.7 and 8.1±1.3 mmol/l in treatment and control group respectively during the first 220 mins (p<0.0001, ANOVA), see figure 12. No glucose value below 4.4 mmol/l was recorded during surgery. The total operation time differed, which implied the start of resection occurred at different times points from start of anesthesia. When comparing glucose levels at same surgical phases, the start of anesthesia, the start of liver resection and the end of resection, the glucose levels were still significantly higher in the control group (p<0.0001, ANOVA), see figure 13, in chapter 14.7. The elevation in blood glucose levels was predominantly seen between the start of surgery and the start of resection. However, the alterations between the groups, during the resection phase, were not significantly different (p=0.21, ANOVA).

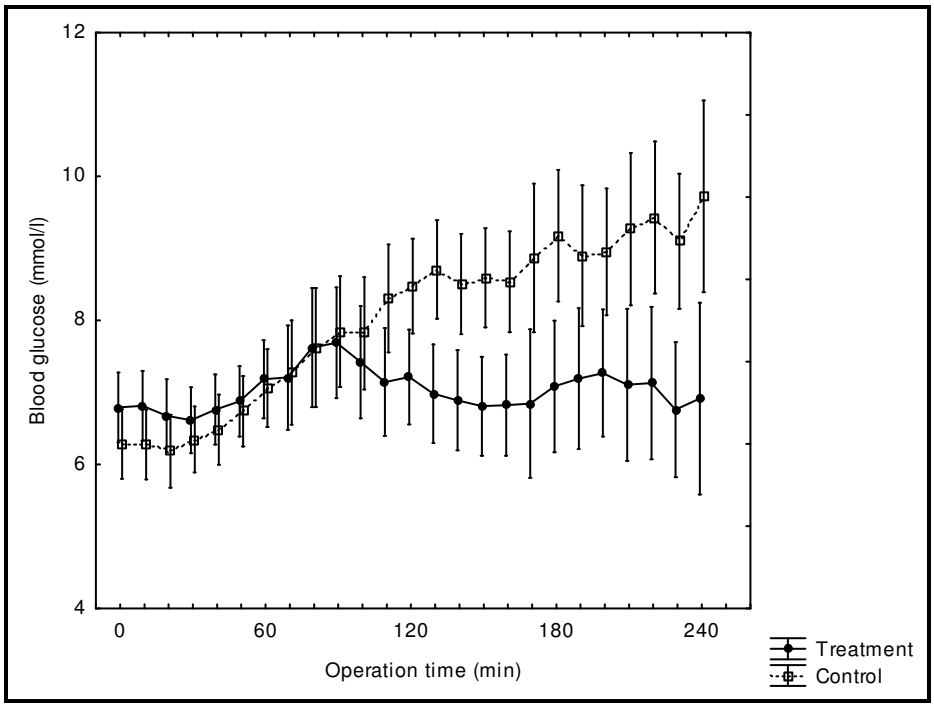

Figure 12: Combined data of the intraoperative blood glucose concentration during the first 220 min, in the treatment (n=18) and the control group (n=17) respectively (p<0.0001, ANOVA).

The preoperative M-values, 5.9 and 5.0 mg/kg/min (p=0.61, Mann-Whitney), declined to 2.3 and 0.9 mg/kg/min (p=0.004, Mann-Whitney) in the treatment and control group respectively,

see figure 14. The decrease over time between the groups, was significantly different ($p<0.001$, ANOVA on log-transformed data). The relative reduction, the ratio, between pre- and postoperative values was significantly different, 0.41 vs 0.12 ($p=0.003$ Mann-Whitney) in the treatment and control group respectively. Perioperative median M-values and mean glucose values (for the first 220 mins), for paper III, IV and combined data, are presented in table 2.

Moreover, the M-ratio, in all patients, was strongly correlated to the randomization of insulin treatment and total mean intraoperative glucose values ($r=0.50$, $p=0.002$ and $r=-0.46$, $p=0.005$ respectively). There were more men included in the trial, 27 vs 8 patients and the median M-ratio was 0.49(0.28-0.61) and 0.31(0.11-0.45) for women and men respectively ($p=0.1$, Mann-Whitney). Both BMI and gender showed a trend to significant correlation with insulin resistance ($r=-0.31$ and $r=0.31$ respectively, $p=0.07$ for both parameters). Overall, the total mean perioperative glucose values correlated to the Pringle maneuver, resection time and amount of bleeding ($p=0.05$, 0.008 and 0.03 respectively). As expected, the size and total time of surgery are correlated to the amount of bleeding and the resection time, though not to the Pringle maneuver. Correlations are presented in table 3, in chapter 14.7.

Nevertheless, in a forward stepwise logistic regression analysis, neither factors (BMI or gender) were significant correlated to the reduction of insulin resistance, nor were age, Pringle maneuver, amount of bleeding, blood transfusion, noradrenaline or time or size of surgery. In the control group, no perioperative or baseline parameters had any correlation to the M ratio.

In conclusion, the combined data confirmed that glucose control during liver surgery significantly reduced the development of postoperative insulin resistance, with a M-ratio of 0.41 vs 0.12, or if presented as percentage, glucose control maintained 41% vs 12% of preoperative insulin sensitivity.

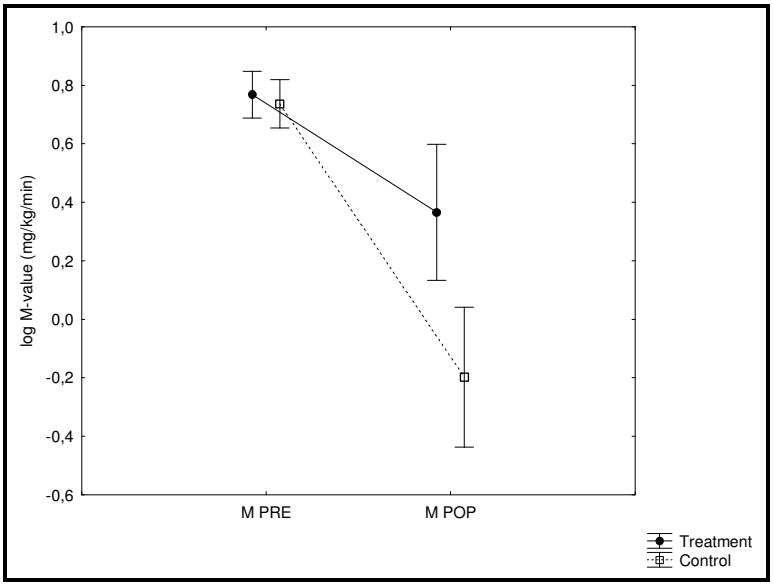

Figure 14: Combined data of the M-PRE vs M-POP values for the treatment (n=18) and the control group (n=17) ($p< 0.001$, ANOVA).

	Treatment group						Control group						p-value		
	Paper 3		Paper 4		Combination		Paper3		Paper4		Combination		Paper 3	Paper 4	Comb
Patients	n =9	Range	n=9	Range	n=18	Range	n=8	Range	n=9	Range	n=17	Range			
Age (yrs)¶	64 (4)	57-70	71 (10)	46-79	68 (8)	46-79	68 (10)	54-81	67 (13)	42-81	67 (11)	42-81	0.35	0.46	0.92
BMI (kg/m^2)¶	25.7 (4.8)	19-35.6	23.7 (2.5)	19.5-27.7	24.7 (3.8)	19.0-35.6	27.5 (2.8)	22.8-31.2	25.8 (2.7)	21.9-30.8	26.6 (3)	22-31	0.37	0.11	0.11
Gender (m/f)	6/3		6/3		12/6		8/0		7/2		15/2		0.21	1.00	0.23 β
Operation time (min) ¶	214 (66)	122-315	277 (120)	147-470	246 (99)	122-470	242 (29)	140-350	262 (106)	122-481	253 (93)	122-481	0.40	0.78	0.83
Blood loss (ml) €	900	500-1350	400	250-500	500	300-1100	1000	500-2300	600	200-2200	700	500-2200	0.96	0.22	0.30 £
Pringle (pats)	0		1		1		2		2		4		0.21	1.00	0.34 β
Resection time (min) ¶	45	31-53	50	29-60	47	29-55	60	44-85	50	41-117	58	41-105	0.07	0.49	0.1
>2 segments (pats)	5		3		8		4		4		8		1.00	1.00	1.00 β
Transfusion (pats)	4		1		5		4		2		6		1.00	1.00	0.73 β
RBC/pats (units) ¶	4.3	1-8	4	4	4.2	1-8	2	1-3	3	3	2.3	1-3	0.18	1.00	0.12
Noradrenaline (µg/kg/min)	0.04 (0.02)	0.01-0.08	0.06 (0.02)	0.03-0.11	0.05 (0.03)	0.01-0.11	0.04 (0.01)	0.01-0.06	0.04 (0.02)	0.01-0.08	0.04 (0.02)	0.01-0.08	0.89	0.02	0.06
Insulin (mU/m^2/min) ¶	24.3(11.2)	14.0-45.8	19.4(12.7)	0-45.6	21.8(11.9)	0-45.8	xxx		xxx		xxx				
Insulin (mU/kg/min) ¶	0.60 (0.25)	0.31-1.12	0.49 (0.29)	0-0.36	0.55 (0.27)	0-1.12	xxx		xxx		xxx				
Comorbidity:															
IHD	1		2		3		0		0		0		1.00	0.47	0.23 β
Hypertonia	1		2		3		4		3		7		0.13	1.00	0.15 β
Pulmonary	0		1		1		0		1		1		1.00	1.00	1.00 β

Table 1: Baseline characteristics of paper III and IV as well as of the combined data. Abbreviations: packed red blood cells (RBC) and ischemic heart disease (IHD). Values are presented as ¶ mean(SD) and range (min-max), or € median and range (lower-upper quartile). P-values: Student´s t-test except for £: Mann-Whitney or ß: Fischer´s exact test.

	Treatment group						Control group						p-value		
	Paper 3		Paper 4		Combination		Paper3		Paper4		Combination		Paper 3	Paper 4	Comb
Patients	**n =9**	Range	**n=9**	Range	**n=18**	Range	**n=8**	Range	**n=9**	Range	**n=17**	Range			
M PRE €	**7.4**	5.0-9.2	**4.6**	4.4-6.8	**5.9**	2.9-10.4	**6.5**	4.7-9.6	**4.6**	4.1-5.0	**5.0**	4.3-6.9	0.96	0.55	0.61 £
M POP €	**3.1**	1.8-4.5	**2.1**	1.2-2.6	**2.3**	0.6-5.9	**1.1**	0.4-2.8	**0.6**	0.1-1.8	**0.9**	0.1-1.8	0.01	0.03	<0.001 γ
M-ratio €	**0.55**	0.34-0.62	**0.35**	0.26-0.51	**0.41**	0.13-0.92	**0.19**	0.06-0.35	**0.11**	0.02-0.41	**0.12**	0.02-0.39	0.02	0.11	0.003 £
Start glucose ¶	**6.7 (0.7)**	5.7-8.1	**6.7 (0.9)**	5.5-8.4	**6.7 (0.8)**	5.5-8.4	**6.6 (0.9)**	5.3-7.9	**6.3 (0.7)**	5.4-7.4	**6.4 (0.8)**	5.3-7.9	0.69	0.35	0.27
Mean glucose 220(min) ¶	**6.9 (0.7)**	4.4-9.4	**7.0 (0.8)**	4.6-9.4	**7.0 (0.7)**	4.4-9.4	**8.4 (1.4)**	4.9-15.6	**7.7 (1.1)**	5.3-16	**8.1 (1.3)**	4.9-16	<0.0001	<0.001	<0.0001 γ

Table 2: M-values and values for perioperative glucose for paper III and IV as well as for combined data. Values are presented as ¶ mean (SD) and range as (min-max), or as € median and range (lower-upper quartile). P-values: Student t-test, except for £: Mann-Whitney or γ: ANOVA.

14 DISCUSSION

The land breaking trial published in 2001 by researchers in Leuven, where tight glucose control turned out to be beneficial to patients´ outcome compared to standard treatment, made glucose control a hot topic and standard care in worldwide ICU and perioperative settings. Consecutive studies were launched with the goal to confirm the outstanding results for these severely ill patients, indicating a reduced mortality of more than 30%. However, by including all types of patients, medical and surgical, as well as diabetics and non-diabetics, to the intensive insulin therapy, an increased risk for hypoglycemic events became more and more obvious[7,8,10]. Furthermore, two multicenter studies were prematurely interrupted, partly because of high incidences of hypoglycemic events[9,11]. In 2009, the NICE-SUGAR trial was published, and demonstrated not only a substantial risk for hypoglycemia by aiming at a tight glucose range compared to standard treatment, but indeed associated the hypoglycemic events to a higher mortality[10]. As a result of the interest in glucose control, various devices for improved quality of measurement were developed, with the intention to continuously monitor glucose concentration.

Consequently, the initial eagerness to the idea of tight glucose control was cooled off. Moreover, further research has suggested that various kinds of fluctuations in glucose concentrations, not only hyper- or hypoglycemia, could add to the negative impact on patients´ outcome[194]. Whether glucose control is beneficial or not to outcome, has been suggested to depend on the genesis to the illness or type of surgery. Surgical patients have possibly a stronger benefit from glucose control, compared to non-surgical patients[180]. In addition, the diabetic status may influence dysglycemia, and thereby suggested to affect outcome. The DM status has been suggested to alter the tolerance for hyperglycemia, which could make the benefit from strict normoglycemia less pronounced[199]. Also, it is not known, whether it is the metabolic effect of insulin or the maintenance of normoglycemia that contributes to beneficial outcomes in certain patient groups. Finally, methods used to perform and measure glucose control in the different hospitals, could have affected both efficiency and safety as well as outcome in several trials. In conclusion, the optimal glucose target, to whom and where, as well as when and how to measure glucose has been vividly and frequently debated.

14.1 ACCURACY OF GLUCOSE MEASUREMENTS

A method to continuously measure and display glucose values is warranted. Ideally, a superior CGMS should combine the safe, swift analyzing and minimal labor effort of a POC-device with the accuracy and reliability of a blood gas analyzer or laboratory analyses.

Proposed ways of reporting accuracy have been according to the International Organization for Standardization (ISO) criteria, where glucose values should be within 20% of reference value in >95% of the times over 4.2 mmol/l[274]. The standards have lately been revised, valid from 2016 the accuracy should be the same over a value of 5.6 mmol/l[275]. In contrast, the FDA (Food and Drug Administration) recommends higher accuracy, 95% of the values should be

within 15% of reference value, or 99% within 20%[276]. In addition, the even more stricter ICU-consensus criteria from 2013 recommend over 98% of the readings should be within 12.5 % of the reference standard measurements[219]. Yet, another standard has been proposed, a mean absolute relative difference (MARD) of <10%[277]. Readings could also be presented in a Clark Error Grid, intended for single point of accuracy, or in a Bland-Altman plot.

Sensor or sampling positioning seem to improve the measurements, though in general, subcutaneous CGMS report lower accuracy, quantified as MARD, than intravascular devices[278]. Trials on intravascular devices often report close to 100% of readings within the former ISO-standards and in the "non-dangerous" zones (A and B) in a Clark Error Grid. Other aspects of CGMS performances concern the need and frequency of calibration, which can reduce systematic errors, as well as the set-up time and reliability.

In addition, the site of intravascular access is of interest. The difference between arterial and venous glucose levels has been demonstrated to be minimal, venous displayed 97% and arterial 100% of the readings within 20% of laboratory reference measurements[252]. Despite this, arterial sampling accuracy is considered superior to venous. Moreover, a peripheral positioning could produce inferior readings due to vasospasm or hypothermia. Consequently, it makes intravascular sampling site preferred in the ICU settings. Moreover, irrespectively of the sampling site (arterial or venous) continuous monitoring of exact values as well as trends over time is suggested beneficial for reducing different types of dysglycemia and work load[164]. In contrast, it has been argued that an experienced and dedicated staff could well compensate for inferior methods of monitoring and sampling[279], as demonstrated in the Leuven trials, and glycemic variability is not convincingly reduced by more intense monitoring[235].

14.2 MICRODIALYSIS TECHNIQUE

Nevertheless, various CGMS have been developed over the last couple of years, presenting different degrees of agreement to plasma reference values. One of the applied techniques is measurements by microdialysis. The technology has the advantage of performing continuous sampling of interstitial fluid over a membrane, without extracting any fluid, in this case plasma. The drawback is the analysis is performed at a distance from the site of collection, resulting in a lag-time. This can also be an advantage, since the risk of misreading due to clotting of the analyzing part of the device is not an issue. Instead, clotting of the semi-permeable membrane is a potential hazard.

As previously mentioned, Rooyackers et al. presented data for the feasibility of a MD-catheter in ICU patients over 5 days and in healthy volunteers[258]. However, the major conclusion was divergent reliability of the measurements, some readings showed high accuracy, whereas others was obviously insufficient. Another important observation was the difficulty to find adequate peripheral venous access in the severely ill patients. Possibly, a too large diameter of the microdialysis catheter could produce misreadings, by affecting the blood flow, which indicated the need for larger veins for insertion of the catheter. Moreover, it was suggested the catheter should preferably have a central position, as a distal position could imply differences in glucose

levels depending on which tissue the vein drains. This was illustrated by diverging glucose levels between different peripheral sampling sites, in the same subject.

Another paper on the subject was published at about the same time, having investigated the agreement of glucose measurements by a peripheral intravenous microdialysis catheter in 14 patients in a cardiac ICU during 3 days[280]. The measurements were performed in intermittent sampling periods during one hour each day. This study demonstrated a MARD of 14.7% and only 82% of the readings were within 20% of the reference. In addition, it reported a concern about the reliability, in 4 of 14 patients the readings were below reference values, which was speculated being caused by a reduced blood flow around the membrane. Another possibility for reduced agreement could have been the fluid rate, 1 µl/min, which was higher than the optimal rate reported in paper I.

14.3 PAPER I AND II-INTRAVENOUS MICRODIALYSIS

Consequently, paper I and II were designed to further investigate glucose measurements by intravenous microdialysis catheters. In the first study, the diameter of the vessel was evaluated by ultrasound, and no vessel with a diameter less than 3 mm was used, to avoid the catheter occluding the lumen and thereby reducing the surrounding blood flow. The two protocols evaluated the effect of the membrane length and the rate of the perfusion fluid separately. The best agreement to plasma reference values was demonstrated by the longest membrane, 30 mm, in combination with the lowest perfusion fluid rate, 0.5µl/min. The deviation from the line of equality can be handled by calibration. These findings were in line with the general principles for microdialysis measurements in other tissues, where increasing length, which gives a larger membrane area, and lower perfusion rate augments the agreement. The previous paper indicated that a shorter membrane length (10 mm) gives far lower agreement to plasma glucose readings than the longer membranes in paper I[258]. Thus, the conclusion was that the technique needed to be evaluated in a central vein, which by its size allows for even larger MD-catheter size and area of the membrane, thereby permitting higher fluid rate without losing degree of recovery. In addition, this shortens the lag time for analyses. Consequently, the criteria for on-line real-time continuous glucose measurement systems could better be fulfilled.

The larger microdialysis catheter studied in paper II was under refinement, and therefore delivered as a separate catheter, making the clinical handling somewhat more complicated. The distance between the two catheter tips were measured to avoid misreading due to concurrent glucose infusion in the standard central venous catheter (CVC). In later versions, the membrane has been integrated with the CVC, demonstrating similar agreement to plasma readings to the one we investigated. The catheter was evaluated over 20 hours, during and after surgery, without any signs of membrane failure, thereby indicating good feasibility. The device demonstrated a close agreement with an absolute difference of 0.6±0.8mM/l, and a MARD of 6.8±9.3%. The readings disclosed a limit of agreement of 24.2%, with a line of equality close to zero. Eight of ten patients showed a good agreement to plasma values and the remaining two demonstrated an accurate trend to the changes in plasma glucose levels, but the exact

microdialysis values were lower than in plasma. The reason could have been rapid changes in glucose levels due to concurrent glucose infusions. Even though the two catheter tips were separated with a mean of 59 (39-82) mm, confirmed by X-ray, movements by the patient in the postoperative ward could have temporarily shortened the distance. In addition, the changes in the central vein and the artery (reference sampling) might not be completely synchronized during faster changes in glucose levels. Excluding these outliers, 95% and 98% of the readings were within 12.5% and 20% of reference values, respectively, which would imply the criteria stated in the 2013 consensus could be fulfilled. Perhaps a more frequent sampling could have improved the agreement. Furthermore, reference values were sampled in an arterial line and microdialysis values were venous, which could have added to the difference between MD and reference values.

Two ways of calibration, a single calibration and every eight hour, as recommended by the manufacturer, were compared. Frequent calibrations are labor intense, and implementing continuous measurement systems have been suggested beneficial for the work load of the staff. In paper II, no significant difference between single and repeated calibration could be demonstrated, which implies that the technique is indeed applicable and reliable for this purpose. However, the device was only evaluated in 10 patients and for a relative short period, 20 hours, and it is impossible to comment on the agreement during longer periods.

No matter the technique, microdialysis or not, the advantages of a central positioning of the catheter have been clearly demonstrated in these trials. In paper I, the diameter of the vessel was assessed by ultrasound, and still possible outliers could have been explained partly by the catheter´s positioning. For instance, the catheter can position itself in to the vessel wall and block part of the membrane and blood flow. In the following paper, using a separate microdialysis catheter, the placement in the vessel and compared to the adjacent CVC, could also have been factors for misreading.

Consequently, an integrated catheter has been developed and extensively evaluated in cardiac surgery patients, in gradually more advanced versions. A previous version of the MD-catheter as in paper II, was demonstrated having 93% of the MD-values within 20% of arterial reference values, though less agreement was shown to laboratory and venous values, 92 and 89% respectively[252]. In this version of the catheter, the microdialysate was collected in vials every 5 minutes and did not represent a single-point value as the blood gas analysis.

The MD-catheter, in the same version as in paper II, was later evaluated in 48 cardiac surgery patients, demonstrating a close agreement with a MARD of 5%, and 99.2% of all values within 20% of standard reference[253]. The device was further developed and integrated within a CVC, which would be an advantage since two separate catheters could be difficult to handle, and increase the risk for misreading if the two tips are misplaced, as suggested in comparison studies. In the improved state, it had a shorter, 5 minute, time-lag and displayed similar MARD of 5.6%[254]. Though, in a study on 12 ICU patients, where the newer MD-device was used for 3 days, the investigators demonstrated a somewhat lower agreement[251], where 93.6% of

readings over 4.2 mmol/l were within 20% of reference, and displayed a MARD of 7.5% and 93.6% of measurements in zone A of a Clark-Error Grid. The latter study was more close to our findings. However, they reported problems with the device, indicated by malfunctioning CVC´s or sensors.

Altogether, the investigators demonstrated promising results, even though some reported malfunctioning sensors, and the main conclusion was that the MD-device is an accurate and reliable system. In comparison with a subcutaneous CGMS, the intravascular MD-system was demonstrated superior, where the subcutaneous CGMS showed a MARD of 6.5% but only 90% within 20% of standard reference[255].

Overall, the main limitation for many CGMS is that the agreement has not been tested in the hypoglycemic range. As for most papers on the subject, also in paper II, there were no values in the hypoglycemic range, which makes it difficult to evaluate the accuracy for hypoglycemic values. For obvious reasons, it is difficult to intentionally perform clinical research on accuracy in this glucose range. However, the intravenous MD-catheter has been evaluated in the hypoglycemic range in an animal model. Hypoglycemia was provoked by insulin administration and restored by rapid glucose infusion[281]. The device was tested in a range where close to 60% of the readings were <4.1 mmol/l, and demonstrated good agreement to venous reference sampling, where 99% of values were in zone A in a Clarke Error Grid, 97.7% of the readings were within 20% of reference values and showed accurate trends. A notable finding, in contrast to other prior results[252], was that the reference arterial values were consistently higher than the MD- or venous reference values, the latter was also previously reported by Rooyackers et al.[258]. A substantial difference between arterial and venous samples may have implications on the accuracy when arterial glucose is considered reference standard, though the impact can be solved by calibration.

In paper I, the intravascular microdialysis glucose measurement using the lowest perfusion rate, over the longest membrane, displayed the closest agreement to plasma reference values, fulfilling the suggested criteria of a MARD <10%. However, glucose measurements could differ up to 6 % between intravenous sampling sites, an interesting finding in paper I to consider before accepting this criterion.

14.4 VARIOUS INTRAVENOUS CGMS

Other manufacturers of intravascular CGMS employ various analyzing methods, with sensor placement inside, or outside the vessel and different vascular accesses. One approach especially interesting for ICU patients, namely an arterial catheter using fluorescence sensing technique, was studied in a trial over 48 hours[256]. However, when compared to subcutaneous measurements, this device could not demonstrate a superior agreement, presenting only 85.8% of the readings within 20% of reference values vs 84.2% for the subcutaneous device. Moreover, when intravenous sampling in a peripheral vein over three days, by using frequent blood sampling over the sensor every 5 minutes, was investigated, the investigators reported a MARD of just above 5% and a mean absolute difference of 0.3 mmol/l[282], compared to 6.8%

and 0.6 mmol/ in paper II. They concluded that the device is both accurate and reliable. However, they also reported initial problems with the catheters, requiring daily replacement, which would certainly imply patient discomfort. This discovery, in combination with the conclusions from our study group, that finding a suitable peripheral venous access in seriously ill patient is problematic, would argue for a central venous placement of the catheters, at least in the ICU-setting. However, since not all patients need, or are possible to provide with a CVC, a device developed for flexible vascular application, arterial, central or peripheral venous access, would be preferable.

Central intravenous measurements, which apply different techniques, have been presented in several trials[283,284]. For example, a system, which uses a fluorescence sensing technique, presenting data every 15 seconds, demonstrated a MARD <10% in two different cohorts[250]. Another near-continuous device, using mid-infrared spectroscopy to measure blood sampled every 15 minute, has been evaluated[248,249]. Though presented with a similar accuracy as the MD-catheter, with a MARD of 7.6-8.0%, it could not reach the newer, stricter standards. However, the device was tested in both hyper- and hypoglycemic range, a strength as the latter is often lacking in most trials.

However, the integrated MD-catheter has still not been a commercial success, why? Agreement to reference values are acceptable to good, and trials have reported reliable trend data, which can be argued more valuable as a single value. Early detection of unfavorable trends could ideally imply that the staff reacts more swiftly to changes in blood glucose levels, which could reduce hypoglycemic events or glucose variability. Whether that could be accomplished by CGMS or not can be debated. By short measurement intervals, up to every hour, a significantly improved glucose metrics was reached, whereas further shortening of that seemed to have less effect[218]. Further reduction could also imply considerable blood loss over time, if glucose is assessed by blood sampling. Interestingly, as previously discussed, high time in range (TIR), has been associated with an improved clinical outcome[200,203,204]. Accordingly, by using frequent arterial blood gas analyses, the Leuven investigators produced impressive results, possibly due to a lower variability and a higher TIR in combination with a dedicated, experienced staff. However, if implementing glucose control in various ICU-settings, with sometimes less skilled personnel, CGMS could improve safety, performance and possibly effect outcome. Though, this should be proven in randomized trials.

Still, the MD-CGMS seems to have limitations, trials have reported malfunctioning sensors and missing data. Ideally, the start-up time until readings are presented on screen, should be as rapid and handling simple as invasive blood pressure measurements, but is reported considerably longer and more complex in comparison. In addition, to our knowledge, trials on clinical outcome are lacking.

14.5 POSTOPERATIVE INSULIN RESISTANCE AND HYPERGLYCEMIA

As previously discussed, a common feature for trauma and critical illness is the development of insulin resistance and subsequent hyperglycemia. The latter has been closely linked to poor

clinical outcome in both surgical[54,74] as well as ICU settings[57,58]. As mentioned, the suggested explanation for the unfavorable hyperglycemia, is the strong associations between high glucose levels and an impaired immune function, delayed surgical wound healing and a higher infection rate[33]. The diabetic status may also to be a factor influencing the risk for adverse events, where non-DM patients seem to be more vulnerable to elevated glucose levels[61,68,69,285]. Consequently, the optimal glucose range may be different in different patient populations, and possibly dependent on the diabetic status.

The degree of postoperative insulin resistance has been linked to the magnitude of surgery, fasting time, pain management and insulin treatment to keep normoglycemia. Abdominal surgery has been demonstrated to reduce insulin sensitivity by 50%, and even if most pronounced on the first postoperative day, it may persist for weeks after surgery[100].

Many studies on the subject are small and not designed to evaluate clinical outcome. In contrast, in a considerably larger prospective study, cardiac patients with poor preoperative blood glucose control showed higher degrees of postoperative insulin resistance (assessed by HNC) and increased incidence of adverse events; infections, need for blood transfusions and longer ICU and hospital stay[53]. In this study, the degree of insulin resistance was linked in a linear relationship to poor postoperative outcome. The authors, along with other investigators, propose HbA1c, indicative of poor glucose control, as a an useful tool to predict the development of postoperative insulin resistance[62]and adverse events[210]. Though being an interesting parameter, HbA1c was not included in the protocols for paper III and IV.

14.6 PAPER III AND IV-EFFECT OF INTRAOPERATIVE GLUCOSE CONTROL ON INSULIN RESISTANCE

Paper III and IV were designed to evaluate the effect of perioperative glucose control on postoperative insulin resistance, assessed by an hyperinsulinemic normoglycemic clamp, in a surgical (liver resection) research model. The intraoperative insulin infusion rate varied depending on the blood glucose values, sampled every 10 minutes. To our knowledge, few studies have used a similar protocol. If used at all, the HNC was often proceeded during surgery[120], or glucose control pursued by using an artificial pancreas[166]. Moreover, insulin may have been administered according to a preset protocol, where blood glucose was measured at longer intervals[193]. In some studies, with focus on evaluating postoperative outcome, a so-called GIN- or GIK-infusions with glucose and insulin in various combinations, have been used for glucose control[6]. In addition, some investigators have chosen to assess postoperative insulin resistance by indirect measurements, like HOMA-IR[81]. Even though time and labor consuming, we chose the HNC, which is considered the gold standard.

In paper III the main conclusion was, that by keeping near-normoglycemia (6-8 mmol/l) during the intraoperative period, the postoperative insulin resistance, assessed within 2 hours after surgery, can be significantly reduced. The absolute postoperative reduction in insulin sensitivity was different between the groups, and the relative reduction, the ratio, was significantly attenuated in the treatment group compared to the control group. For comparison

between paper III and IV, all numbers for M-values are presented as the median value in the combined data, as data for one group in paper IV was non-normally distributed. Therefore, the numbers given in paper III differs from those given in the combined data, see table 2.

After we observed a significant relative reduction of postoperative insulin sensitivity in paper III, we hypothesized that, by repeating the protocol and adding an isotopic tracer of glucose, we could discriminate the predominant site of the disturbed glucose turn-over, i.e. whether an increased glucose production or a reduced disposal was responsible for the alterations in insulin resistance. In paper IV, we confirmed that glucose control partly prevents postoperative insulin resistance, as the absolute reduction (M-PRE to M-POP) was significantly different between the groups, whereas the relative reduction in postoperative insulin sensitivity, did not reach significance. The relative reduction (the M-ratio) could be regarded as more accurate than the absolute values, as the value are suggested to remain constant between individuals, and has been demonstrated to correlate to the size of operations and the length of stay[99]. In paper III and IV, parameters for insulin resistance were significantly or close to significantly different between the groups, which may have been a result of small sample sizes.

14.7 COMBINED DATA FROM PAPER III AND IV – GLUCOSE CONTROL AND POSTOPERATIVE INSULIN RESISTANCE

We acknowledge that the main limitation in paper III and IV was the small number of patients included in each group, 9 vs 8 in paper III and 9 vs 9 in paper IV. Thus, the combined data from paper III and IV compiled data from 35 patients, and resulted in a more considerable number of patients subjected to open liver surgery. The improved statistical power increased the possibility to detect and determine mechanisms in the development of postoperative insulin resistance during liver surgery. Baseline characteristics of the combined treatment and control groups were comparable for the size of the resection, for the time of operation and resection and for blood loss. Though the groups were statistical comparable for most characteristics, most Pringle maneuvers were performed in the control group, see table 1.

The combined results demonstrated a significant difference in the absolute reduction in M-value, as well as in the M-ratio, between the treatment and the control group ($p<0.001$ ANOVA and $p=0.003$, Mann-Whitney, respectively).

Unfortunately, in one parameter, the control groups in paper III and IV were not comparable. The treatment and the control group in paper IV presented lower median M-PRE values than the corresponding groups in paper III, and the two control groups was statistically different. Obviously, the patients in paper IV can be regarded as having less insulin sensitivity, even though handled in the same way preoperatively, and they were possibly closer to a pre-diabetic state. It could also have been a result of studying small sample sizes, since no other parameter differs significantly between papers III and IV. The only exception noted was the noradrenaline dosage, though this difference was only noted between the two treatment groups.

In addition, in paper III, preoperative glucose sampling during the HNC was collected in a peripheral venous catheter. Venous sampling often produces lower readings than arterial sampling. This is demonstrated in the combined data, where venous preoperative glucose values in paper III are 3.9% lower than the corresponding arterial values in paper IV, and this could partly explain the differences in numbers of M-values between paper III and IV. Mean glucose values at start of the preoperative HNC were 5.6±0.8 and 5.4±0.5 mmol/l in paper III, in treatment and control group respectively (p=0.55, t-test). Corresponding values in paper IV were 6.0±0.5 and 5.6±0.4 mmol/l, in the treatment and control groups respectively (p=0.08, t-test). Moreover, the differences between the corresponding treatment and control groups, in paper III and IV, were not statistical different (p=0.26 vs 0.54, t-test), nor had any patient a preoperative fasting blood glucose value >7.0 mmol/l, which is the limit for diagnosing diabetes. Consequently, no apparent explanation for the diverging M-PRE values could be found.

Since the individual times between the start of anesthesia and the start of resection differed between patients, the glucose values were plotted at the same phases, see fig 13. It revealed a significant difference between the groups (p<0.001, ANOVA), where the treatment group remained within glucose target, whereas the control group developed hyperglycemia, predominantly between the start of anesthesia and the start of resection. A similar elevation was also identified in paper IV, where an additional sampling point also revealed that the major increase in glucose levels occurred after the start of surgery, thereby indicated substantial alterations in glucose kinetics within this phase.

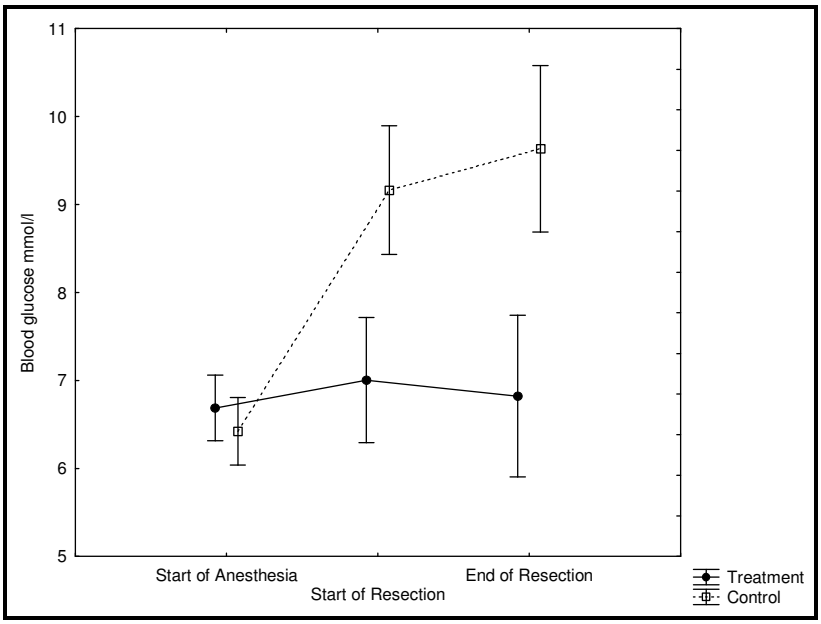

Figure 13. Combined data of the blood glucose concentrations for the treatment (n=18) and the control group (n=17) at different intraoperative phases; the start of anesthesia, the start of liver resection and at the end of resection (p<0.001, ANOVA).

The amount of hemorrhage was similar in the two groups, 500 vs 700 ml (p=0.30, Mann-Whitney). In the data for all subjects, hemorrhage was positively correlated to the mean glucose value during surgery (p=0.03), and a trend for a correlation to the maximal glucose value was identified (p=0.06), see table 3. These findings confirmed the same association between bleeding per se and postoperative hyperglycemia, as Hager et al demonstrated in an animal model[110]. Perioperative hemorrhage, the need for blood transfusion, vascular clamping and/or vasopressors could be regarded as a cross-linked phenomenon. During liver surgery, hemorrhage induces hypotony, which can be corrected by either vasopressors or fluid (in major hemorrhage - RBCs), or the hemorrhage could be reduced by vascular clamping. All these factors are associated with the development of hyperglycemia, which may be corrected with insulin.

The non-metabolic properties of insulin affect the NO-production, which may induce a vasodilating effect. This effect could theoretically induce a vicious circle, where insulin administration induces vasodilation in need of a vasopressor, in this case a catecholamine, which may cause hyperglycemia, and in turn again a need for more insulin. The interaction between insulin and noradrenaline has previously been demonstrated by Baron et al, where insulin sensitive subjects reacted less to a vasopressor than insulin resistant subjects[286], and this could possibly explain the differences between the groups in noradrenaline dosage in paper IV. However, the dosage only differed for the insulin group in paper IV, see table 1. In paper III, the groups had comparable dosage of noradrenaline, as well as in the combined data, so it may have been a coincidental finding in our data. Another explanation for the diverting findings in the two papers, could have been the time elapsed between the trials. Even if no apparent change has been made in the anesthetic handling of these patients, a minor change in the use of vasopressors could have been introduced, with the intent of reducing the fluid administration.

Even though the groups were comparable in most aspects, and no strong correlation could be found between the M-ratio or M-POP with the parameters amount of bleeding, operation time, resection time, transfusion or BMI, see table 3, the glucose control still positively influenced the postoperative insulin resistance. Interestingly, the strongest correlation was the correlation between M-ratio and the absolute M-POP value. As previously mentioned, the relative reduction of insulin sensitivity remained relatively constant between individuals and has been associated to the size of surgery[99]. However, this finding suggests the M-POP-value in itself could be indicative for the size of the reduction of insulin sensitivity, and therefore suggested useful in a simpler assessment of the development of postoperative insulin resistance.

After correlating the parameters for all patients, the size of the resection showed no additional effect on the M-POP, M-ratio, BMI, age or dosage of noradrenaline. Moreover, there was no correlation between the amount of bleeding and the relative reduction of postoperative insulin sensitivity. Thus, the combined data was unable to confirm previous findings, which firmly correlated hyperglycemia and insulin resistance to time of operation[287] and blood loss[110], see table 3.

	Random	>2 segments	Operation	Blood loss	Pringle	Resection	Transfusion	M PRE	M POP	M ratio	Gender	Age	BMI	Noradrenaline
>2 segment	-.0262													
	p=.881													
Operation	-.0373	.3401												
	p=.832	p=.046												
Blood loss	-.1752	.2946	.4447											
	p=.314	p=.086	p=.007											
Pringle	-.2567	-.0468	.0041	.0934										
	p=.137	p=.789	p=.981	p=.594										
Resection	-.3089	.1629	.5064	.4148	.2082									
	p=.071	p=.350	p=.002	p=.013	p=.230									
Transfusion	-.0809	.1200	.1258	.6565	-.1005	.1604								
	p=.644	p=.492	p=.471	p=.000	p=.566	p=.357								
M PRE	.0957	-.0627	-.3617	-.1270	-.0354	-.1727	-.0676							
	p=.585	p=.720	p=.033	p=.467	p=.840	p=.321	p=.699							
M POP	.3877	.0024	-.2800	-.1683	.0106	-.1576	-.0095	.6685						
	p=.021	p=.989	p=.103	p=.334	p=.952	p=.366	p=.957	p=.000						
M ratio	.5005	-.0901	-.2074	-.2470	.0510	-.1348	-.0532	.2363	.8443					
	p=.002	p=.607	p=.232	p=.153	p=.771	p=.440	p=.762	p=.172	p=.000					
Gender	.2567	.0468	.0505	-.2288	-.0278	-.1717	-.0754	-.0143	.2415	.3129				
	p=.137	p=.789	p=.773	p=.186	p=.874	p=.324	p=.667	p=.935	p=.162	p=.067				
Age	.0168	-.1227	-.0882	.0412	.0685	.0787	.2169	-.1060	-.1294	-.0525	-.0871			
	p=.924	p=.483	p=.614	p=.814	p=.696	p=.653	p=.211	p=.544	p=.459	p=.765	p=.619			
BMI	-.2765	-.0940	.1422	.2726	-.1007	.0869	.2646	-.1905	-.2918	-.3112	-.4241	-.1911		
	p=.108	p=.591	p=.415	p=.113	p=.565	p=.620	p=.125	p=.273	p=.089	p=.069	p=.011	p=.272		
Noradrenaline	.3218	.0163	.4914	.0439	.0041	.2180	-.0185	-.3268	.0308	.2750	.1647	.1880	-.2465	
	p=.059	p=.926	p=.003	p=.802	p=.981	p=.208	p=.916	p=.055	p=.860	p=.110	p=.344	p=.279	p=.153	
Mean glucose	-.5723	.0966	.1652	.3727	.3381	.4435	.3240	-.0356	-.3177	-.4632	-.1899	.1323	.2854	-.1625
	p=.000	p=.581	p=.343	p=.027	p=.047	p=.008	p=.058	p=.839	p=.063	p=.005	p=.275	p=.449	p=.097	p=.351

Table 3. Correlation table for the combined data, marker (red) correlations are significant at p<0.05

In general, more men are subjected to liver surgery, a ratio which was reflected in our data of 27 men and 8 women. Whether the gender matters for the insulin resistance is unclear. In our data, BMI and gender showed a correlating trend to the development of insulin resistance. Though, the higher relative postoperative insulin resistance observed in the women in our data, 0.49 vs 0.31 (p=0.1, Mann-Whitney) failed to reach significance. The women had lower BMI than the men, 22.9±2.9 vs 26.4±3.3 (p=0.01, t-test), whereas no difference in age could be demonstrated, 66 vs 68 years (p=0.62, t-test). According to previously results reported from HNC performed in 70 subjects, no correlation between insulin resistance and gender, BMI or age could be found[113]. However, other authors suggested insulin resistance and perioperative glucose control to worsen with increasing BMI[288], which in turn can be associated with aging[289]. In addition, pre-menopausal women were suggested to have higher insulin sensitivity[149]. In our trial, the menopausal status was not recorded, but all women but one, were by age post-menopausal. Even though no strong correlation between insulin resistance and age, gender or BMI could be found, there was a trend in the correlation of M-ratio to BMI and gender (p=0.07), that indicated the parameters may be associated with the development of insulin resistance.

As mentioned, data for the combined groups were comparable in most parameters. In the control group four patients had a Pringle maneuver performed and in the treatment group only one patient (p=0.18). Vascular clamping has been demonstrated to increase the perioperative glucose level[87], and it could not be excluded that the procedure could have induced higher glucose values in the subjects, which were predominantly found in the control group. Though the maneuver was performed later during the surgery, when hyperglycemia had already developed in this group, it was correlated to the maximal glucose level in the control group (p=0.03). Nevertheless, the Pringle maneuver had no correlation to the M-ratio.

Five patients in the treatment group, and six patients in the control group, received blood transfusions. The indication for transfusion, and the amount, was the choice of the anesthesiologist in charge, and the subgroup which received RBCs, had a blood loss of 1545(600-2000) ml. The administered number of RBCs was similar between the groups (p=0.12, t-test), with a range of 1-8 units (250 ml/unit). Red pack blood cells (RBC) are stored in SAG-MAN solution (Saline–Adenosine–Glucose–Mannitol), which contains a considerable amount of glucose, approximately 15-25 mmol/unit[111]. The exact concentration of glucose is depending on the storage time, during which glucose is consumed. The storage time, and the exact timing of transfusion during surgery, were unfortunately not recorded. However, generally the main bleeding during liver surgery occurs during the resection phase. In attempt to reduce the central blood pressure and the risk of further bleeding, transfusion is often started at the end or after the resection. Moreover, as illustrated in figure 11, most patients had already developed hyperglycemia before this stage of the operation. The M-ratio, for all patients who received RBCs, was 0.30(0.07-0.55), which was equal to patients not receiving any blood

transfusion, 0.35(0.15-0.49) (p=0.79, Mann-Whitney). Thus, no relation between the M-ratio and transfusion of blood products could be demonstrated, see table 3.

14.8 PAPER IV - GLUCOSE KINETICS

In paper IV, the isotope tracer dilution technique was added to evaluate intra- and postoperative glucose kinetics. The endogenous glucose production (EGP) is assumed suppressed during the HNC, and thereby the amount of exogenous glucose administered at steady state, is equal to the whole-body glucose disposal (WGD). However, in a state of insulin resistance, the endogenous production might not be fully suppressed, even if a higher insulin dose is used, as in our protocol, and the tracer dilution technique is used to discriminate the endogenous contribution to the blood glucose level.

Glucose kinetics measured during the HNC before and after surgery, indicated a development of a hepatic insulin resistance, as the EGP increased in both groups. In contrast, the WGD was significantly reduced, compared to preoperative values. However, the differences between the groups were subtle and failed to reach statistical significance. Possibly, both parts contributed, with the net result of a significantly reduced insulin resistance, a suggestion supported by the correlations between EGP, WGD and M-ratio (r=-0.74 and r=0.56 for EGP and WGD respectively).

Few, if any, have investigated the alterations in postoperative glucose kinetics during liver surgery. Our results showed in absolute numbers, that the decrease in WGD was larger than the alterations in EGP, thereby indicating that the impaired glucose disposal was the major contributor to early postoperative insulin resistance in liver surgery. Though, it is possible that postoperative insulin resistance and glucose kinetics alters over time, as investigators have presented diverging results. Brandi et al. demonstrated an impaired response to insulin in the liver as well as peripheral tissues 6-8 hours after surgery[290]. Though in line with our findings, other investigators reported an impaired glucose disposal as being the major contributor to early, as early as two hours after operation, decreased postoperative insulin resistance and hyperglycemia[39,113,124]. In addition, in the same postsurgical time span, Lattermann et al. have repeatedly showed depressed levels of glucose clearance, interpreted as a sign of impaired WGD, where an additional EDA attenuated the increased EGP[138,144,291]. The suggested effect on EGP by a functional EDA, could possibly account for the insignificant changes in EGP in our postoperative data. A later study, where insulin resistance was assessed three hours after colorectal surgery, perioperative glucose control was demonstrated to influence both glucose production and disposal[292]. However, this paper confirmed, together with another trial[132], our findings regarding the inability of insulin to fully suppress EGP and attenuate the decline in WGD in the postoperative setting.

Later in the postoperative period, after the first postoperative day, the hepatic glucose production is probably more attenuated, whereas the reduction in glucose disposal could be considered consolidated[114], even though contradictive results exist, where a depressed glucose disposal, even 2 days after colorectal surgery, have been reported[139].

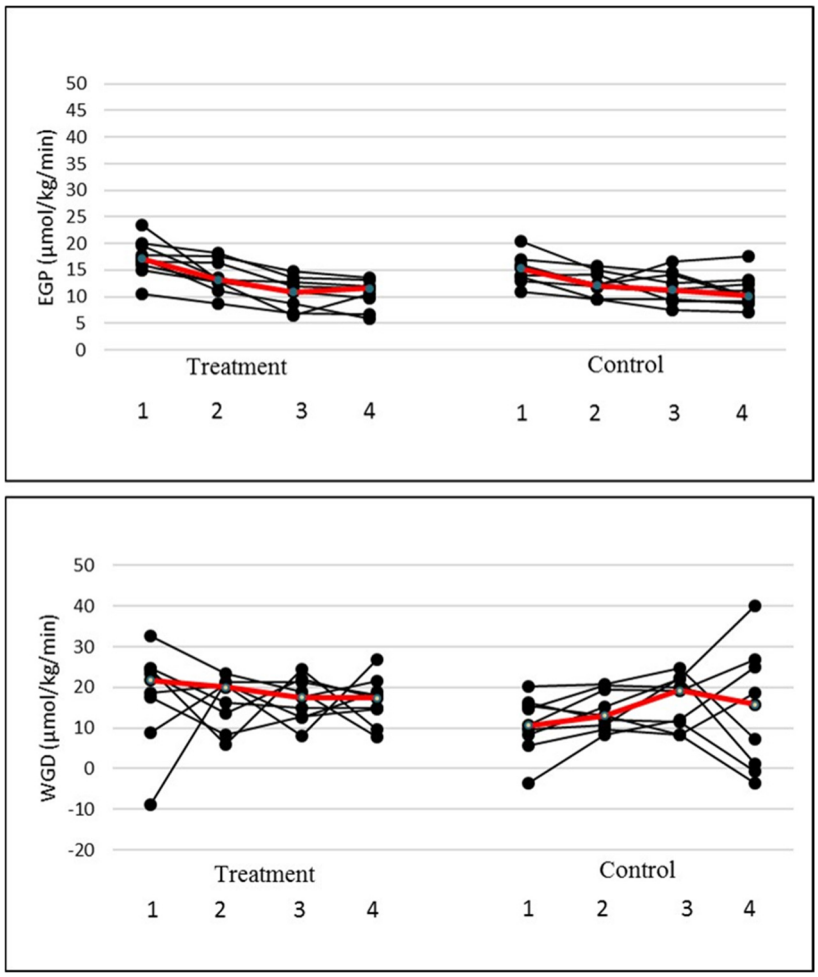

Figure 11: (Paper IV) Individual and median values (red) for intraoperative glucose kinetics, in the treatment and the control group. Numbers represent sampling at timepoints: 1. Start of Anesthesia 2. Start of Operation 3. Start of Resection 4. End of resection. Upper figure: Intraoperative values for endogenous glucose production, EGP (µmol/kg/min) (p = 0.02, ANOVA). Lower figure: Intraoperative values for the whole-body glucose disposal, WGD (µmol/kg/min) (p=0.67, ANOVA).

However, these data were derived later in the postoperative phase. In paper IV, intraoperative glucose kinetics during liver surgery revealed a decreased EGP in both groups, with a significantly higher reduction in the insulin group (p=0.02, ANOVA), see figure 11. The decline started immediately after the induction of anesthesia, though the concurrent glucose infusion, in addition with the insulin infusion, may contributed to the alterations. In contrast, WGD remained unaltered during the operation, in both groups, which is puzzling considering the fact the control group developed hyperglycemia. The findings of a reduced EGP during surgery were in line with the data from Schricker et al., where intraoperative hyperglycemia developed during colorectal surgery, despite a decreased glucose production, which was assumed a result of diminishing glucose disposal[293,294]. The latter conclusion could not be

confirmed in our data, where the measured WGD remained unchanged. The main elevation in blood glucose occurred between the start of surgery and the start of liver resection, whereas prior, and after these phases glucose level was relatively stable. Obviously, substantial alterations in glucose kinetics occurred between these time points, which we unfortunately did not measure.

14.9 LIMITATIONS

In paper I, only volunteers were included, thereby the healthy subjects all presented within the normal glucose range. Moreover, the catheters were only tested over a short time-period, so no conclusion on the long-term performance can be made, which could have implications on the accuracy or validity in an ICU-setting, where patients present a wider glucose range.

The MD-device investigated in paper II, was tested for 20 hours, with hourly sampling points, whereas a reduced sampling interval may have increased the accuracy. In addition, the measurement may have been affected by rapid changes in the concurrent glucose infusion, despite a mean distance between the catheter tips of 59 mm. The accuracy of a device with a venous positioning, compared to arterial reference sampling, can differ considerably, as Rooyackers et al. reported a limit of agreement of 12 % between arterial and venous values[258]. Moreover, of the 195 individual samples, no one was found in the hypoglycemic range, and the test-period was too short for making any further conclusions beyond that time.

In the early postoperative phase, Svanfeldt et al. demonstrated an attenuated glucose production by administration of preoperative beverage, whereas the glucose disposal remained unchanged[132]. However, in paper III, the standard routine at the hospital was to fast the patient overnight. Therefore, we chose to maintain this for the study in paper IV, though fasting time has been demonstrated to increase postoperative insulin resistance, whereas preoperative beverage has been repeatedly proven to be beneficial[123,124].

Some patients had RBC transfusions administered, which could have increased the glucose levels. Unfortunately, the timing was not noted in relation to the level of hyperglycemia. In addition, there was a mismatch in gender, though it did not seem strongly correlated to the M-ratio.

The rate of the insulin infusion was not handled according to a preset protocol, instead manually directed by the investigators, depending on blood glucose values sampled every 10 minutes. Blood glucose measurements were performed by a POC-device. Though regarded to have an inferior accuracy from a blood gas analyzer, the POC device provided swift measurements and easy handling. A correlation analysis of the plasma reference and POC values showed acceptable accuracy, $r=0.9$ ($p<0.001$), and a limit of agreement (±1.96SD CI 95%) of 22.3% (1.6 mmol/l), with a line of equality close to zero. Though it did not meet current standards for POC devices, for the tight glucose measurements required in the protocol, it was vital to have rapid handling of samples, and the trend was as important as the point values.

Though having presented significant results in paper III, we acknowledge the sample size being small. To confirm the results, we included the same number of patients in paper IV. The combined data, with a sample size of 35, improve power to find correlations between the parameters. However, we could only find significant correlations for the randomization, M-POP value and the mean glucose concentration to the relative reduction of insulin sensitivity. For other parameters, the study was underpowered, or correlations did not exist. Most clearly observed in paper IV, some patients in the control group remained fairly normoglycemic despite surgery and did not receive any insulin. This reaction could have influenced the outcome in paper IV, in which no significant difference in the M-ratio could be found.

The diabetic preoperative status[53] may predict the degree of perioperative insulin resistance. However, in paper III and IV all patients with a history of diabetes mellitus were excluded. Though, many patients received preoperative chemotherapy, which itself, or in combination with glucocorticoids, may exacerbate insulin resistance, thereby inducing overt diabetes mellitus[295]. To avoid effects from the glucocorticoid treatment, we allowed for a four-week wash-out period before inclusion. In addition, no patient presented with a glucose value >7.0 mmol/l, i.e. the limit for diabetic diagnosis, before the start of preoperative HNC.

The insulin dosage during the HNC in paper III and IV, 80 mU/m^2/min or 2.0 mU/kg/min, differed in amount from that used in studies by Thorell, Nygren and Soop et al., 0.8 mU/kg/min[39,113,126]. In contrast, other investigators have used even higher amounts during HNC[118,129,136,287]. It is assumed that EGP is suppressed at a plasma insulin level of 60 µU/ml, whereas higher levels only increases glucose disposal[270]. However, administering an insulin dosage, which rendered mean plasma insulin levels >150 µU/ml, failed in our trials to fully suppress the endogenous glucose production. This was demonstrated in several patients, which presented a M-POP of 0.1, and noted in the glucose kinetics data, where EGP was not fully inhibited. In contrast, it indicated that higher plasma insulin levels than suggested, were insufficient to completely suppress EGP in our patients, subjected to major abdominal surgery.

Regarding the glucose kinetics data, baseline values are regrettably lacking. An explanation is that the protocol, which is time and labor consuming, made recruitment challenging. In addition, since tracer sampling only occurred intermittently in four specified intraoperative episodes, certain acute changes in glucose level would be missed, if occurring between measurements.

It seems liver surgery is an immense stress for the human body. The mean M-ratio, for all patents, was 0.33±0.23, which represented a considerably higher reduction of preoperative insulin sensitivity, 67%, than previously reported in other major abdominal surgery[100]. Perhaps insulin resistance, induced by the type of surgery, is already maximized in the individual patient, and no additional parameter could have any further effect, as for example blood loss and time or size of resection. The suggestion could be exemplified by one patient in the control group, who was subjected to a minor, short-time resection, with a blood loss of 110 ml, and who displayed a M-ratio of 0.02. This means, the patient had lost 98% of its preoperative

insulin sensitivity. The identical M-ratio, 0.02, was found in another patient, subjected to a resection time of 128 min and 2200 ml of bleeding. In addition, the hypothesis can be illustrated by findings in the treatment group, where one patient had a minor resection and 550 ml of bleeding, presented a M-ratio of 0.55, while another patient which had a longer resection time, 3100 ml bleeding and required blood transfusion, ended up with an M-ratio of 0.64. Obviously, these are only examples from single patients, but illustrates well the randomness in the M-ratio in our patients.

However, despite its limitations, the combined data represent results from 35 patients subjected to liver surgery, where insulin sensitivity has been assessed by the gold standard, hyperinsulinemic normoglycemic clamp, before and after operation. Thereby it constitutes a substantial sample size for evaluating the effect of glucose control on postoperative insulin resistance. The patients were all handled according to standard routines for upper abdominal surgery in our hospital, and presented comparable baseline data.

14.10 IS THE GLUCOSE CONTROL-REGIMEN BENEFICIAL - AND FOR ALL PATIENTS?

Dysglycemia has repeatedly been associated to poor clinical outcome, and introducing glucose control has been suggested to improve both morbidity and mortality. However, an association is not the same as causality. It has been argued that non-survivors in the ICU are more prone to have higher variability, harder to keep within glycemic targets, and are more susceptible to hypoglycemia, therefore making the glucose control regimen unsafe and perhaps even unnecessary[296]. Though, recently in a retrospective study, the insulin sensitivity and its change over time, assessed by a model-based index, was analyzed in ICU patients. The trial concluded that glucose control could be doable and beneficial for clinical outcome after all, as the patients´ metabolic condition seemed of less importance, since the non-survivors and survivors had equal variability[297], whereas the non-survivors was less insulin resistant. The fact that non-survivors presented less insulin resistance, which was speculated due to a reduced immune response, suggests the belief of a "beneficial" insulin resistance and hyperglycemia, originally developed to improve survival in animals, could be a justified theory[48].

Nevertheless, by minimizing the glucose variability, while maintained in a preset glucose range, and avoiding hyper- and hypoglycemia, the clinical outcome may be improved[200,203]. A recent meta-analysis suggested a blood glucose limit of <8.3 mmol to reduce the risk for surgical site infections[298]. However, the rationale raised the risk for hypoglycemia, even though no increase in severe adverse events or mortality was presented. Instead, individualization of glucose targets, depending on premorbid glycemic status or on the reason for ICU admittance, have been suggested beneficial[164]. As mentioned, DM-patients may be adapted to higher glucose levels, [299], whereas non-DM-patients seem to be more vulnerable to hyperglycemia[60,184]. In addition, cardiac surgical patients seem to have more advantage of a tight glucose control. Consequently, by combining any optimal CGMS-systems with an adapted insulin protocol in a closed loop system, glucose control may be improved and

workload reduced. However, the new devices may also create additional costs for disposable parts. As larger studies on the effect of CGMS on clinical outcome are lacking to our knowledge, this field could be the next interesting target for the researchers.

In paper III and IV, we included only non-DM patients, or at least those assumed to be non-DM. In the first Leuven trial, non-DM post-surgical patients had the most benefit from maintaining normoglycemia, as septic episodes were reduced almost by half and markers for kidney and liver function were significantly decreased. However, those patients had substantially longer insulin treatment, whereas our intervention was terminated after the end surgery. However, Mita et al. presented positive results on postoperative kidney function using a similar period of treatment[168], while other findings of attenuated liver dysfunction and cytokine expression have been based on a prolonged treatment up to 24 hours[169,170]. In other trials, in which HNC was performed during and after cardiac surgery, an attenuated and delayed inflammatory and neuroendocrine stress response was demonstrated, until the clamp was terminated, where after the stress levels were restored[117,118]. Thus, glucose control has been associated with positive effects on postoperative myocardial and kidney function and on stress levels in general, despite insulin administration only in the intra- or in the early postoperative phase.

The current study, was only designed for detecting differences in glucose kinetics and was underpowered to reveal alterations in postoperative outcome. However, perioperative tight glucose control, 4.1-6 mmol/l, in liver surgery, has previously been demonstrated to reduce the rate of surgical infections, postoperative kidney failure and hospital-length of stay[166]. On a cellular level, insulin treatment has been associated with reduced cell apoptosis and necrosis, as well as improved liver glycogen content[169,170]. The beneficial outcome may be explained by the findings that insulin treatment was associated with improved liver dysfunction, lowered levels of transaminases and creatinine[167,168]. Our study aimed for a higher treatment target than in some of the similar trials[169,170,300], yet we displayed a mean glucose value of 8.1 mmol/l in combined control group, which is still below 10 mmol/l, a suggested limit to reduce the risk for hepatocyte injury[45]. Nevertheless, we demonstrated a significant difference in insulin resistance between the groups, and perhaps the effect would have been even stronger, with a lower glucose target in the intervention group.

The correlation was strong between the M-ratio and the randomization ($r=0.50$, $p=0.002$), which suggests insulin treatment do have an important impact on the reduction of insulin resistance. The mean and max glucose values were also strongly correlated to the M-ratio, though the values were probably a result of the insulin treatment. However, some patients in the control group "failed" to develop high glucose levels. When comparing patients, irrespectively, of randomization, which had a max glucose value of < or > 8.2 mmol/l, 5 of 14 patients within the lower group belonged to the control group. The M-ratio was significantly different between the groups, as the lower group had a M-ratio of 0.40(0.32-0.50) and the higher a M-ratio of 0.19(0.05-0.45) ($p=0.02$, Mann-Whitney). In contrast, this result indicated that maintaining normoglycemia by itself may be an important factor in the development of

postoperative insulin resistance. This suggestion is in line with the findings in a post-hoc analysis by the Leuven group, where the beneficial outcome depended on normoglycemia rather than the insulin dosage[206].

The magnitude of insulin resistance and postoperative hyperglycemia may have an important impact on the clinical outcome in general. Thorell et al. demonstrated in combined results from several studies, that postoperative insulin resistance correlated well to the hospital length of stay[99]. In addition, in a study by Umpierrez et al., cardiac patients were randomized to glucose control in range of 5.6-7.8 or 7.8-10 mmol/l, which started in the ICU and continued for 3 months[163]. The cohort of non-DM patients seemed to benefit most from the regimen, having almost 40 % less complications than the DM cohort.

In line with these findings, by implementing a near-normoglycemic target as in paper III and IV, from start from the of surgery and proceed further in the postoperative recovery phase, perhaps even maintained during the entire hospital-stay, the postoperative insulin sensitivity could be preserved better, as demonstrated in our data, and postoperative hyperglycemia reduced. As an improved glucose control, has been suggested to reduce adverse events in several patient populations, primarily in non-DM patients, this regimen could be proven beneficial for clinical outcome in liver surgery.

15 CONCLUSIONS

- The principles for intravenous microdialysis measurements of glucose concentration, are the same as for prior interstitial findings. In paper I, by reducing the velocity of the perfusion fluid, the accuracy to plasma reference values improved. By increasing the length on the semi-permeable membrane, the accuracy was additionally improved. The best agreement to reference values was demonstrated in the longer 30 mm catheter using the lower perfusion fluid rate of 0.5µl/min.

- A larger intravenous microdialysis catheter placed in a central vein, demonstrated close agreement to reference plasma during a continuous on-line measurement period of 20 hours, in patients subjected to upper major abdominal surgery.

- Intraoperative glucose kinetics, assessed by isotopicly labelled glucose, demonstrated a reduced endogenous glucose production due to perioperative glucose control using insulin, whereas peripheral glucose uptake remained unchanged. Despite the data, patients did develop hyperglycemia during surgery, which could be explained by undetected rapid changes in kinetics in-between sampling periods. Glucose kinetics during postoperative insulin reistance assessed during a hyperinsulinemic normoglycemic clamp, revealed a trend towards an increased postoperative glucose production, with an additional reduced whole-body glucose disposal. The findings suggested that the major contributor to the early postoperative insulin resistance in liver surgery was impaired peripheral glucose disposal.

- During liver surgery, irrespective the size of surgery, level of hemorrhage, blood transfusion, time of operation or resection, vasopressor therapy, gender, BMI or age, a substantial absolute and relative reduction of insulin sensitivity was demonstrated. In our study, for all patients, only 33% of preoperative insulin sensitivity were maintained. However, by maintaining glucose levels between 6-8 mmol/l under surgery, the development of postoperative insulin resistance was significantly reduced. The treatment group retained 41% and the control group 12% of the preoperative insulin sensitivity, assessed by a hyperinsulinemic normoglycemic clamp.

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