DISSERTATIONES
MEDICINAE
UNIVERSITATIS
TARTUENSIS
223

LIIVI MADDISON

Tissue perfusion and metabolism during intra-abdominal hypertension

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
223

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
223

LIIVI MADDISON

Tissue perfusion and metabolism during intra-abdominal hypertension

UNIVERSITY OF TARTU
PRESS
1632

Department of Anaesthesiology and Intensive Care, University of Tartu, Estonia

Dissertation is accepted for the commencement of the degree of Doctoral of Philosophy (in Medicine) on March 19, 2014 by the Council of the Faculty of Medicine, University of Tartu, Estonia

Supervisors: Joel Starkopf, MD, PhD, Professor of Anaesthesiology and Intensive Care
Department of Anaesthesiology and Intensive Care,
University of Tartu, Tartu University Hospital, Estonia

Juri Karjagin, MD, PhD, Senior Assistant,
Research Fellow in Anaesthsiology and Intensive Care
Department of Anaesthesiology and Intensive Care,
University of Tartu, Tartu University Hospital, Estonia

Reviewers: Aavo Lang, MD, PhD, Senior Lecturer in Human Physiology,
Department of Physiology, Institute of Biomedicine and Translational Medicine University of Tartu

Peep Talving, MD, PhD, FACS, Visiting Professor
Department of Surgery, University of Tartu,
Tartu University Hospital, North-Estonian Medical Centre

Opponent: Vladimir Cerny, MD, PhD,
Professor of Anaesthesiology and Intensive Care
Department of Anaesthesiology and Intensive Care,
Department of Research and Development
Charles University in Prague, Faculty of Medicine in Hradec Kralove,
University Hospital Hradec Kralove, Czech Republic

Adjunct Professor of Anaesthesiology
Department of Anaesthesia, Pain Management and Perioperative Medicine,
Microcirculation Diagnostics and Applied Studies Research Group
Dalhousie University, Halifax, Canada

Commencement: Linkberg's Hall, Puusepa 8, Tartu, on June 9, 2014, at 15.00

This research was supported by the European Union through the European Social Fund.

ISSN 1024–395X
ISBN 978–9949–32–543–6 (print)
ISBN 978–9949–32–544–3 (pdf)

University of Tartu Press
www.tyk.ee

In memory of my great-uncle
Jack Hansmann

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

I Maddison L, Karjagin J, Tenhunen J, Starkopf J. Moderate intra-abdominal hypertension is associated with an increased lactate-pyruvate ratio in the rectus abdominis muscle tissue: a pilot study during laparoscopic surgery. Annals of Intensive Care 2012, July 5; 2(Suppl 1), S14

II Maddison L, Riigor KM, Karjagin J, Starkopf J. Sublingual microcirculatory changes during transient intra-abdominal hypertension – a prospective observational study in laparoscopic surgery patients. Clinical Hemorheology and Microcirculation xx (20xx) x–xx. DOI 10.3233/CH-131791

III Maddison L, Karjagin J, Buldakov M, Mäll M, Kruusat R, Lillemäe K, Kirsimägi Ü and Starkopf J. Sublingual microcirculation in patients with intra-abdominal hypertension: a pilot study in 15 critically ill patients. Journal of Critical Care 2014 Feb; 29(1):183.e1–6

IV Maddison L, Karjagin J, Tenhunen J, Kirsimägi Ü, Starkopf J. Moderate intra-abdominal hypertension leads to anaerobic metabolism in the rectus abdominis muscle tissue of critically ill patients: A prospective observational study. BioMed Research International 2014, Article ID 857492, 8 pages, 2014. doi:10.1155/2014/857492

Other relevant publications:

V Maddison L, Tamme K, Kitus R, Reintam A, Starkopf J. Abdominaalse kompartmentsündroomi levimus ning ravitulemused Tartu Ülikooli Kliinikumi üldintensiivravi osakonnas aastatel 2004 kuni 2006. Eesti Arst 2009; 88(4):234−241

Contributions by Liivi Maddison. In all papers Liivi Maddison participated in the design of the study, data acquisition and analysis and wrote the first draft of the manuscript.

ABBREVIATIONS

ACS	abdominal compartment syndrome
APACHE	Acute Physiology and Chronic Health Evaluation (score)
APP	abdominal perfusion pressure
ARF	acute renal failure
ASA score	American Society of Anaesthesiologists score
AVA	automated vascular analysis
BE	base excess
BMI	body mass index (kg/m^2)
CVI	cumulative vasopressor index
CO	cardiac output
HI	heterogeneity index
IAH	intra-abdominal hypertension
IAP	intra-abdominal pressure
ICU	intensive care unit
IQR	interquartile range
L/G ratio	lactate-to-glucose ratio
L/P ratio	lactate-to-pyruvate ratio
LED	light-emitting diode
MAP	mean arterial pressure
MFI	microvascular flow index
MODS	multiple organ dysfunction syndrome
NO	nitric oxide
OPS	*Orthogonal Polarization Spectral* (imaging)
PPV	proportion of perfused vessels (%)
PVD	perfused vessel density (n/mm^2)
RAM	abdominal rectus muscle
RBC	red blood cell
ROS	reactive oxygen species
SDF	*Sidestream Dark Field* (imaging)
SMC	smooth muscle cell
SOFA	Sequential Organ Failure Assessment (score)
TVD	total vascular density (n/mm^2)
WSACS	World Society of Abdominal Compartment Syndrome

1. INTRODUCTION

Progress of modern intensive care medicine has made it possible to save more and more lives of critically ill patients. Nowadays we seldom have the patients not surviving the initial phase of illness. However, the development of multiple organ dysfunction syndrome (MODS) in the later course of the disease remains still a challenge for the caregivers and medical research. Adequate tissue perfusion and metabolism are mandatory for normal organ functioning. Hypoperfusion is the main reason for the MODS, which means that altered organ function is present in acutely ill patients so that homeostasis cannot be maintained without intervention. It usually involves two or more organ systems. MODS is a main cause of mortality in critically ill patients. Abdominal compartment syndrome (ACS) was first described as a phenomenon by Emerson in 1911 by demonstrating an association between increased intra-abdominal pressure (IAP) and decreased central venous return (Emerson, 1911). Nowadays ACS is the well-known risk factor for the development of MODS. Intra-abdominal pressure (IAP) was first measured via the urinary bladder by Kron et al., 1984; they also concluded that an IAP above 25 mmHg and low urinary output are an indication for abdominal re-exploration and decompression (Kron et al., 1984). There are several hypotheses that attempt to explain the role of intra-abdominal hypertension (IAH) in the pathogenesis of MODS: one of them is the splanchnic hypoperfusion and the subsequent mucosal ischaemia cause structural changes and alterations in cellular function (Moore, 1999). This may result in increased gut permeability, altered immune function of the gut and increased translocation of bacteria (Deitch, 1989).

Hypoperfusion is difficult to measure; one possibility is to observe the microcirculation by means of intravital microscopy. Another possibility is to measure the metabolism in the tissue of interest. Both techniques are described in experimental studies in animals and humans, but measuring hypoperfusion of splanchnic organs is still considered to be very invasive for routine clinical practice. Thus, some surrogate measuring sites have been proposed: sublingual microscopy for microcirculation and rectus abdominis muscle for metabolism measurements.

The main aim of the present dissertation was to test the hypothesis that elevated IAP is associated with tissue hypoperfusion and the prevalence of anaerobic metabolism.

2. REVIEW OF THE LITERATURE

2.1. Intra-abdominal hypertension

Intra-abdominal hypertension (IAH), sustained or repeated pathological elevation of the intra-abdominal pressure (IAP) above 12 mmHg, affects approximately one third of intensive care patients. Epidemiological studies have repeatedly shown that patients with IAH have impaired outcomes (Regueira et al., 2008; Santa Teresa et al., 2012; Reintam et al., 2008). Several clinical conditions are associated with the increased risk of IAH (Kirkpatrick et al., 2013). Obesity, positive fluid balance abdominal surgery, and pancreatitis are the most commonly appearing indipendent risk factors in different studies (Holodinsky et al., 2013).

Normal values for IAP in intensive care patients are between 5 and 7 mmHg (Kirkpatrick et al., 2013). The IAP is usually measured via a urinary bladder catheter, at end-expiration in the supine position (Malbrain, 2004), after instillation of 25 ml of sterile saline into the bladder. The mid-axillary line is taken as a zero level for IAP readings (Malbrain, 2004) and, if required, patients are given bolus sedation/relaxation to avoid excessive pressure artefacts during measurements. On elevating the head of bed from 10 to 30 to 45 degrees, IAP is increased progressively (Yi et al., 2012), and therefore a full supine position is recommended for correct measurements.

2.1.1. Abdominal perfusion pressure

The abdominal perfusion pressure (APP), calculated as the difference between the mean arterial pressure (MAP) and the IAP (APP = MAP - IAP), determines the blood flow into abdominal organs and tissues Verdant et al., 2009; Cheatham et al., 2007). The APP is suggested to be the best predictor of patient outcome when compared with MAP, IAP, arterial pH, base deficit, arterial lactate or hourly urinary output (Cheatham et al., 2000). APP has been suggested for use as a surrogate marker to assess haemodynamic changes related to IAH (Cheatham et al., 2000). A minimally sufficient level for APP is still not unequivocal; there have been suggestions to keep it at a level of above 60 mmHg at least (Malbrain et al., 2006). The latest consensus definitions and clinical practice guidelines from the World Society of Abdominal Compartment Syndrome (WSACS), however, do not recommend using APP as the resuscitation or management endpoint because of the lack of a sufficient level of positive evidence (Kirkpatrick et al., 2013).

2.1.2 Consequences of IAH

The effect of elevated IAP on end-organ function has been described repeatedly, both in experimental animals and in human studies. Among the earliest clinical signs of increased IAP are oliguria and renal dysfunction. Dalfino et al. showed that elevated IAP and low APP are factors that promote acute renal failure (ARF) in critically ill patients after shock (Dalfino et al., 2008). The risk of ARF is already increased at IAP levels above 12 mmHg (Dalfino et al., 2008; Mohmand & Goldfarb, 2011). The cardiopulmonary system is also frequently affected by IAH. Increased IAP leads to elevated diaphragms, raised intrathoracic pressures, decreased lung and chest wall compliance, and to hypoxia and hypercapnia as a result (Vegar-Brozovic et al., 2008). Its direct compression of the heart and the vena cava system elevates venous pressures and decreases heart volumes (Carlotti & Carvalho, 2009). Elevated venous pressure and increased intrathoracic pressure cause a secondary increase in intracranial pressure because of obstruction of the cerebral venous blood outflow. Therefore IAH can lead to brain oedema and ischaemia and subsequent brain deterioration and damage (Vegar-Brozovic et al., 2008).

Few data are available on splanchnic perfusion and gut microcirculation in connection with elevated abdominal pressures. In an animal model of IAH, Moore-Olufemi et al. showed that an increased IAP of 20 to 25 mmHg leads to gut oedema in spite of intact lymphatics (Moore-Olufemi et al., 2005). Doty et al. investigated whether IAH causes bacterial translocation through the gut mucosal barrier in pigs. Bacterial translocation was not convincingly proven, but significant gut mucosal acidosis and ischaemia were found to be caused by high levels (up to 30 mmHg) of IAP (Doty et al., 2002)

There are also some studies indicating involvement of cytokines in the fatal cascade leading to multiple organ failure in IAH (Avraamidou et al., 2012; Rezende-Neto et al., 2002). Whether IAH has an impact on the microcirculation is not known. The present thesis addresses this question.

2.1.3. Severity of IAH

According to its severity, IAH is graded into four levels. Grade I refers to IAP levels from 12 to 15 mmHg, Grade II 16 to 20, Grade III 21 to 25 and Grade IV above 25 mmHg, respectively. The most severe form of IAH is abdominal compartment syndrome (ACS). ACS is a life-threatening syndrome, defined as a sustained IAP $\geq$ 20 mmHg (with or without an APP of $\leq$ 60 mmHg) associated with a new onset or worsening of existing organ failure (Malbrain et al., 2006). The reported prevalence of ACS varies from 1 to 12%, depending on the study population (Vidal et al. 2008; Malbrain et al. 2005). Higher incidence has been reported in trauma and burns (Balogh et al., 2003; Balogh et al., 2003; Tuggle et al., 2007), while our studies in mixed critically ill patients have shown an incidence of approximately 2% (Reintam Blaser et al., 2013; Reintam Blaser et al., 2011; Maddison et al., 2009; Reintam et al., 2008). Awareness and

treatment of ACS has been addressed more and more frequently (Carr, 2013; Kirkpatrick et al., 2013) and, as a result, the incidence and mortality have decreased about twofold in the last decade (Carr, 2013; Reintam Blaser et al., 2013).

In contrast to ACS, the consequences of IAH grade I and II are not uniformly understood and treatment recommendations are either absent or imprecise. At the same time, most intensive care patients suffering from IAH express IAP levels between 12 and 18 mmHg, i.e., IAH Grades I and II (Malbrain et al., 2013). The present work was undertaken first of all to address the tissue perfusion and metabolism at the lower grades of IAH. We hypothesized that even a moderate increase in IAP attenuates vascular perfusion in the abdominal area with consequent disturbances in tissue metabolism. This might explain the development of organ dysfunction later in the course of IAH.

2.1.4. IAH during laparoscopic surgery

During laparoscopic surgery pneumoperitoneum is generated by insufflation of CO^2; as a result, IAP is elevated above the normal level. The European Practice Guidelines for Pneumoperitoneum in laparoscopic surgery state that IAP levels higher than 12 mmHg should be avoided and that the duration of the procedure must be kept as short as possible (Neudecker et al., 2002). Still, pneumoperitoneum serves as an excellent model for investigating the effects of mild and relatively short-time IAH on patients.

2.2. Microcirculation and IAH

2.2.1. Importance of microcirculation

The microcirculation is that part of the organism's circulation in which oxygen, nutrients and other substances are exchanged between the circulating blood and parenchymal cells (Ince, 2005). Adequate blood flow in capillaries is mandatory for normal organ perfusion and functioning (den Uil et al. 2008). In different critical conditions, an aggressive correction of volume status, perfusion pressures and cardiac output (CO) by means of volume resuscitation and vasoactive agents is the primary therapy. Still, we observe the cases where patient dies despite of clear improvement in MAP, arterial lactate, and/or venous oxygen saturation levels. Research on microcirculation has repeatedly demonstrated weak correlations of macrocirculatory indices (CO, MAP) with ones from micovasculatory beds (vascular density, proportion of perfused microvessels (PPV), etc.)(De Backer et al., 2010). Further, recently De Backer and coworkers demonstrated that characteristics of microcirculation are independent prognostic factors of outcome in critical conditions, especially in septic shock (De Backer et al., 2013). On that background it has been suggested that microcirculatory indices (PPV, microvascular flow index (MFI), etc.) rather than

macrocirculatory parameters (blood pressure, heart rate, urine output) should be used as an endpoint in shock resuscitation (Trzeciak et al., 2008; De Backer et al., 2013).

The practical problem in such approach, however, is the reliability of the methodology for the bed-side assessment of microcirculation. The sublingual region is the most readily accessible mucosal area in humans. The blood supply comes from the external carotid artery – lingual artery – sublingual artery. Only a limited number of sublingual arterioles are present, but there are many capillaries and venules.

2.2.2. Visualisation of microcirculation

In experimental cases, the intravital microscopy is regarded as the golden standard for visualization of microcirculation (Cerny, 2012). This technique makes it possible to investigate thin tissues that allow transillumination and requires fluorescent dyes (den Uil et al., 2008); therefore, it is not applicable to humans.

In clinical settings, several methods are used to assess the microcirculation. For a long time, capillary microscopy was the only method available to assess the human microcirculation. The main disadvantage was the usage of this technique only in restricted surfaces such as the skin or nailfold (Cerny, 2012). Laser-Doppler flowmetry is also an option, but it provides the flow characteristics only for local situation (den Uil et al., 2008). The human microcirculation can be visualized directly with various videomicroscopes: the first-generation device used orthogonal polarization spectral (OPS) imaging, then a more advanced device was produced – the sidestream dark field (SDF) imaging device and now the latest development of the Braedius cytocam. The main principle of these devices is basically the same, but the image quality has improved significantly with every new development. The most studied site is a sublingual microcirculation, but the microcirculation can be studied in different organs and tissues covered with an epithelial layer, and also in the microcirculation in the skin of preterm babies.

A schematic representation of the SDF is shown in Figure 1. Captured images are saved for later analysis.

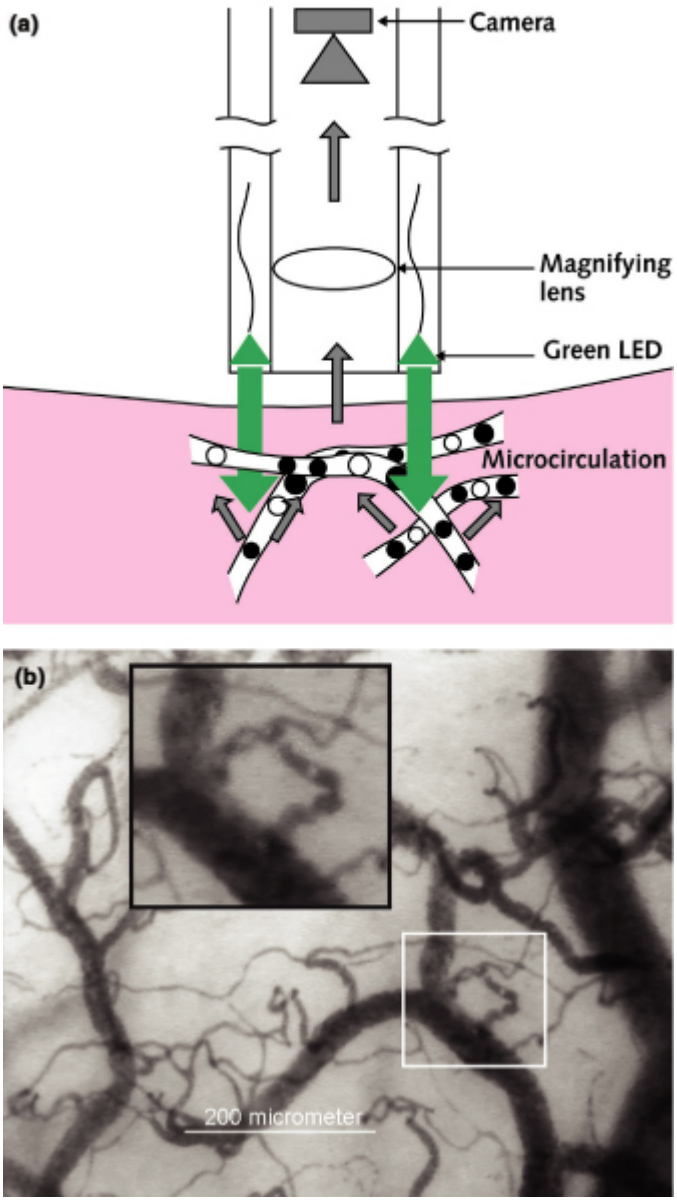


Figure 1. SDF imaging. (a) SDF imaging consists of a light guide surrounded by green light-emitting diodes (LEDs; wavelength 530 nm) whose light penetrates the tissue and illuminates the microcirculation. The light is absorbed by haemoglobin in the red blood cells and scattered by leucocytes. A magnifying lens projects the image onto a video-camera. Placed on organ surfaces, SDF imaging provides images of the red blood cells and leucocytes flowing through the microcirculation. (b) An example of the image quality provided by SDF imaging. (From Ince, 2005 with the permisson from BioMed Central; Critical Care).

2.2.3. Microcirculation, shock and multiple organ failure

Shock refers to the life-threatening medical condition characterised by inadequate oxygen transport to the tissues. As microcirculation is the part of vascular bed where gas exchange between blood and tissues occurs, one may consider the shock as a disorder of microcirculation. It has been shown that in early phases of sepsis, a persistent microcirculatory deficit is associated with a poor outcome (De Backer, 2002), and there are several pathogenic mechanisms which affect the microcirculation.

During the septic shock there is a cascade of pathophysiological mechanisms which lead to microcirculatory failure. The leukocytes and red blood cells normal functioning is disturbed (Eppihimer & Lipowsky 1994; Baskurt et al., 1998). Circulating inflammatory mediators cause vasodilatation and endothelial injury which leads to capillary leakage. The heterogeneous shutdown of capillaries occurs because of vasodilatation and endothelial injury (Ince & Sinaasappel 1999). When global flow returns after fluid resuscitation, the microcirculation becomes even more heterogeneous, probably because of inflammatory response associated with reperfusion (DeBacker 2010). Increased heterogeneity results in severe tissue hypoxia and decreased oxygen extraction (Klijn et al., 2008).

The microcirculatory changes in sepsis can occur in the presence of normal systemic haemodynamics. On the other hand, in hypovolemic shock, the microcirculation and systemic haemodynamics seem to follow each other more closely (De Backer et al., 2010). The difference is probably in the fact that autoregulatory mechanisms are still functioning in hypovolemic shock but not in sepsis (Klijn et al., 2008).

Taking together on may suggest that correction of macrocirculatory parameters in treatment of shock might be insufficient as the uncorrected microcirculation can lead to the multiple organ dysfunction (Klijn et al., 2008).

2.2.4. Microcirculation and IAH

Whether or not the splanchnic blood flow is altered at increased IAP, especially at low degrees of IAH, is not known. Direct measurements of the blood flow or gut microcirculation are not applicable in everyday clinical practice. Verdant et al. demonstrated in a porcine model of severe sepsis that the severity and time course of microcirculatory changes are similar in the sublingual and gut regions (Verdant et al., 2009). Sublingual perfusion reflects blood flow in the splanchnic region due to the same embryonic origin (Kärner, 1997) and there are studies that support this hypothesis and show a good correlation between sublingual and gastric CO^2 pressures (Marik, 2001; Jin et al., 1998). Thus we believe that the sublingual assessment of the microcirculation may be relevant in this clinical setting.

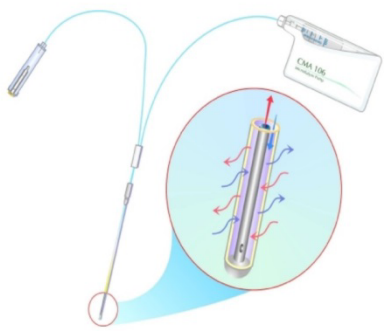
2.3. Tissue metabolism and IAH

Alterations in the organ microcirculation may lead to tissue anaerobic metabolism. Microdialysis methodology has been successfully used in animal experiments to assess metabolic changes during IAH. Meier and co-workers used a rectus abdominis muscle (RAM) tissue microdialysis in a rat model of IAH (Meier et al., 2007). The RAM is surrounded by a tight sheet of fascia, which makes the muscle-fascia compartment relatively non-compliant. Therefore, the pressure in the intra-abdominal cavity directly influences the muscle tissue and its perfusion. Recent animal experiments further support this notion and suggest that microdialysis of the RAM could serve as a readily accessible site for early detection of subclinical organ dysfunction (Benninger et al., 2012).

2.3.1. Microdialysis methodology

The microdialysis technique has been used for over 60 years. It is a minimally invasive sampling technique that is used for *in vitro* experiments and *in vivo* sampling of drugs, metabolites or endogenous substances from body fluids or from the extracellular fluid of selected tissues (Plock & Kloft, 2005).

The microdialysis system consists of the microdialysis pump, the microdialysis catheter and a microvial in which the probe is collected. The microdialysis catheter consists of a thin dialysis tube with an inner diameter in the range of 0.15–0.3 mm and a semipermeable membrane at the tip of the catheter (Plock & Kloft, 2005). A schematic representation of a microdialysis system is shown in Figure 2.



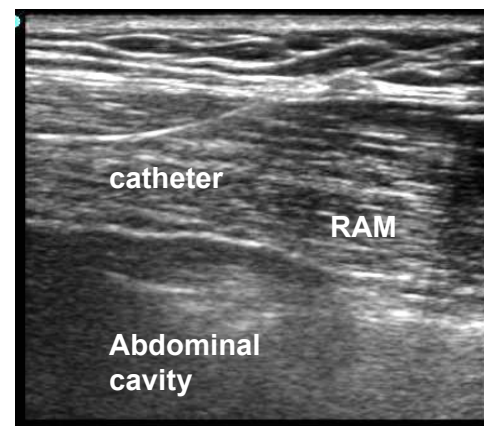


Figure 2. The microdialysis pump, the microdialysis catheter and a microvial in which the catheter is collected. On the tip of the catheter is a semipermeable membrane. Blue arrows indicate perfusate and red arrows dialysate. Ultrasound picture shows the microdialysis catheter position in RAM tissue.

The perfusion fluid runs through inner tubing at a constant rate (0.5–5 μmol/min), passes through the membrane and is then transported through the outlet tubing and collected in a microvial. The diffusion process is dependent on the concentration gradient.

2.3.2. Measured metabolites and their meaning

The main endogenous compounds and metabolites measured in the extracellular fluid in muscle tissue are glucose, pyruvate, lactate, glycerol and glutamate. To great extent they characterize the state of aerobic metabolism of the tissue the samples are drawn.

Glucose is the basic energy substrate for tissue functioning. It is broken down into pyruvate and then, through the Krebs cycle and oxidative phosphorylation, into water and carbon dioxide. This reaction requires the presence of oxygen. Under anaerobic conditions, the mitochondria are unable to oxidize pyruvate into water and carbon dioxide; therefore, the lactate concentration increases and thus lactate is a good marker of hypoxia. The tissue lactate-to-pyruvate ratio (L/P ratio) reflects a reaction to changed oxygen and glucose supplies. It is a specific marker of anaerobic conditions (Birke-Sorensen & Andersen, 2010).

Setälä et al. used microdialysis after microvascular flap transfer to monitor flap perfusion postoperatively. They found that tissue glucose and lactate concentrations, and especially their relationship (lactate-to-glucose ratio), can demonstrate the presence of ischaemia. A high lactate-to-glucose ratio can predict total or partial flap necrosis (Setälä & Gudaviciene, 2013). The mathematical ratios L/P and lactate-to-glucose (L/G) are more sensitive for differentiation between arterial and venous ischaemia than lactate, pyruvate or glucose values analysed alone (Setälä et al., 2004). Glycerol is most likely released from damaged cells due to ischaemia and tissue injury; it indicates the degradation of the glycerophospholipids of the cell membranes (Liu et al., 2010).

Glutamate is a proteinogenic amino acid which may act as a neurotransmitter or metabolic substrate. Elevated extracellular concentrations may reflect excitotoxicity and membrane breakdown (Korth et al., 2003). Glutamate release may be regarded as a surrogate of ongoing ischaemia (Kawada et al., 2005; Liu et al., 2010).

Thus, all those metabolites are relevant to investigate metabolic derangements in tissues where perfusion and/or oxygen supply is compromised.

2.4. Summary of the review of the literature

In summary, there is still a ‘grey zone’ in IAP, ranging from 12 mmHg to 18 mmHg, where the clinical consequences of IAH are not clearly evident and treatment recommendations are inconsistent. This is in contrast to ACS, the clinical course of which is unequivocally detectable and where early causative

treatment is directly connected with a positive outcome. Thus, there is still no clear trigger at which level IAP active treatment should commence. The deterioration of tissue metabolism in the abdominal area may occur well before the clinical signs of organ dysfunction related to IAH are evident. The present work was undertaken to clarify these issues.

3. AIMS

The main aim of the present study was to investigate whether intra-abdominal hypertension is associated with alterations in tissue perfusion and metabolism. We focused on the sublingual microcirculation and abdominal rectus muscle metabolism as possible sites of reflection of splanchnic perfusion, which may have deteriorated first, already from moderate, Grade I-II IAH (IAP from 12 to 18 mmHg).

The specific aims were to test the following hypotheses:

1. A mild and short-term increase in IAP during pneumoperitoneum compromises blood flow in the splanchnic area, which is reflected in in microcirculatory changes in the sublingual area.
2. Moderate and prolonged increases in IAP in critically ill patients cause alterations in the sublingual microcirculation and these changes are related to the severity of IAH.
3. Mild and short-term increases in IAP during pneumoperitoneum lead to an accumulation of metabolites of anaerobic metabolism in the RAM, which reflects insufficient tissue perfusion in the abdominal area.
4. An elevated IAP and a decreased abdominal perfusion pressure are associated with a prevalence of anaerobic metabolism in RAM tissue, thereby indicating abdominal hypoperfusion in critically ill patients.

4. MATERIALS AND METHODS

To test the above hypotheses, four subsequent studies were conducted.
Two prospective observational studies focused on microcirculatory changes:

1. Mild and short-term increases in IAP in elective laparoscopic surgery patients (Study I)
2. Moderate and prolonged increases in IAP in intensive care patients (Study II)

Two prospective observational studies investigated abdominal rectus muscle tissue metabolism:

3. Mild and short-term increases in IAP in elective laparoscopic surgery patients (Study III)
4. Moderate and prolonged increases in IAP in intensive care patients (Study IV)

All studies were approved by the University of Tartu Ethics Review Committee on Human Research (protocol nos. 170/T-11 28.04.2008 and 181/T-12 20.04. 2009) and were conducted in accordance with the Helsinki Declaration. Informed consent was obtained from the patients or from the next-of-kin of ICU patients prior to inclusion in the study.

4.1. Patients

4.1.1. Mild and short-term increases in IAP (Studies I and III)

Sixteen patients (14 females, 2 males, Table 1) scheduled for elective laparoscopic cholecystectomy were included in Study I, which investigated the sublingual microcirculation, clinical data are presented in Table 2. SDF measurements were performed as described below. The exclusion criteria were other laparoscopic surgery than cholecystectomy, refusal to participate, existing comorbidities (chronic or acute cardiac failure, ischaemic myocardial disease, coagulopathy, diabetes) and poor dental status.

Table 1. Number of patients enrolled in the studies of current work.

Table 1	Microcirculation	Tissue metabolism
Short-term increase in IAP (laparoscopic surgery patients)	16	6
Prolonged increase in IAP (ICU patients)	15	10

In Study III, six patients scheduled for elective laparoscopic gastric fundoplication were included to investigate RAM tissue metabolism. The exclusion criteria were patient's refusal to participate, BMI ≥ 32 kg/m^2 and other surgery than laparoscopic gastric fundoplication.

Pneumoperitoneum was part of the surgical procedure in both studies. IAP was held between 12 and 14 mmHg using an automated insufflator.

4.1.2. Moderate and prolong increases in IAP (Studies II and IV)

Consecutive patients admitted to the Department of General Intensive Care (ICU) at Tartu University Hospital were screened for inclusion in the study during the first three days of their treatment if they satisfied the following criteria:

– ≥ 18 years of age
– Measurement of IAP was possible (i.e., the urinary bladder catheter had been positioned)
– At least one of the following risk factors for the development/presence of IAH at admission:
  - acute pancreatitis
  - liver failure with cirrhosis and ascites
  - gastrointestinal haemorrhage
  - use of vasopressors/inotropes
  - body mass index ≥ 30 kg/m^2
  - PaO_2/FiO_2 ≤ 300 mmHg

These risk factors for IAH were previously identified by Reintam Blaser et al. in the study conducted at the same department and, therefore, the case-mix is similar and the risk factors relevant (Reintam Blaser et al., 2011).

In all screened patients, IAP was measured at intervals of at least 6 hours.

If the patient had an IAP ≥ 12 mmHg for at least 12 hours, he/she was included in the study.

Exclusion criteria were as follows: patient's or next of kin's refusal to participate, thrombocytopenia or bleeding disorders, and BMI ≥ 32 kg/m^2.

Fifteen adult, mechanically ventilated patients (4 females, 11 males) were included. In all patients, the sublingual microcirculation was measured, while microdialysis probes for RAM tissue metabolism assessment were inserted in 10 patients.

4.2. Microcirculation measurements

In 31 patients the sublingual microcirculation was visualized using an SDF imaging device (Microscan, Microvision Medical, Amsterdam, The Netherlands).

The optical probe was applied to the sublingual mucosa after removal of saliva with a gauze swab. The videos were captured with sufficient precaution

to minimize motion and pressure artefacts; altogether, nine videos were recorded at one time point.

Elective laparoscopic surgery patients (Study I) were enrolled to investigate the changes during short-term increases in IAP. Microcirculatory videos were recorded before administering anaesthesia, at least 15 minutes after the initiation of pneumoperitoneum and 1 hour after the end of pneumoperitoneum.

Intensive care patients with IAH (Study II) were included. The SDF measurements were performed at baseline and at 1, 2, 3 and 12 hours, and then twice daily for seven consecutive days or until extubation or death. The maximum number of recordings per patient was 17. For the final analysis, six time points were selected: 0, 12, 24, 48, 72 and 144 h. The SDF measurements in these patients were performed with the head of the bed elevated at least 30°.

Microcirculatory videos, each 20 to 25 s, were analysed with the aid of specialized software by two different investigators who were unaware of the patient data.

4.2.1. Microcirculatory values and indexes

Microcirculatory videos were analysed using Automated Vascular Analysis (AVA 3.02, Academic Medical Centre, University of Amsterdam, The Netherlands) software. The microcirculation cut-off value for the vessels was 20 μm.
AVA software gives the following two parameters automatically:

1) Total vascular density (TVD) – total lengths of the small vessels divided by the total area of the image (De Backer et al., 2007).
2) The De Backer Score – the density of the vessels is proportional to the number of vessels crossing arbitrary lines. In this score, three equidistant horizontal and vertical lines are drawn on the screen, and then the De Backer Score is calculated as the number of small vessels crossing the lines divided by the total length of the lines (De Backer et al., 2007).

Subsequent parameters are derived after the subjective analysis of bloodflow in the microcirculatory videos. First, perfusion is evaluated as continuous (uninterrupted flow for at least 15 seconds), sluggish (slow, but continuous flow), intermittent (no flow ≤ 50% of the time) or absent (no flow ≥ 50% of the time) (De Backer et al., 2007). The flow parameters are as follows:

3) Proportion of perfused vessels (PPV), calculated as 100 × (total number of vessels – [no flow + intermittent flow]) divided by total number of vessels (De Backer et al., 2007).
4) Perfused vessel density (PVD), calculated by multiplying the vessel density by the proportion of perfused vessels (De Backer et al., 2007).
5) Microvascular flow index (MFI) – the bloodflow is characterized as absent, 0, intermittent, 1, sluggish, 2 or normal, 3, in four separate quadrants, and then the mean is calculated (De Backer et al., 2007).
6) Heterogeneity index (HI) – calculated as the highest site flow velocity minus the lowest site flow velocity, divided by the mean flow velocity of all sublingual sites (Trzeciak et al., 2007) (De Backer et al., 2007)

4.3. Microdialysis sampling

Two hours before surgery (laparoscopic surgery patients, Study III) or at the beginning of the study (ICU patients, Study IV), the microdialysis catheter (CMA 60, Solna, Sweden) was inserted into the RAM tissue under local (2% lidocaine) or general (ICU patients) anaesthesia. The catheter was placed using ultrasound (Sonosite MicroMaxx, Bothell, WA, USA). The microdialysate perfusion rate was set at 0.3 μl/min.

In laparoscopic surgery patients the dialysate was collected hourly prior to pneumoperitoneum, during pneumoperitoneum and for 2 hours after the resolution of pneumoperitoneum.

In ICU patients the sample specimens were collected at 26 time points: hourly for the first eight hours, every two hours for the next eight hours and then every four hours until the end of the study. The duration of the study was 72 hours.

The specimens were stored in a freezer at –80°C at Tartu University Hospital in Estonia and were sent in a single shipment at the end of both studies to Tampere University Hospital in Finland for additional analysis. The glucose, lactate, pyruvate, glycerol and glutamate contents of the microdialysates were measured with a CMA 600 analyser (Solna, Sweden). The L/P ratio and the L/G ratio were calculated.

4.4. Statistical analysis

The statistical analysis was performed using GraphPad Prism 5.02 (GraphPad Software, Inc., San Diego, CA, USA), STATISTICA 10 (Software System Statsoft, Inc., Tulsa, Oklahoma, USA) and StatsDirect 2.7.9 software (StatsDirect Ltd, Cheshire, UK). Normal distribution was determined by Kolmogorov-Smirnov test.

In Study I, repeated measurements analysis of variance with Tukey's *post hoc* test was used to test the differences between the time points.

In Study II, repeated measurements analysis of variance with Dunn's *post hoc* test was used to test the differences between the time points in normally distributed data. Friedman´s test, along with Dunn's *post hoc* test, was used for repeated comparisons of non-normally distributed data. For the correlation analysis, consecutive single measurements were used. The analysis was performed using STATISTICA 10 software; correlations within the subjects (using the method of Bland and Altman) were used because of the presence of multiple measurements from one patient (Bland, MJ, 1995). To eliminate the variation caused by the subjects, we performed an analysis of covariance to evaluate the relationship between the microdialysate contents and clinical variables, all of the patients being treated simultaneously as a categorical factor. Interobserver variability was calculated separately for each parameter by means of the Bland-Altman analysis for assessing agreement between two opinions (Bland & Altman, 1986) using StatsDirect 2.7.9 software.

In Study III, the Wilcoxon matched-pairs test (non-parametric) was used to test the median differences in paired data.

In Study IV, for the RAM tissue metabolite concentrations (non-normally distributed data), Friedman's test was used to test the change over the observation period. The patients' clinical characteristics were averaged for 6 time points (baseline and from 3 to 6 hours, from 7 to 12 hours, 12 to 24 hours, 24 to 48 hours and 48 to 72 hours) and analysed using a repeated-measurements analysis of variance (normally distributed data) or Friedman's test. Dunn's test was used for the *post hoc* analysis. Correlation analyses were performed as in Study II.

The data are presented as medians with interquartile ranges (IQRs) if not stated otherwise. Differences were considered significant at $p \leq 0.05$.

5. RESULTS

5.1 Clinical characteristics

The main clinical characteristics of the study patients are shown in Table 2. All data are presented as medians (IQR). In laparoscopic surgery patients (Studies I, III), clinical data are given during the pneumoperitoneum period; in ICU patients (Studies II, IV) the clinical data describe the baseline situation.

Table 2	Study I (during pneumo-peritoneum)	Study II (baseline)	Study III (during pneumo-peritoneum)	Study IV (baseline)
Patient cohort	Laparoscopic cholecystectomy	Critically ill	Laparoscopic fundoplication	Critically ill
Patient numbers (F/M)	16 (14/2)	15 (4/11)	6 (3/3)	10 (1/9)
Intervention	SDF	SDF	Microdialysis	Microdialysis
Age (years)	52 (from 29 to 81)	67 (from 19 to 89)	36 (from 27 to 44)	65 (from 19 to 89)
ASA/APACHE II score	2 (2–3)	28 (13–36)	2 (1–2)	29 (21–37)
BMI kg/m^2	29.7 (24.9–34.7)	28.5 (24.6–33.0)	26 (23–28)	26.9 (24.6–33.0)
IAP (mmHg)	12.5 (12–13)	14.5 (12.5–16.0)	12 (12–13)	14.5 (12.5–17.8)
MAP (mmHg)	86 (69–93)	90 (78–97)	77 (74–94)	83 (75–89)
APP (mmHg)	73 (57–81)	72 (66–81)	65 (62–82)	70 (58–74)
Heart rate (beats/min)	65 (55–70)	105 (86–117)	61 (56–73)	106 (94–121)
Arterial lactate (mmol/l)		1.8 (1.3–3.4)	0.7 (0.6–1.5)	1.9 (1.3–3.75)
Arterial pH		7.38 (7.34–7.47)	7.43 (7.37–7.45)	7.42 (7.34–7.47)
Arterial BE		1.9 (-1.0–2.9)	-1.1 (-2.8–0.1)	1.9 (-1.3–2.8)
CVI		3 (0–4)		3.5 (0–4.3)
SOFA		8 (6–9)		7 (6–9)

F, female; M, male; SDF, Sidestream dark field imaging; ASA, American Society of Anesthesiologists (physical status classification system); APACHE, Acute Physiology and Chronic Health Evaluation (score); IAP, intra-abdominal pressure; MAP, mean arterial pressure; APP, abdominal perfusion pressure; CVI, cumulative vasopressor index (Trzeciak et al., 2008); SOFA, Sequential Organ Failure Assessment (score).

5.2. Arterial and intra-abdominal pressures in ICU patients (Studies II, IV)

Changes in arterial and intra-abdominal pressures in intensive care patients are shown in Figures 3 and 4.

The IAP decreased significantly in both studies (Figures 3A and 4A). While MAP remained unchanged (Figures 3B and 4B), the requirement for vasopressors decreased significantly over the time period. APP increased in microdialysis (Study IV) ($p \leq 0.03$) (Figure 4C), but not in the microcirculation study (Study II).

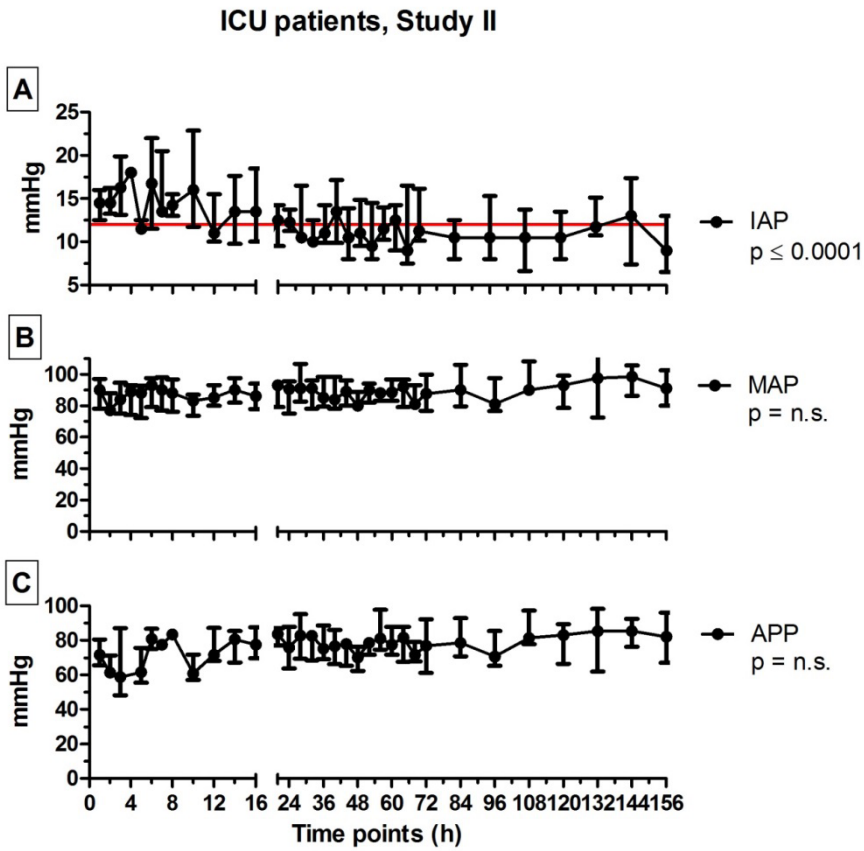


Figure 3. Changes in intra-abdominal (IAP, panel A), mean arterial (MAP, panel B) and abdominal perfusion pressures (APP, panel C) in intensive care (ICU) patients, included for assessment of the sublingual microcirculation study (n = 15, Study II). Data are presented as medians (IQR) and p-values indicate the significance of differences between the time points.

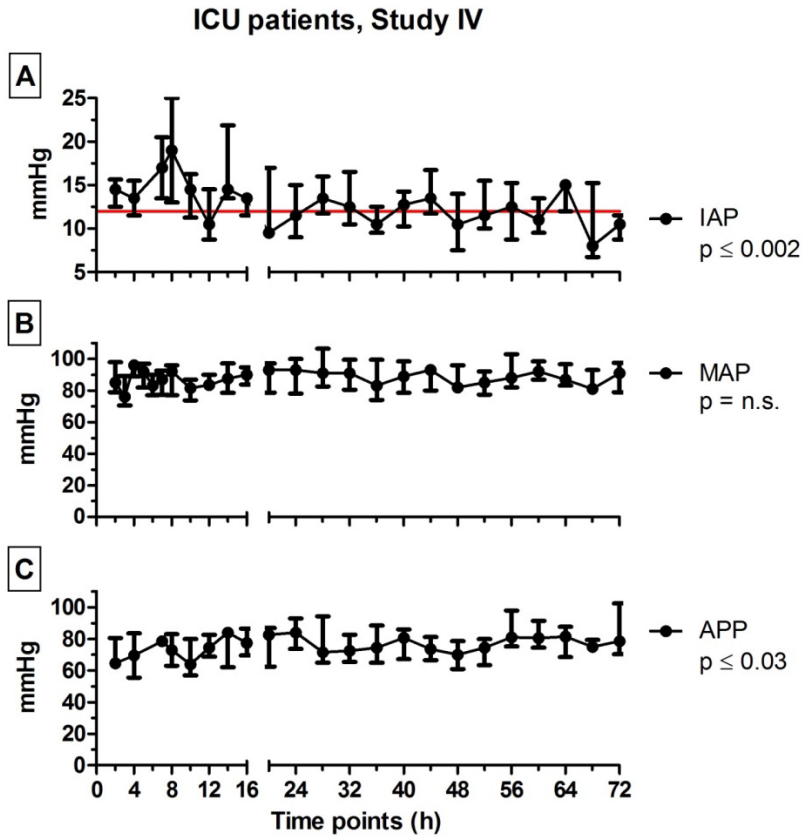


Figure 4. Changes in intra-abdominal (IAP, panel A), mean arterial (MAP, panel B) and abdominal perfusion pressures (APP, panel C) in intensive care unit (ICU) patients included for assessment of abdominal rectus muscle (RAM) tissue metabolism (n = 10, Study IV). Data are presented as medians (IQR), and p-values indicate the significance of differences between the time points.

5.3. Sublingual microcirculation (Studies I, II)

The dynamics of the sublingual microcirculation are shown in Figure 5. No significant changes were observed between the time points, neither in laparoscopic surgery nor in ICU patients. However, the overall density of microvessels, if measured before laparoscopy (Figure 5, panel A, left graph) was significantly higher than in critically ill patients at inclusion in the study (Figure 5, panel A, right graph; $p \leq 0.0001$). In contrast, the proportion of perfused sublingual microvessels (PPVs) was significantly better ($p \leq 0.0001$) in critically ill patients, which may reflect their relatively better fluid status (Figure 5, panel C). The microvascular flow index (Figure 6, panel E, right graph) in ICU patients was also significantly higher ($p \leq 0.0001$) and the flow was less heterogeneous (Figure 6, panel F; $p = 0.02$).

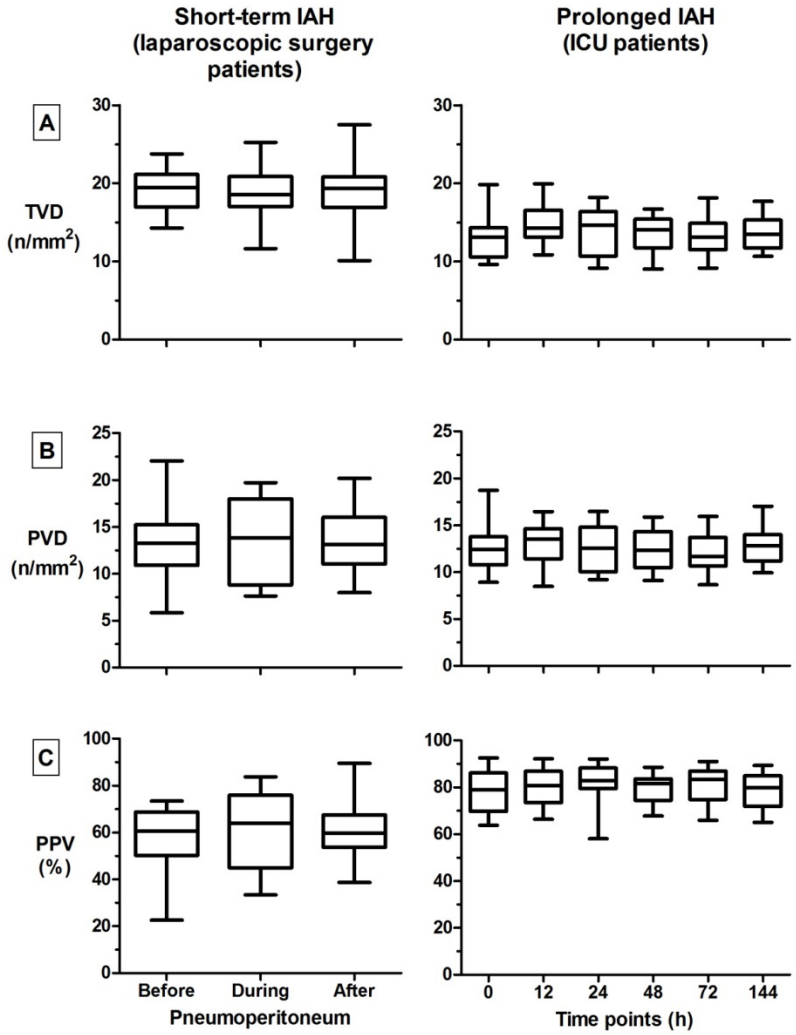


Figure 5. Dynamics of the sublingual microcirculation during the short-term increase in IAP (Study I) and during the moderate and prolonged increases of IAP (Study II). TVD indicates the total vascular density, PVD the perfused vessel density, PPV the proportion of perfused vessels. Data are presented as medians with the IQR and min-max. No differences were observed between the time points in both studies.

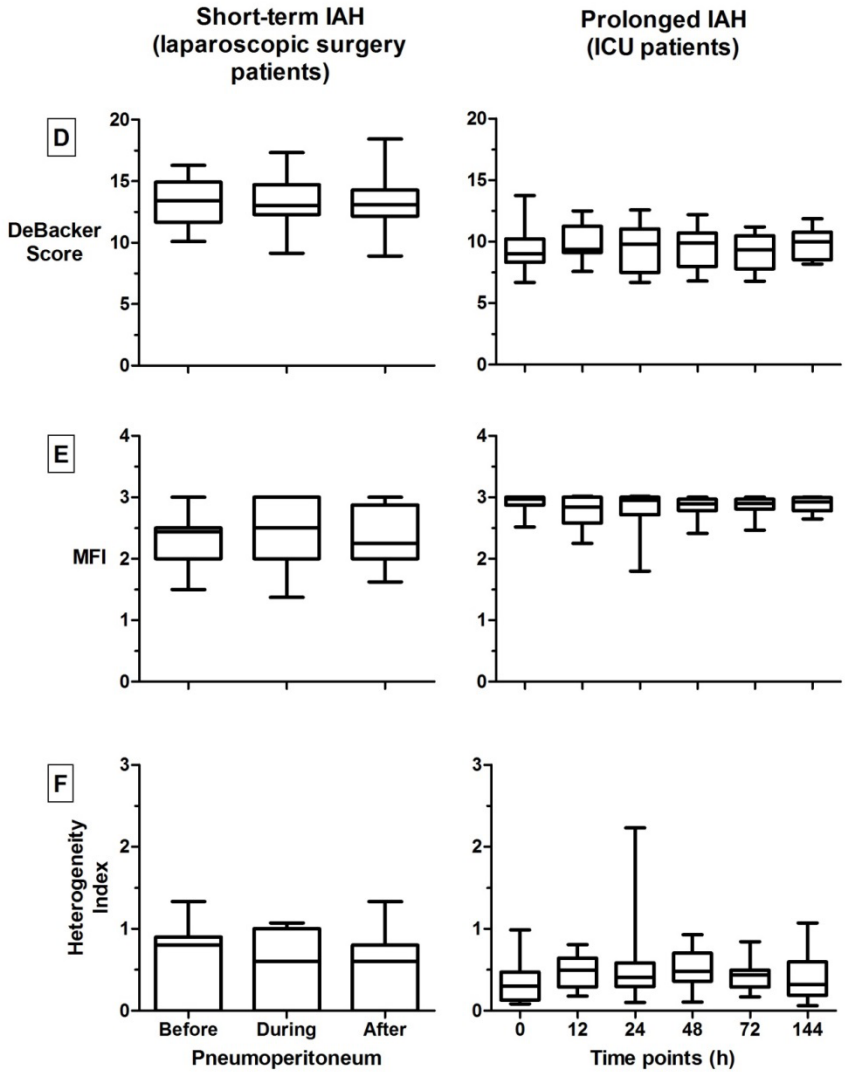


Figure 6. Dynamics of the sublingual microcirculation during the short-term increase in IAP (Study I) and during the moderate and prolonged increases of IAP (Study II). MFI indicates the microvascular flow index. Data are presented as medians with the IQR and min-max. No differences were observed between the time points in both studies.

The correlation analysis, adjusted for repeated measurements, revealed weak positive correlations between TVD and IAP, MFI and MAP and between MFI and APP (Table 3) in critically ill patients. The heterogeneity index was negatively correlated with both MAP and APP; however, no correlation was detected between microcirculatory parameters and pH, lactate or the cumulative vasopressor index (CVI) (Trzeciak et al., 2008).

Table 3. Main data of correlation analysis of sublingual microcirculatory parameters and mean arterial pressure, intra-abdominal pressure and abdominal perfusion pressure. The correlation is considered significant at $p \leq 0.05$. Only significant correlations are shown.

	MAP		IAP		APP	
	R	p	R	p	R	p
TVD	0.07	0.56	**0.29**	**0.02**	0.008	0.94
MFI	**0.23**	**0.05**	–0.15	0.22	**0.26**	**0.03**
Heterogeneity Index	**–0.29**	**0.01**	0.02	0.86	**–0.29**	**0.01**

TVD, total vascular density; MFI, microvascular flow index; MAP, mean arterial pressure; IAP, intra-abdominal pressure; APP, abdominal perfusion pressure; R, correlation coefficient.

5.4. RAM tissue metabolism during mild and short-term increase in IAP (laparoscopic surgery patients, Study III)

The dynamics of RAM tissue metabolites in laparoscopic surgery patients are shown in Figure 7. The tissue lactate (Figure 7B) and L/P ratio (Figure 7C) rose significantly after 1 hour of pneumoperitoneum; both levels returned to baseline after the cessation of pneumoperitoneum. Glucose (Figure 7A) showed a trend towards a decrease and glycerol (Figure 7C) rose during the pneumoperitoneum, but they did not reach the level of significance.

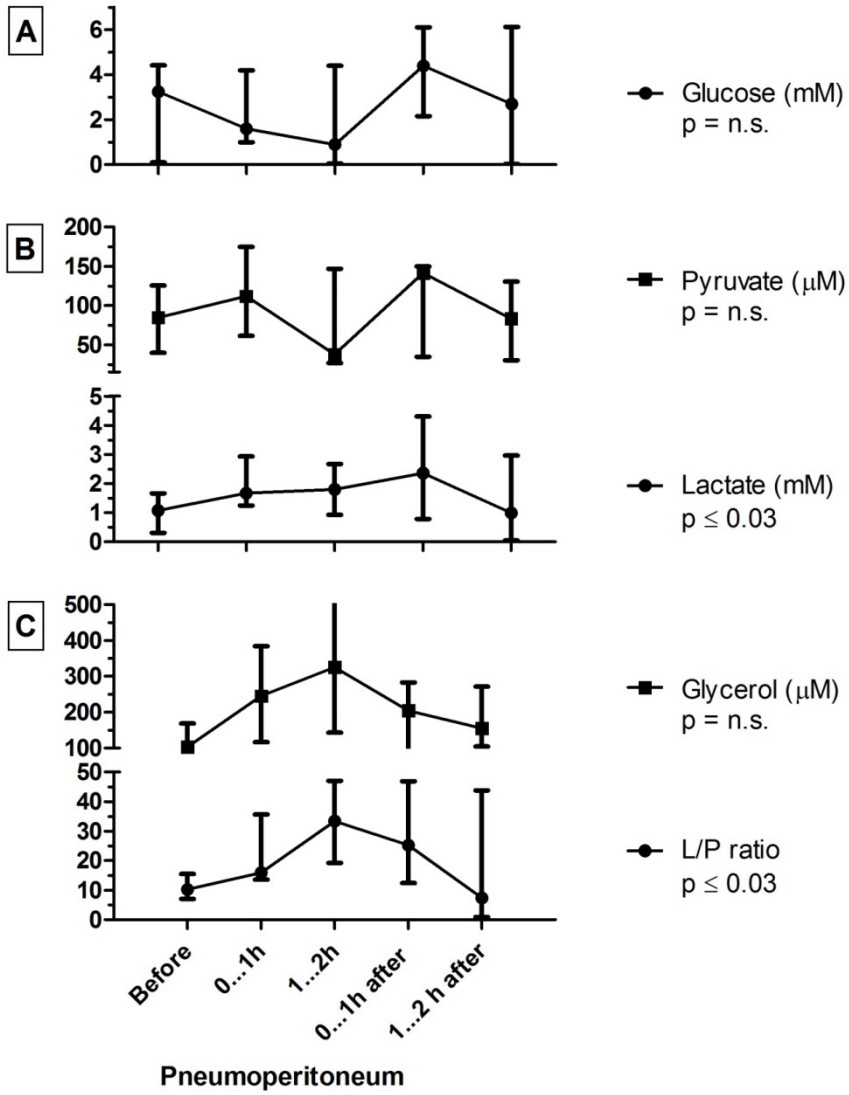


Figure 7. Dynamics of tissue metabolites in the abdominal rectus muscle during the short-term increase in IAP (laparascopic surgery, Study III, n = 6). Data are presented as medians (IQR). L/P ratio stands for lacatate-to-pyruvate ratio; L/G ratio for lactate-to-glucose ratio.

5.5. RAM tissue metabolism during moderate and prolonged increases in IAP (intensive care patients, Study IV)

The dynamics of RAM tissue metabolites in ICU patients are shown in Figure 8. Significant changes were observed in tissue lactate (Figure 8B), L/P ratio (Figure 8C) and glutamate levels (Figure 8D). Tissue pyruvate elevated at the beginning of study but this change did not reach the significance (Figure 8B). L/G ratio level (Figure 8C) changed significantly ($p = 0.003$) during first 40 hours but not then comparing the whole study period ($p = 0.08$).

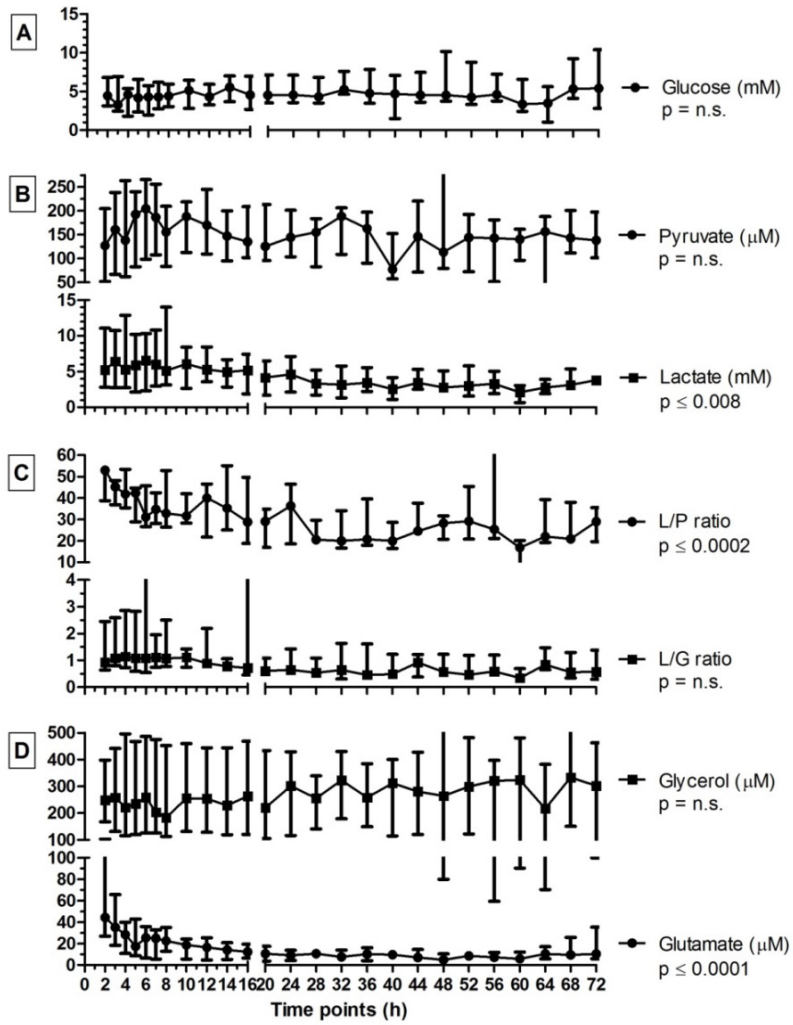


Figure 8. Dynamics of tissue metabolites in the abdominal rectus muscle during moderate and prolonged increases in IAP (ICU patients, n = 10, Study IV). Data are presented as medians (IQR). L/P ratio stands for lactate-to-pyruvate ratio; L/G ratio for lactate-to-glucose ratio.

The correlation analysis revealed an association between higher MAP and APP levels and lower tissue pyruvate concentrations and between the noradrenaline dose and RAM tissue pyruvate concentrations (Table 4). The elevated L/G ratio significantly correlated with a higher dose of noradrenaline (Table 4). There was also an association between elevated IAP levels and higher tissue glutamate concentrations (Table 4). RAM tissue glycerol concentrations did not change significantly during the study period; however, a simultaneous correlation analysis suggested that higher MAP and APP values were associated with lower glycerol concentrations (Table 4).

Table 4. Correlation analysis between RAM metabolites and mean arterial pressure (MAP), intra-abdominal pressure (IAP), abdominal perfusion pressure (APP) noradrenaline dose (μg/kg/min) and serum lactate concentration. The correlation is considered significant at $p \leq 0.05$. R stands for correlation coefficient.

	MAP		IAP		APP		Nor-adrenaline	
	R	p	R	p	R	p	R	p
Glucose	-0.04	0.5	-0.1	0.3	0.02	0.9	0.1	0.1
Pyruvate	**-0.3**	**0.0003**	-0.08	0.4	**-0.3**	**0.009**	**0.2**	**0.04**
Lactate	-0.1	0.09	-0.09	0.4	-0.4	0.3	0.1	0.08
L/P ratio	0.03	0.7	0.1	0.3	0.1	0.4	**0.2**	**0.02**
L/G ratio	0.09	0.2	-0.08	0.4	0.05	0.6	**0.6**	**0.0000**
Glycerol	**-0.2**	**0.008**	-0.04	0.7	**-0.2**	**0.03**	0.008	0.9
Glutamate	0.04	0.6	**0.2**	**0.02**	-0.02	0.8	-0.06	0.5

We also performed correlation analyses between microcirculatory parameters (Study II) and RAM tissue metabolites (Study IV), measured concomitantly in the ICU patients, but did not observed any correlation between these parameters.

6. DISCUSSION

The main finding of the present work is the prevalence of anaerobic metabolism in RAM tissue during grade I and II IAH. This result is supported by the results of tissue microdialysis during mild and moderate IAH in critically ill patients and during laparoscopic surgery. However, at the same time we could not demonstrate significant microcirculatory changes either in critically ill patients or during laparoscopic surgery. According to our results, the reason for anaerobic metabolism was not the alteration of the microcirculation.

6.1. Microcirculation

6.1.1. Mild and short-term increase in IAP (Study I)

Microcirculation measurements during laparoscopic surgery showed that the short-term elevation of the IAP caused by pneumoperitoneum has no significant effect on the sublingual microcirculation. At the same time, the microcirculatory perfusion indices were relatively low at baseline and did not improve during the study period. Based on this study, we may speculate that a short-term moderate increase in IAP has no significant impact on the splanchnic circulation.

Patients in the current study were scheduled for elective surgery; they were relatively healthy and had no serious organ dysfunctions. Their baseline PPV was, however, only 59% and remained unchanged during the pneumoperitoneum. This could be explained by preoperative stress and concomitant adrenergic stimulation. A slight improvement in PPV during anaesthesia supports this theory.

It is more likely that the relative hypovolaemia explains this observation. Patients were on a nil per os regimen from midnight prior to surgery. Considering that that they did not received intravenous fluids before the anaesthesia and many of them had surgery late in the morning or in the afternoon, dehydration could easily develop (Keane & Murray, 1986; Holte & Kehlet, 2002). Although the patients received, on an average, 1550 ml of intravenous fluids during anaesthesia and the recovery period, this did not improve their perfusion indices at all. This may suggest that perioperative fluid management was suboptimal.

Potential interference of arterial hypertension and smoking with the microcirculatory blood flow (Henriksson et al., 2012) could also be considered, but their effect is most likely negligible – the patients did not smoke for at least two hours prior to anaesthesia, while hypertension was well treated and stable in all cases.

Another factor to consider of microcirculation is volatile anaesthetics. Özarslan et al. have recently shown that isoflurane increases the microcirculatory flow after cardiopulmonary bypass in cardiac surgery patients, while desflurane has no effect and sevoflurane a negative effect on the micro-

circulation (Özarslan et al., 2012). These effects were not evident, however, if microcirculatory values after the induction of anaesthesia were compared with baseline values; therefore, it is unlikely that the choice of volatile anaesthetic has influenced the results of the present study.

6.1.2. Moderate and prolonged increases in IAP (Study II)

In line with the Study I, the microcirculation measurements in the heterogeneous group of critically ill patients revealed that moderate and prolonged increases in IAP of up to 24 hours have only a negligible influence on the sublingual microcirculation.

These patients had a median IAP of 14.5 mmHg measured in the supine position on entering the study entry and 10 out of 15 were in circulatory shock. It was somewhat unexpected that we did not detect significant alterations in the sublingual microcirculation. There are several explanations for this. First, it may appear that SDF technique used would detect only the changes in the most critical disorders. As tissue perfusion was already relatively good at study entry, no improvement can be substantiated by the further decrease in IAP. The relatively good microcirculation at study entry might also be regarded as indirect proof of an appropriate treatment of shock directed by macrocirculatory indices in our patients. Secondly, it could be that an IAP between 12 and 20 mmHg is not associated with haemodynamic changes that are transmitted to produce detectable alterations in the peripheral circulation. Relatively stable mean arterial pressures throughout the study period support this claim. On the contrary, decreases in the lactate concentrations and vasopressor requirements would indicate improved tissue perfusion during the study. Another explanation for our findings is that our patients were not recruited immediately after admittance to the ICU but, on average, on day 2 of their stay. They had all undergone primary fluid-resuscitation, but 10 out of 15 patients still needed vasopressor infusions. The main obstacles to earlier inclusion were logistical reasons, primary among which was difficulty in obtaining the desired informed consent, but, most importantly, the frequent development of IAH 24 to 72 hours after admission to the ICU, especially in cases of secondary IAH (Reintam et al., 2008), and therefore we believe the inclusion on the second day, on the average, is clinically relevant.

Based on the correlation analysis, a higher IAP indicates higher vascular density, a result that is difficult to interpret, as it is the opposite of what was expected. One possible explanation is that direct compression of the vena cava causes hyperaemia in the upper vena cava system, which may result in redistribution of the bloodflow, and, accordingly, an increased TVD.

The observed positive correlations between MFI, MAP and APP and negative correlations between HI, MAP and APP are more in accord with the idea that the microvascular bloodflow is impaired at lower levels of MAP and higher levels of IAP. The surprising result was that if we compared the previous two studies' microcirculation results, the laparoscopic surgery sublingual perfusion

was significantly altered. The overall vascular density was higher in laparoscopic surgery patients, but the perfusion indices were significantly lower. A probable explanation for this is the preoperative stress and concomitant adrenergic stimulation during anaesthesia and surgery and suboptimal fluid therapy during the perioperative period. This is in accord with Holte & Kehlet's conclusions (Holte & Kehlet, 2002).

6.2. RAM tissue metabolism

6.2.1. Mild and short-term increases in IAP (Study III)

The main finding of the microdialysis measurements during the laparoscopic surgery is that a short-term elevation of the IAP caused by pneumoperitoneum is associated with metabolic changes in the abdominal wall muscle tissue. This indicates that anaerobic metabolism and, thereby, possible RAM tissue hypoperfusion occurs after very modest increases in IAP. Several studies have shown that an increase in the mean IAP is associated with adverse ICU outcomes (Malbrain et al., 2005; Reintam Blaser et al., 2011) However, the time course of IAP-related adverse events in humans is poorly understood. To address this issue, we used microdialysis-aided sample collections from the extracellular space of the RAM. The RAM is surrounded by a tight sheet of fascia, which makes the muscle-fascia compartment relatively non-compliant. Thus, the pressure in the intra-abdominal cavity is reflected to the muscle tissue and its perfusion. Meier and co-workers used a similar microdialysis approach in a rat model of IAH (Meier et al., 2007). They showed that during a 3-h period of IAP at 20 mmHg, the L/P ratio in the RAM tissue increased significantly, indicating ischaemia and energy failure.

The results of the current laparoscopic surgery study, in which an increased L/P ratio in the RAM tissue of our patients was observed after only 1 hour of mild IAH, are surprising. However, it remains unclear whether the observed metabolic changes in RAM are directly related to the altered perfusion of intra-abdominal organs, whether they are related to the clinical outcome and whether patient treatment should be modified based on these metabolic changes. The results indicate that IAP levels of 12 mmHg are associated with unfavourable metabolic conditions, and they therefore support the recommended IAH grading (Malbrain et al., 2006). Our results also support the European practice guidelines for pneumoperitoneum in laparoscopic surgery which state that IAP levels higher than 12 mmHg should be avoided and that the duration of the procedure must be kept as short as possible (Neudecker et al., 2002).

6.2.2. Moderate and prolonged increases in IAP (Study IV)

The main finding of the microdialysis measurements during elevated IAP after initial resuscitation from shock was the prevalence of anaerobic metabolism in the RAM tissue indicates hypoperfusion in critically ill patients. Our finding of a negative correlation between APP, pyruvate and glycerol indicates a likely relevance of APP as a resuscitation end-point. The use of vasopressors contributed to keeping the MAP of our patients virtually unchanged and well above the target range of 75 mmHg. The fact that APP changed significantly and was associated with unfavourable changes supports the notion that IAP and APP should be considered when setting the targets for MAP and vasopressor therapy.

In Study III, we observed that an IAP of 12–13 mmHg led to significant elevations of the L/P ratio during the short procedure. The investigations in critically ill patients corroborate findings in laparoscopic surgery patients: the L/P ratio and tissue lactate and glutamate are significantly elevated during IAH. When we compared the present results with the absolute values of RAM metabolites that are available from clinical studies, our patients appeared to have a markedly elevated L/P ratio and lactate and glycerol levels already at the beginning of the study (Table 5).

Hörer and co-authors measured intraperitoneal lactate, pyruvate and glycerol in patients with an endovascular repair of a ruptured aortic aneurysm (Hörer et al., 2013). They observed an elevated L/P ratio and glycerol level (and IAP) in patients who were subjected to decompressive laparotomy because of clinically evident ACS. At the beginning of Study IV, 7 of the 10 patients were in shock. It is unclear from our microdialysis results whether the observed changes occurred because of shock resolution or because of dynamics in IAP and APP. The positive correlation between glutamate and IAP strongly indicates the importance of IAP in observed metabolite changes.

Table 5. Microdialysis parameters from different studies. Microdialysis catheter was placed either in RAM tissue or in peritoneal cavity (Hörer et al).

Parameter	Patient group	Baseline/at inclusion	During/max values	At end of study
IAP (mmHg)	Laparoscopic surgery	normal[(1)]	12...13[(1)]	
	IAH, decompressed	16 (CI 12–20)[(2)]	19 (CI 12–23)[(2)]	
	IAH, non-decompressed	15 (CI 11–18)[(2)]	14 (CI 7–15)[(2)]	
	ICU	14.5 (12.5–17.8)[(3)]		9.8 (9.3–14.9)[(3)]
L/P ratio	Laparoscopic surgery	10.3 (7.1–15.5)[(1)]	20.2 (13.1–45.5)[(1)]	
	IAH, decompressed	20 (CI 17–25)[(2)]	24 (CI 17–36)[(2)]	
	IAH, non-decompressed	12 (CI 11–16)[(2)]	13 (CI 10–19)[(2)]	
	ICU	49 (36–54)[(3)]		24 (22–40)[(3)]
Glucose (mM)	Laparoscopic surgery	3.3 (0.1–4.4)[(1)]	0.9 (0.1–4.4)[(1)]	
	IAH, decompressed	11.0 (8.0–14.1)[(2)]	10.0 (6.4–13.6)[(2)]	
	IAH, non-decompressed	8.7 (6.1–11.3)[(2)]	8.9 (CI 6.0–12.5)[(2)]	
	ICU	4.1 (3.2–6.5)[(3)]		4.4 (3.0–6.3)[(3)]
Lactate (mM)	Laparoscopic surgery	1.1 (0.3–1.7)[(1)#]	1.7 (1.2–2.9)[(1)#]	
	IAH, decompressed	10.1 (4.8–12.4)[(2)]	6.5 (3.7–11.2)[(2)]	
	IAH, non-decompressed	2.7 (1.7–3.4)[(2)]	2.6 (CI 1.6–4.2)[(2)]	
	ICU	4.7 (2.7–10)[(3)]		3.4 (2.3–4.6)[(3)]
Pyruvate (μM)	Laparoscopic surgery	85 (40–125)[(1)#]	112 (61–175)[(1)#]	
	ICU	129 (57–189)[(3)]		134 (75–167)[(3)]
Glycerol (μM)	Laparoscopic surgery	103 (65–169)[(1)]	326 (144–730)[(1)]	
	IAH, decompressed	274.6 (172.5–475.4)[(2)]	245.5 (117.8–512.9)[(2)]	
	IAH, non-decompressed	121.7 (62.4–212.7)[(2)]	135(CI 76.2–272.9)[(2)]	
	ICU	251 (173–408)[(3)]		330 (174–361)[(3)]

[(1)] Maddison et al., 2012, 6 patients, RAM microdialysis (MD) during elective laparoscopic surgery; [#]part of the data unpublished.

[(2)] Hörer et al., 2013, 15 patients, Intraperitoneal MD during endovascular repair of RAA, non-decompressed (9) and decompressed (6) patients, 95% CI.

[(3)] Maddison et al., 2014, 10 patients, RAM MD in critically ill patients with IAH.

6.3. Methodological considerations

6.3.1. Microcirculation studies

There were several limitations of Studies I and II: first, the reproducibility and exactness of SDF imaging in assessments of the sublingual microcirculation have been questioned (Sallisalmi et al., 2012). We carefully followed the international consensus recommendations for this technique, such as sufficient training, more than one independent assessor of videos, etc. (De Backer et al., 2007), but it still remains possible that this does not ensure a proper methodology for research. Interobserver variability showed that one observer systematically underestimated vascular density parameters and overestimated perfusion parameters or vice versa. We believe that using the median values in this case describes the real value in the best way.

The absence of baseline videos (at admission to the ICU) and a control group could be regarded as a second limitation. For logistic reasons, we were not able record admission videos for all patients screened for inclusion. However, development of IAH 24 to 72 hours after ICU admission is common, especially in cases of secondary IAH (Reintam et al., 2008), and therefore we believe the inclusion on the second day, on the average, is clinically relevant. Due to the observational nature of both studies, a control group was not considered. The microcirculatory characteristics of healthy volunteers were recently published by Vellinga et al (Vellinga et al. 2012).

The absence of cardiac output measurements for a comparative assessment of the systemic bloodflow is a third limitation of the study, especially in Study II. However, several studies have shown that relationships between the macro- and microcirculation in critically ill patients are surprisingly poor, especially after initial fluid resuscitation (De Backer et al., 2006; Arnold et al., 2012; Jhanji et al., 2010). Since the patients were enrolled in the microcirculation study after initial treatment of shock, we did not expect to see strong (IAP-related) associations between cardiac output and the sublingual microcirculation and cardiac output measurements were not implemented.

6.3.2. Tissue metabolism studies

The main limitations of both microdialysis studies include small sample size and microdialysis-related problems, specifically catheter displacement before or during laparoscopic surgery in two patients. The first limitation is expected, given that we are reporting these results as a pilot trial (Study III). The latter reflects the limitation of the method *per se*. Microdialysis is an invasive, relatively expensive and time-consuming procedure.

The second limitation was the absence of control samples of non-abdominal origin, for example, from the extremities. It would have been desirable to address this question; however, due to ethical and cost concerns, we inserted only one MD catheter per patient. Indirect support of our findings is derived

from animal experiments by Meier and Benninger, who demonstrated that other tissues/locations are not influenced by IAP as fast as RAM is (Meier et al., 2007; Benninger et al., 2012). Furthermore, the positive correlation between glutamate and IAP indicates the importance of IAP in observed metabolite changes.

7. CONCLUSIONS

1. Mild and short-term increases in IAP in elective laparoscopic surgery patients is not associated with significant changes in the sublingual microcirculation. A low proportion of perfused vessels suggests some alteration of circulation during the perioperative period.
2. Moderate and prolonged increases in IAP (IAH grades I and II (IAP 12 to 18 mmHg)) cause mild microcirculatory changes in previously resuscitated ICU patients, lasting up to 24 hours. The correlation analysis indicates better microvascular bloodflow at higher MAP and APP levels.
3. Mild and short-term increases in IAP during elective laparoscopic surgery are associated with unfavourable metabolic changes in the rectus abdominis muscle.
4. Moderate and prolonged increases in IAP lead to RAM tissue anaerobic metabolism suggestive of hypoperfusion in critically ill patients. The correlation analysis supports the concept of using APP as a primary endpoint of resuscitation in addition to MAP and IAP.

The clinical implication of the present work is that mild to moderate IAH (IAP from 12 to 18 mmHg) cannot be ignored in critically ill patients. We support the use of APP as a primary endpoint of resuscitation in addition to MAP and IAP. The feasibility of RAM microdialysis as a diagnostic tool for early-stage tissue damage related to IAH requires additional investigation. Similarly, further studies are needed to clarify whether higher levels of IAP are associated with microcirculatory changes and whether preoperative fluid therapy would improve the sublingual microcirculation in laparoscopic surgery patients.

8. REFERENCES

Arnold, R.C. et al., 2012. Discordance between microcirculatory alterations and arterial pressure in patients with hemodynamic instability. *Journal of Critical Care*, 27(5), pp. 531.e1–7.

Avraamidou, A. et al., 2012. The impact of ischemic preconditioning on hemodynamic, biochemical and inflammatory alterations induced by intra-abdominal hypertension: an experimental study in a porcine model. *Langenbeck's Archives of Surgery / Deutsche Gesellschaft für Chirurgie*, 397(8), pp. 1333–41.

De Backer, D. et al., 2007. How to evaluate the microcirculation: report of a round table conference. *Critical Care*, 11(5), p. R101.

De Backer, D. et al., 2013. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome*. *Critical Care Medicine*, 41(3), pp. 791–9.

De Backer, D., 2002. Microvascular blood flow is altered in patients with sepsis. *American Journal of Respiratory and Critical Care Medicine*, 166(1), pp. 98–104.

De Backer, D. et al., 2006. The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects*. *Critical Care Medicine*, 34(2), pp. 403–8.

De Backer, D. et al., 2010. Coupling microcirculation to systemic hemodynamics. *Current Opinion in Critical Care*, 16(3), pp. 250–4.

Balogh, Z. et al., 2003. Both primary and secondary abdominal compartment syndrome can be predicted early and are harbingers of multiple organ failure. *The Journal of Trauma*, 54(5), pp.848–59; Discussion 859–61.

Balogh, Z. et al., 2003. Supranormal trauma resuscitation causes more cases of abdominal compartment syndrome. *Archives of Surgery*, 138(6), pp.637–42; Discussion 642–3.

Baskurt, O.K., Gelmont, D. & Meiselman, H.J., 1998. Red blood cell deformability in sepsis. *American Journal of Respiratory and Critical Care Medicine*, 157(2), pp. 421–7.

Benninger, E. et al., 2012. Early detection of subclinical organ dysfunction by microdialysis of the rectus abdominis muscle in a porcine model of critical intra-abdominal hypertension. *Shock*, 38(4), pp. 420–8.

Birke-Sorensen, H. & Andersen, N.T., 2010. Metabolic markers obtained by microdialysis can detect secondary intestinal ischemia: an experimental study of ischemia in porcine intestinal segments. *World Journal of Surgery*, 34(5), pp. 923–32.

Bland, J.M. & Altman, D.G., 1986. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*, 1(8476), pp. 307–10.

Bland MJ, A.D., 1995. Calculating correlation coefficients with repeated observations: Part 1-correlation within subjects. *British Medical Journal*, 310:446

Carlotti, A.P.C.P. & Carvalho, W.B., 2009. Abdominal compartment syndrome: a review. *Pediatric Critical Care Medicine,* 10(1), pp. 115–20.

Carr, J.A., 2013. Abdominal compartment syndrome: a decade of progress. *Journal of the American College of Surgeons*, 216(1), pp. 135–46.

Cerny, V., 2012. Sublingual microcirculation. *Applied Cardiopulmonary Pathophysiology*, 16, pp. 229–48.

Cheatham, M.L. et al., 2000. Abdominal perfusion pressure: a superior parameter in the assessment of intra-abdominal hypertension. *The Journal of Trauma*, 49(4), pp. 621–6; Discussion 626–7.

Cheatham, M.L. & Malbrain, M.L.N.G., 2007. Cardiovascular implications of abdominal compartment syndrome. *Acta Clinica Belgica. Supplementum*, (1), pp. 98–112.

Dalfino, L. et al., 2008. Intra-abdominal hypertension and acute renal failure in critically ill patients. *Intensive Care Medicine*, 34(4), pp. 707–13.

Deitch, E.A., 1989. Simple intestinal obstruction causes bacterial translocation in man. *Archives of Surgery*, 124(6), p. 699.

Doty, J.M. et al., 2002. The effects of hemodynamic shock and increased intra-abdominal pressure on bacterial translocation. *The Journal of Trauma*, 52(1), pp. 13–7.

Eppihimer, M.J. & Lipowsky, H.H., 1994. Leukocyte sequestration in the microvasculature in normal and low flow states. *American Journal of Physiology - Heart and Circulatory Physiology*, 267(3), pp. H1122–34.

Emerson H., 1911. Intra-abdominal pressures. *Archives of Internal Medicine,* 7, pp. 754–84

Henriksson, P. et al., 2012. Microvascular reactivity in response to smoking and oral antioxidants in humans. *Microcirculation*, 19(1), pp. 86–93.

Holodinsky, J.K. et al., 2013. Risk factors for intra-abdominal hypertension and abdominal compartment syndrome among adult intensive care unit patients: a systematic review and meta-analysis. *Critical Care*, 17(5), p. R249

Holte, K. & Kehlet, H., 2002. Compensatory fluid administration for preoperative dehydration – does it improve outcome? *Acta Anaesthesiologica Scandinavica*, 46(9), pp. 1089–93.

Hörer, T.M. et al., 2013. Intra-peritoneal microdialysis and intra-abdominal pressure after endovascular repair of ruptured aortic aneurysms. *European Journal of Vascular and Endovascular Surgery*, 45(6), pp. 596–606.

Ince, C., 2005. The microcirculation is the motor of sepsis. *Critical Care*, 9 Suppl 4, pp. S13–9.

Ince, C. & Sinaasappel, M., 1999. Microcirculatory oxygenation and shunting in sepsis and shock. *Critical Care Medicine*, 27(7), pp. 1369–77.

Jhanji, S. et al., 2010. Haemodynamic optimisation improves tissue microvascular flow and oxygenation after major surgery: a randomised controlled trial. *Critical Care*, 14(4), p. R151.

Jin, X. et al., 1998. Decreases in organ blood flows associated with increases in sublingual PCO2 during hemorrhagic shock. *Journal of Applied Physiology*, 85(6), pp. 2360–64.

Kawada, T. et al., 2005. Myocardial interstitial choline and glutamate levels during acute myocardial ischaemia and local ouabain administration. *Acta Physiologica Scandinavica*, 184(3), pp. 187–93.

Keane, P.W. & Murray, P.F., 1986. Intravenous fluids in minor surgery. Their effect on recovery from anaesthesia. *Anaesthesia*, 41(6), pp. 635–7.

Kirkpatrick, A.W. et al., 2013. Intra-abdominal hypertension and the abdominal compartment syndrome: updated consensus definitions and clinical practice guidelines from the World Society of the Abdominal Compartment Syndrome. *Intensive Care Medicine*, pp. 1190–1206.

Klijn, E. et al., 2008. The heterogeneity of the microcirculation in critical illness. *Clinics in Chest Medicine*, 29(4), pp. 643–54.

Korth, U. et al., 2003. Intestinal ischaemia during cardiac arrest and resuscitation: comparative analysis of extracellular metabolites by microdialysis. *Resuscitation*, 58(2), pp. 209–17.

Kron, I.L. et al., 1984. The measurement of intra-abdominal pressure as a criterion for abdominal re-exploration. *Annals of Surgery*, 199(1), pp. 28–30.

Kärner J., 1997. Sissejuhatus arengubioloogiasse. Tartu: Tartu Ülikooli kirjastus.

Liu, Z. et al., 2010. Glutamate release predicts ongoing myocardial ischemia of rat hearts. *Scandinavian Journal of Clinical & Laboratory Investigation*, 70, pp. 217–24.

Maddison, L. et al., 2009. Abdominaalse kompartmentsündroomi levimus ning ravitulemused TÜ Kliinikumi üldintensiivravi osakonnas aastatel 2004–2006. Eesti Arst, 88(4), pp. 234–41.

Malbrain, M.L.N.G**.** et al., 2013. A systematic review and individual patient data meta-analysis on intra-abdominal hypertension in critically ill patients: The Wake-Up Project World Initiative on Abdominal Hypertension Epidemiology, a Unifying Project (WAKE-Up!). *Minerva Anestesiologica,* 80(3), pp. 293–306

Malbrain, M.L.N.G., 2004. Different techniques to measure intra-abdominal pressure (IAP): time for a critical re-appraisal. *Intensive Care Medicine*, 30(3), pp. 357–71.

Malbrain, M.L.N.G. et al., 2005. Incidence and prognosis of intraabdominal hypertension in a mixed population of critically ill patients: a multiple-center epidemiological study*. *Critical Care Medicine*, 33(2), pp. 315–22.

Malbrain, M.L.N.G. et al., 2006. Results from the International Conference of Experts on Intra-abdominal Hypertension and Abdominal Compartment Syndrome. I. Definitions. *Intensive Care Medicine*, 32(11), pp. 1722–32.

Marik, P.E., 2001. Sublingual capnography *. *CHEST Journal*, 120(3), p. 923.

Meier, C. et al., 2007. Microdialysis of the rectus abdominis muscle for early detection of impending abdominal compartment syndrome. *Intensive Care Medicine*, 33(8), pp. 1434–43.

Mohmand, H. & Goldfarb, S., 2011. Renal dysfunction associated with intra-abdominal hypertension and the abdominal compartment syndrome. *Journal of the American Society of Nephrology : JASN*, 22(4), pp. 615–21.

Moore, F.A., 1999. The role of the gastrointestinal tract in postinjury multiple organ failure. *American Journal of Surgery*, 178(6), pp. 449–53.

Moore-Olufemi, S.D. et al., 2005. Effects of primary and secondary intra-abdominal hypertension on mesenteric lymph flow: implications for the abdominal compartment syndrome. *Shock*, 23(6), pp. 571–5.

Neudecker, J. et al., 2002. The European Association for Endoscopic Surgery clinical practice guideline on the pneumoperitoneum for laparoscopic surgery. *Surgical Endoscopy*, 16(7), pp. 1121–43.

Plock, N. & Kloft, C., 2005. Microdialysis – theoretical background and recent implementation in applied life-sciences. *European Journal of Pharmaceutical Sciences,* 25(1), pp. 1–24.

Regueira, T. et al., 2008. Intra-abdominal hypertension: incidence and association with organ dysfunction during early septic shock. *Journal of Critical Care*, 23(4), pp. 461–7.

Reintam, A. et al., 2008. Primary and secondary intra-abdominal hypertension – different impact on ICU outcome. *Intensive Care Medicine*, 34(9), pp. 1624–31.

Reintam Blaser, A. et al., 2013. Expanded measurements of intra-abdominal pressure do not increase the detection rate of intra-abdominal hypertension: a single-center observational study. *Critical Care Medicine*, (c), pp. 3–9.

Reintam Blaser, A et al., 2011. Risk factors for intra-abdominal hypertension in mechanically ventilated patients. *Acta Anaesthesiologica Scandinavica*, 55(5), pp. 607–14.

Rezende-Neto, J.B. et al., 2002. Systemic inflammatory response secondary to abdominal compartment syndrome: stage for multiple organ failure. *The Journal of Trauma*, 53(6), pp. 1121–8.

Sallisalmi, M. et al., 2012. Evaluation of sublingual microcirculatory blood flow in the critically ill. *Acta Anaesthesiologica Scandinavica*, 56(3), pp. 298–306.

Santa-Teresa, P. et al., 2012. Incidence and prognosis of intra-abdominal hypertension in critically ill medical patients: a prospective epidemiological study. *Annals of Intensive Care*, 2 Suppl 1(Suppl 1), p.S3.

Setälä, L. & Gudaviciene, D., 2013. Glucose and lactate metabolism in well-perfused and compromised microvascular flaps. *Journal of Reconstructive Microsurgery*, 29(08), pp. 505–10.

Setälä, L.P. et al., 2004. Glucose, lactate, and pyruvate response in an experimental model of microvascular flap ischemia and reperfusion: a microdialysis study. *Microsurgery*, 24(3), pp. 223–31.

Trzeciak, S. et al., 2008. Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Intensive Care Medicine*, 34(12), pp. 2210–17.

Trzeciak, S. et al., 2007. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Annals of Emergency Medicine*, 49(1), pp. 88–98, 98.e1–2.

Tuggle, D. et al., 2007. The abdominal compartment syndrome in patients with burn injury. *Acta Clinica Belgica. Supplementum*, (1), pp.136–40.

Den Uil, C. a et al., 2008. The microcirculation in health and critical disease. *Progress in Cardiovascular Diseases*, 51(2), pp. 161–70.

Vegar-Brozovic, V., Brezak, J. & Brozovic, I., 2008. Intra-abdominal hypertension: pulmonary and cerebral complications. *Transplantation Proceedings*, 40(4), pp. 1190–2.

Vellinga, N. a R. et al., 2012. Study design of the microcirculatory shock occurrence in acutely ill Patients (microSOAP): an international multicenter observational study of sublingual microcirculatory alterations in intensive care patients. *Critical Care Research and Practice*, 2012, pp. 1–7.

Verdant, C.L. et al., 2009. Evaluation of sublingual and gut mucosal microcirculation in sepsis: a quantitative analysis. *Critical Care Medicine*, 37(11), pp .2875–81.

Vidal, M.G. et al., 2008. Incidence and clinical effects of intra-abdominal hypertension in critically ill patients. *Critical Care Medicine*, 36(6), pp. 1823–31.

Özarslan, N.G. et al., 2012. Comparison of the effects of sevoflurane, isoflurane, and desflurane on microcirculation in coronary artery bypass graft surgery. *Journal of Cardiothoracic and Vascular Anesthesia*, 26(5), pp. 791–8.

Yi, M. et al., 2012. The evaluation of the effect of body positioning on intra-abdominal pressure measurement and the effect of intra-abdominal pressure at different body positioning on organ function and prognosis in critically ill patients. *Journal of Critical Care*, 27(2), pp.222. e1–6.

SUMMARY IN ESTONIAN

Kudede perfusioon ja metabolism intra-abdominaalse hüpertensiooniga patsientidel

Intra-abdominaalne hüpertensioon (IAH) on püsiv või korduv intra-abominaalse rõhu (IAP) tõus üle 12 mmHg. IAH esineb umbes kolmandikul intensiivravi haigetel ja on seotud halvema elulemusega (Regueira et al., 2008; Santa-Teresa et al., 2012; Reintam et al., 2008).

Tõusnud IAP põhjustab erinevaid muutuseid inimorganismis. Esmaseks IAH sümptom on enamasti langenud neerude funktsioon (Dalfino et al. 2008). Samuti halvendab tõusnud IAP oluliselt südameveresoonkonna tööd, hingamisfunktsiooni (Vegar-Brozovic et al., 2008). Sekundaarselt põhjustab tõusnud IAP ka ajusisese rõhu tõusu, mis omakorda võib viia ajuturse ja tõsise ajukahjustuse tekkele (Vegar-Brozovic et al. 2008). Samuti tekib IAH ajal erinevate põletikumediaatorite vabanemine, mis võib kokkuvõtteks põhjustada hulgiorganpuudulikkuse tekke kriitilises seisundis haigel (Avraamidou et al. 2012; Rezende-Neto et al. 2002).

IAH olulisus splanhikuse piirkonna verevarustuse muutuste ning soole mikrotsirkulatsiooni häirete juures on siiani kinnitust leidnud vaid loomkatsetes (Moore-Olufemi et al. 2005; Doty et al. 2002). Siiani puuduvad andmed selle kohta, kas ja kuidas mõjutab IAH kudede mikrotsirkulatsiooni ja metabolismi inimestel.

Uurimistöö eesmärgid

Uurimistöö peamiseks eesmärgiks oli teada saada, kas IAH põhjustab verevoolu ja ainevahetuse muutusi splanhikuspiirkonna kudedes. Keskendusime oma uuringutes keelealusele mikrotsirkulatsioonile ja kõhu sirglihase metabolismile, sest mõlemad piirkonnad peegeldavad splanhikuspiirkonna verevarustust ning võiksid olla esimestena mõjutatud mõõdukast, I–II astme IAH-st (IAP vahemikus 12–18 mmHg).

Konkreetsed eesmärgid:

1. Kas kerge ja lühiaegne IAP tõus pneumoperitoneumi ajal halvendab verevoolu splanhikuspiirkonnas, mis avaldub keelealuse piirkonna mikrotsirkulatsiooni häirumises.
2. Kas mõõdukas ja pikaaegne IAP tõus kriitilises seisundis haigetel põhjustab keelealuse piirkonna mikrotsirkulatsiooni halvenemise, mis on seotud IAH raskusastmega.
3. Kas kerge ja lühiaegne IAP tõus pneumoperitoneumi ajal viib anaeroobse metabolismi produktide kuhjumisele kõhu sirglihases, mis väljendab ebapiisavat kudede verevarustust.
4. Kas tõusnud IAP ja langenud abdominaalne perfusioonirõhk (APP) on seotud anaeroobse metabolism prevaleerumisega kõhu sirglihases, mis omakorda viitab kudede hüpoperfusioonile kriitilises seisundis haigetel.

Patsiendid ja metoodika

Kokku viisime läbi 4 prospektiivset kliinilist uuringut. Kaks uuringut keskendusid keelealuse piirkonna mikrotsirkulatsiooni muutustele: 1. kerge ja lühiaegne IAP tõus plaanilise laparoskoopilise kirurgia ajal (Uuring I); 2. mõõdukas ja pikaaegne IAP tõus intensiivravi haigetel (Uuring II). Kaks uuringut keskendusid kõhu sirglihase metabolismile: 3. kerge ja lühiaegne IAP tõus plaanilise laparoskoopilise kirurgia aja (Uuring III); 4. mõõdukas ja pikaaegne IAP tõus intensiivravi haigetel (Uuring IV).

Kokku kaasasime 37 patsienti.

Mikrotsirkulatsiooni uurimiseks kasutasime *Sidestream Dark Field (SDF)* metoodikat, mis seisneb videomikroskoobiga mikrotsirkulatsiooni visualiseerimises. SDF-metoodika võimaldab uurida erinevaid kudesid, mis on kaetud õhukese epiteeli kihiga. Esimesesse uuringusse kaasasime plaanilised laparoskoopilise sapipõie kirurgia patsiendid, kellel oli operatsiooni ajal IAP lühiaegselt tõstetud 12 kuni 14 mmHg-ni. Teise uuringusse kaasasime IAH intensiivravihaiged, kes olid hospitaliseeritud SA TÜK üldintensiivravi osakonda.

Kudede metabolismi uurimiseks kasutasime mikrodialüüsi metoodikat, mis seisneb koevedeliku analüüsil. Kõikidele patsientidele sisestasime mikrodialüüsikateetri kõhu sirglihasesse ning määrasime koevedelikust hiljem glükoosi, püruvaadi, laktaadi, glütserooli, glutamaadi ning arvutasime laktaadi/püruvaadi ja laktaadi/glükoosi suhte. Esimese uuringugrupi moodustasid plaanilised laparoskoopilise fundoplikatsiooni patsiendid (IAP tõstetud operatsiooni ajal 12 kuni 14 mmHg-ni) ning teise grupi IAH-ga intensiivravi haiged.

Tulemused

Mikrotsirkulatsiooniuuringud: laparoskoopilise sapipõie kirurgia ajal ning IAH-ga intensiivravi haigetel mikrotsirkulatsioon uuringu perioodi vältel oluliselt ei muutunud. Küll aga oli laparoskoopilise kirurgia patsientide keelealuste veresoonte perfusioon oluliselt halvem, kui eelnevalt vedelikravi saanud intensiivravi haigetel. Korrelatsioonanalüüsist selgus, et kõrgem keskmine arteriaalne vererõhk (MAP) ja kõrgem APP on mõlemad seotud parema mikrovaskulaarse verevoolu indeksiga ning verevoolu väiksema heterogeensusega. Koe metabolismiuuringud: mõlemas uuringugrupis tekkis kõhu sirglihases tõusnud IAP ajal laktaat-püruvaadi suhte (L/P) oluline tõus, mis viitab anaeroobse metabolismi prevaleerumisele. Intensiivravi haigete grupis esines lisaks laktaadi ja glutamaadi tõus, mis viitab tõsisele koekahjustusele. Intensiivravi haigete grupis teostasime ka korrelatsioonianalüüsid. Nendest ilmnes, et kõrgem MAP ja APP on seotud madalama püruvaadi- ja glütseroolikontsentratsiooniga RAM-koes. Samal ajal kõrgem noradrenaliini annus viitas tõusnud püruvaadi kontsentratsioonile; kõrgemale laktaat-glükoosi suhtele, mis viitab isheemia olemasolule ning ka tõusnud L/P suhtele. Tõusnud IAP korreleerus kõrgema glutamaadi sisaldusega. Kõik eelnev kinnitab, et IAH ajal prevaleeruvub kõhu sirglihases anaeroobne metabolism ning see võiks omakorda viidata kudede hüpoperfusioonile.

Uurimuse järeldused

1. Kerge ja lühiaegne IAP tõus pneumoperitoneumi ajal ei ole seotud keelealuse piirkonna mikrotsirkulatsiooni muutuste tekkega. Vähese verevooluga veresoonte suur osakaal võiks viidata vereringe mõningasele halvenemisele perioperatiivses perioodis.
2. Mõõdukas ja pikaaegne IAP (I ja II astme IAH (IAP vahemikus 12–18 mmHg)) põhjustab kergeid mikrotsirkulatsiooni muutusi eelnevalt vedelikravi saanud kriitilises seisundis haigetel. Korrelatsioonanalüüsid viitavad sellele, et mikrovaskulaarne verevool on seda parem, mida kõrgem on keskmine arteriaalne vererõhk (MAP) ja APP.
3. Kerge ja lühiaegne IAP tõus plaanilise laparoskoopilise fundoplikatsiooni ajal viib kõhu sirglihase metabolismi halvenemisele.
4. Mõõdukas ja pikaaegne IAP viib anaeroobse metabolismi prevaleerumisele kõhu sirglihases kriitilises seisundis haigetel, mis omakorda viitab võimalikule kudede hüpoperfusioonile. Korrelatsioonanalüüsid toetavad põhimõtet, et APP normaliseerimine peaks olema esmaseks ravieesmärgiks lisaks senisele MAP-ile ja IAP-le.

Antud uurimuse peamine kliiniline olulisus on see, et kerget kuni mõõdukat IAH ei tohi kriitilises seisundis haigetel ignoreerida. Piisava APP tagamine on meie tulemuste põhjal oluline osa selliste haigete esmasest ravist.

Kindlasti on vajalikud edasised uuringud selgitamaks, kas kliinilises praktikas oleks kõhu sirglihase mikrodialüüs varajane diagnostiline vahend IAH-ga seotud koekahjustuse tekke identifitseerimisel. Samuti on vajalikud täpsemad uuringud selleks, et selgitada kas kõrgemad IAP tasemed on seotud mikrotsirkulatsiooni muutustega ning kas operatsioonieelne vedelikravi parandab keelealust mikrotsirkulatsiooni laparoskoopilise kirurgia haigetel.

ACKNOWLEDGEMENTS

This work was performed at the Department of Anaesthesiology and Intensive Care, Tartu University Hospital. The research was supported in part, by the Estonian Science Foundation Grants 8717 and 7761, target financing from the Ministry of Education and Science of Estonia (SF0180004s12) and external funding for Critical Care Medicine Research Group in Tampere (J.T.) and also by the European Union through the European Social Fund.

The publications presented in this thesis are based on teamwork, with the contributions of many people to whom I would like to express my sincere gratitude.

In particular, I would like to thank the following people:

- My supervisors, Professor Joel Starkopf and Juri Karjagin for inspiring and supporting and encouraging me all this time and most of all for their patience
- All the people working in the General ICU of Tartu University Hospital, especially the nurses, who helped me with microdialysis probing
- Kadri, Kairi-Marie, Maksim, Merilin and Rein for their huge contribution on microcirculation recordings and analysis.
- Ülle Kirsimägi for her time and patience with all that statistics
- My colleges at the Department of Neuro-Intensive Care for their understanding and friendliness, especially my chief Veronika for providing such a good possibilities for writing these thesis
- My babysitters, Mirjam and Greete for giving me free time for science then I needed it most
- My mother and father, for demanding more from me and for your support in all the things I have ever done
- My brother and sister for being as they are and understanding my efforts.

Finally, and above all, I would like to thank my family, my husband Martin and my children Helena and Rasmus for their love, patience, support and understanding through all those years.

PUBLICATIONS

CURRICULUM VITAE

Name: Liivi Maddison (formerly Liivlaid)
Date of birth: 9 May, 1980, Haapsalu, Estonia
Citizenship: Estonia
Telephone: +372 522 9094
E-mail: Liivi.Maddison@kliinikum.ee

Education:

1986–1998	Haapsalu High School
1998–2004	University of Tartu, Faculty of Medicine, MD
2004–2008	University of Tartu, Residentship in anaesthesiology and intensive care
2008–2014	University of Tartu, Faculty of Medicine, Anaesthesiology and Intensive Care Clinic, PhD student

Professional career:

2004–2008	Tartu University Hospital, resident physician
2004–2008	Tartu Emergency Medicine Service, physician
2008–	Tartu University Hospital, Anaesthesiology and Intensive Care Clinic, Department of Neurointensive Care, anaesthesiologist
2014–	Tartu University Hospital, Anaesthesiology and Intensive Care Clinic, Department of General Anaesthesia, anaesthesiologist

Scientific work:
Researsh fields: pathophysiology of intra-abdominal hypertension, microcirculatory changes in critically ill and surgical patients, microdialysis as a diagnostic tool in critical care settings

Memberships:
- Estonian Society of Anaesthesiologists
- Medical Association of Tartu
- International MiDAS Group
- The Doctoral School of Clinical Medicine

ELULOOKIRJELDUS

Nimi: Liivi Maddison (end Liivlaid)
Sünniaeg: Haapsalus, 9. mai 1980
Kodakondsus: Eesti
Telefon: 522 9094
E-post: Liivi.Maddison@kliinikum.ee

Haridus:

1986–1998	Haapsalu Gümnaasium
1998–2004	Tartu Ülikool, arstiteaduskond, arstiteaduse eriala MD
2004–2008	Tartu Ülikool, arstiteaduskond, anestesioloogia ja intensiivravi residentuur
2008–2014	Tartu Ülikool, arstiteaduskond, doktorantuur

Teenistuskäik:

2004–2008	SA Tartu Ülikooli Kliinikum, arst-resident
2004–2008	SA Tartu Kiirabi, kiirabiarst
2008–	SA Tartu Ülikooli Kliinikum, anestesioloogia ja intensiivravi kliinik, neurointensiivravi osakond, arst-õppejõud
2014–	SA Tartu Ülikooli Kliinikum, anestesioloogia ja intensiivravi kliinik, üldanestesioloogia osakond, arst-õppejõud

Teadustegevus:
Peamised uurimisvaldkonnad: intra-abdominaalse hüpertensiooni patofüsioloogia, mikrotsirkulatsioonimuutused intensiivravi ja kirurgilistel haigetel, mikrodialüüsi metoodika kasutamine intensiivravis

Kuuluvus erialaseltsidesse:
- Eesti Anestesioloogide Seltsi liige
- Tartu Arstide Liidu liige
- Rahvusvahelise töögrupi MiDAS (Microcirculation Diagnostics and Applied Studies) liige
- Kliinilise meditsiini doktorikooli liige

DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS

1. **Heidi-Ingrid Maaroos.** The natural course of gastric ulcer in connection with chronic gastritis and *Helicobacter pylori*. Tartu, 1991.
2. **Mihkel Zilmer.** Na-pump in normal and tumorous brain tissues: Structural, functional and tumorigenesis aspects. Tartu, 1991.
3. **Eero Vasar.** Role of cholecystokinin receptors in the regulation of behaviour and in the action of haloperidol and diazepam. Tartu, 1992.
4. **Tiina Talvik.** Hypoxic-ischaemic brain damage in neonates (clinical, biochemical and brain computed tomographical investigation). Tartu, 1992.
5. **Ants Peetsalu.** Vagotomy in duodenal ulcer disease: A study of gastric acidity, serum pepsinogen I, gastric mucosal histology and *Helicobacter pylori*. Tartu, 1992.
6. **Marika Mikelsaar.** Evaluation of the gastrointestinal microbial ecosystem in health and disease. Tartu, 1992.
7. **Hele Everaus.** Immuno-hormonal interactions in chronic lymphocytic leukaemia and multiple myeloma. Tartu, 1993.
8. **Ruth Mikelsaar.** Etiological factors of diseases in genetically consulted children and newborn screening: dissertation for the commencement of the degree of doctor of medical sciences. Tartu, 1993.
9. **Agu Tamm.** On metabolic action of intestinal microflora: clinical aspects. Tartu, 1993.
10. **Katrin Gross.** Multiple sclerosis in South-Estonia (epidemiological and computed tomographical investigations). Tartu, 1993.
11. **Oivi Uibo.** Childhood coeliac disease in Estonia: occurrence, screening, diagnosis and clinical characterization. Tartu, 1994.
12. **Viiu Tuulik.** The functional disorders of central nervous system of chemistry workers. Tartu, 1994.
13. **Margus Viigimaa.** Primary haemostasis, antiaggregative and anticoagulant treatment of acute myocardial infarction. Tartu, 1994.
14. **Rein Kolk.** Atrial versus ventricular pacing in patients with sick sinus syndrome. Tartu, 1994.
15. **Toomas Podar.** Incidence of childhood onset type 1 diabetes mellitus in Estonia. Tartu, 1994.
16. **Kiira Subi.** The laboratory surveillance of the acute respiratory viral infections in Estonia. Tartu, 1995.
17. **Irja Lutsar.** Infections of the central nervous system in children (epidemiologic, diagnostic and therapeutic aspects, long term outcome). Tartu, 1995.
18. **Aavo Lang.** The role of dopamine, 5-hydroxytryptamine, sigma and NMDA receptors in the action of antipsychotic drugs. Tartu, 1995.
19. **Andrus Arak.** Factors influencing the survival of patients after radical surgery for gastric cancer. Tartu, 1996.
20. **Tõnis Karki.** Quantitative composition of the human lactoflora and method for its examination. Tartu, 1996.

21. **Reet Mändar.** Vaginal microflora during pregnancy and its transmission to newborn. Tartu, 1996.
22. **Triin Remmel.** Primary biliary cirrhosis in Estonia: epidemiology, clinical characterization and prognostication of the course of the disease. Tartu, 1996.
23. **Toomas Kivastik.** Mechanisms of drug addiction: focus on positive reinforcing properties of morphine. Tartu, 1996.
24. **Paavo Pokk.** Stress due to sleep deprivation: focus on $GABA_A$ receptor-chloride ionophore complex. Tartu, 1996.
25. **Kristina Allikmets.** Renin system activity in essential hypertension. Associations with atherothrombogenic cardiovascular risk factors and with the efficacy of calcium antagonist treatment. Tartu, 1996.
26. **Triin Parik.** Oxidative stress in essential hypertension: Associations with metabolic disturbances and the effects of calcium antagonist treatment. Tartu, 1996.
27. **Svetlana Päi.** Factors promoting heterogeneity of the course of rheumatoid arthritis. Tartu, 1997.
28. **Maarike Sallo.** Studies on habitual physical activity and aerobic fitness in 4 to 10 years old children. Tartu, 1997.
29. **Paul Naaber.** *Clostridium difficile* infection and intestinal microbial ecology. Tartu, 1997.
30. **Rein Pähkla.** Studies in pinoline pharmacology. Tartu, 1997.
31. **Andrus Juhan Voitk.** Outpatient laparoscopic cholecystectomy. Tartu, 1997.
32. **Joel Starkopf.** Oxidative stress and ischaemia-reperfusion of the heart. Tartu, 1997.
33. **Janika Kõrv.** Incidence, case-fatality and outcome of stroke. Tartu, 1998.
34. **Ülla Linnamägi.** Changes in local cerebral blood flow and lipid peroxidation following lead exposure in experiment. Tartu, 1998.
35. **Ave Minajeva.** Sarcoplasmic reticulum function: comparison of atrial and ventricular myocardium. Tartu, 1998.
36. **Oleg Milenin.** Reconstruction of cervical part of esophagus by revascularised ileal autografts in dogs. A new complex multistage method. Tartu, 1998.
37. **Sergei Pakriev.** Prevalence of depression, harmful use of alcohol and alcohol dependence among rural population in Udmurtia. Tartu, 1998.
38. **Allen Kaasik.** Thyroid hormone control over β-adrenergic signalling system in rat atria. Tartu, 1998.
39. **Vallo Matto.** Pharmacological studies on anxiogenic and antiaggressive properties of antidepressants. Tartu, 1998.
40. **Maire Vasar.** Allergic diseases and bronchial hyperreactivity in Estonian children in relation to environmental influences. Tartu, 1998.
41. **Kaja Julge.** Humoral immune responses to allergens in early childhood. Tartu, 1998.
42. **Heli Grünberg.** The cardiovascular risk of Estonian schoolchildren. A cross-sectional study of 9-, 12- and 15-year-old children. Tartu, 1998.

43. **Epp Sepp.** Formation of intestinal microbial ecosystem in children. Tartu, 1998.
44. **Mai Ots.** Characteristics of the progression of human and experimental glomerulopathies. Tartu, 1998.
45. **Tiina Ristimäe.** Heart rate variability in patients with coronary artery disease. Tartu, 1998.
46. **Leho Kõiv.** Reaction of the sympatho-adrenal and hypothalamo-pituitary-adrenocortical system in the acute stage of head injury. Tartu, 1998.
47. **Bela Adojaan.** Immune and genetic factors of childhood onset IDDM in Estonia. An epidemiological study. Tartu, 1999.
48. **Jakov Shlik.** Psychophysiological effects of cholecystokinin in humans. Tartu, 1999.
49. **Kai Kisand.** Autoantibodies against dehydrogenases of α-ketoacids. Tartu, 1999.
50. **Toomas Marandi.** Drug treatment of depression in Estonia. Tartu, 1999.
51. **Ants Kask.** Behavioural studies on neuropeptide Y. Tartu, 1999.
52. **Ello-Rahel Karelson.** Modulation of adenylate cyclase activity in the rat hippocampus by neuropeptide galanin and its chimeric analogs. Tartu, 1999.
53. **Tanel Laisaar.** Treatment of pleural empyema — special reference to intrapleural therapy with streptokinase and surgical treatment modalities. Tartu, 1999.
54. **Eve Pihl.** Cardiovascular risk factors in middle-aged former athletes. Tartu, 1999.
55. **Katrin Õunap.** Phenylketonuria in Estonia: incidence, newborn screening, diagnosis, clinical characterization and genotype/phenotype correlation. Tartu, 1999.
56. **Siiri Kõljalg.** *Acinetobacter* – an important nosocomial pathogen. Tartu, 1999.
57. **Helle Karro.** Reproductive health and pregnancy outcome in Estonia: association with different factors. Tartu, 1999.
58. **Heili Varendi.** Behavioral effects observed in human newborns during exposure to naturally occurring odors. Tartu, 1999.
59. **Anneli Beilmann.** Epidemiology of epilepsy in children and adolescents in Estonia. Prevalence, incidence, and clinical characteristics. Tartu, 1999.
60. **Vallo Volke.** Pharmacological and biochemical studies on nitric oxide in the regulation of behaviour. Tartu, 1999.
61. **Pilvi Ilves.** Hypoxic-ischaemic encephalopathy in asphyxiated term infants. A prospective clinical, biochemical, ultrasonographical study. Tartu, 1999.
62. **Anti Kalda.** Oxygen-glucose deprivation-induced neuronal death and its pharmacological prevention in cerebellar granule cells. Tartu, 1999.
63. **Eve-Irene Lepist.** Oral peptide prodrugs – studies on stability and absorption. Tartu, 2000.
64. **Jana Kivastik.** Lung function in Estonian schoolchildren: relationship with anthropometric indices and respiratory symptomas, reference values for dynamic spirometry. Tartu, 2000.

65. **Karin Kull.** Inflammatory bowel disease: an immunogenetic study. Tartu, 2000.
66. **Kaire Innos.** Epidemiological resources in Estonia: data sources, their quality and feasibility of cohort studies. Tartu, 2000.
67. **Tamara Vorobjova.** Immune response to *Helicobacter pylori* and its association with dynamics of chronic gastritis and epithelial cell turnover in antrum and corpus. Tartu, 2001.
68. **Ruth Kalda.** Structure and outcome of family practice quality in the changing health care system of Estonia. Tartu, 2001.
69. **Annika Krüüner.** *Mycobacterium tuberculosis* – spread and drug resistance in Estonia. Tartu, 2001.
70. **Marlit Veldi.** Obstructive Sleep Apnoea: Computerized Endopharyngeal Myotonometry of the Soft Palate and Lingual Musculature. Tartu, 2001.
71. **Anneli Uusküla.** Epidemiology of sexually transmitted diseases in Estonia in 1990–2000. Tartu, 2001.
72. **Ade Kallas.** Characterization of antibodies to coagulation factor VIII. Tartu, 2002.
73. **Heidi Annuk.** Selection of medicinal plants and intestinal lactobacilli as antimicrobil components for functional foods. Tartu, 2002.
74. **Aet Lukmann**. Early rehabilitation of patients with ischaemic heart disease after surgical revascularization of the myocardium: assessment of health-related quality of life, cardiopulmonary reserve and oxidative stress. A clinical study. Tartu, 2002.
75. **Maigi Eisen.** Pathogenesis of Contact Dermatitis: participation of Oxidative Stress. A clinical – biochemical study. Tartu, 2002.
76. **Piret Hussar.** Histology of the post-traumatic bone repair in rats. Elaboration and use of a new standardized experimental model – bicortical perforation of tibia compared to internal fracture and resection osteotomy. Tartu, 2002.
77. **Tõnu Rätsep.** Aneurysmal subarachnoid haemorrhage: Noninvasive monitoring of cerebral haemodynamics. Tartu, 2002.
78. **Marju Herodes.** Quality of life of people with epilepsy in Estonia. Tartu, 2003.
79. **Katre Maasalu.** Changes in bone quality due to age and genetic disorders and their clinical expressions in Estonia. Tartu, 2003.
80. **Toomas Sillakivi.** Perforated peptic ulcer in Estonia: epidemiology, risk factors and relations with *Helicobacter pylori*. Tartu, 2003.
81. **Leena Puksa.** Late responses in motor nerve conduction studies. F and A waves in normal subjects and patients with neuropathies. Tartu, 2003.
82. **Krista Lõivukene**. *Helicobacter pylori* in gastric microbial ecology and its antimicrobial susceptibility pattern. Tartu, 2003.
83. **Helgi Kolk.** Dyspepsia and *Helicobacter pylori* infection: the diagnostic value of symptoms, treatment and follow-up of patients referred for upper gastrointestinal endoscopy by family physicians. Tartu, 2003.

84. **Helena Soomer.** Validation of identification and age estimation methods in forensic odontology. Tartu, 2003.
85. **Kersti Oselin.** Studies on the human MDR1, MRP1, and MRP2 ABC transporters: functional relevance of the genetic polymorphisms in the *MDR1* and *MRP1* gene. Tartu, 2003.
86. **Jaan Soplepmann.** Peptic ulcer haemorrhage in Estonia: epidemiology, prognostic factors, treatment and outcome. Tartu, 2003.
87. **Margot Peetsalu.** Long-term follow-up after vagotomy in duodenal ulcer disease: recurrent ulcer, changes in the function, morphology and *Helicobacter pylori* colonisation of the gastric mucosa. Tartu, 2003.
88. **Kersti Klaamas.** Humoral immune response to *Helicobacter pylori* a study of host-dependent and microbial factors. Tartu, 2003.
89. **Pille Taba.** Epidemiology of Parkinson's disease in Tartu, Estonia. Prevalence, incidence, clinical characteristics, and pharmacoepidemiology. Tartu, 2003.
90. **Alar Veraksitš**. Characterization of behavioural and biochemical phenotype of cholecystokinin-2 receptor deficient mice: changes in the function of the dopamine and endopioidergic system. Tartu, 2003.
91. **Ingrid Kalev.** CC-chemokine receptor 5 (CCR5) gene polymorphism in Estonians and in patients with Type I and Type II diabetes mellitus. Tartu, 2003.
92. **Lumme Kadaja.** Molecular approach to the regulation of mitochondrial function in oxidative muscle cells. Tartu, 2003.
93. **Aive Liigant**. Epidemiology of primary central nervous system tumours in Estonia from 1986 to 1996. Clinical characteristics, incidence, survival and prognostic factors. Tartu, 2004.
94. **Andres, Kulla.** Molecular characteristics of mesenchymal stroma in human astrocytic gliomas. Tartu, 2004.
95. **Mari Järvelaid.** Health damaging risk behaviours in adolescence. Tartu, 2004.
96. **Ülle Pechter.** Progression prevention strategies in chronic renal failure and hypertension. An experimental and clinical study. Tartu, 2004.
97. **Gunnar Tasa.** Polymorphic glutathione S-transferases – biology and role in modifying genetic susceptibility to senile cataract and primary open angle glaucoma. Tartu, 2004.
98. **Tuuli Käämbre.** Intracellular energetic unit: structural and functional aspects. Tartu, 2004.
99. **Vitali Vassiljev.** Influence of nitric oxide syntase inhibitors on the effects of ethanol after acute and chronic ethanol administration and withdrawal. Tartu, 2004.
100. **Aune Rehema.** Assessment of nonhaem ferrous iron and glutathione redox ratio as markers of pathogeneticity of oxidative stress in different clinical groups. Tartu, 2004.
101. **Evelin Seppet.** Interaction of mitochondria and ATPases in oxidative muscle cells in normal and pathological conditions. Tartu, 2004.

102. **Eduard Maron.** Serotonin function in panic disorder: from clinical experiments to brain imaging and genetics. Tartu, 2004.
103. **Marje Oona.** *Helicobacter pylori* infection in children: epidemiological and therapeutic aspects. Tartu, 2004.
104. **Kersti Kokk.** Regulation of active and passive molecular transport in the testis. Tartu, 2005.
105. **Vladimir Järv.** Cross-sectional imaging for pretreatment evaluation and follow-up of pelvic malignant tumours. Tartu, 2005.
106. **Andre Õun.** Epidemiology of adult epilepsy in Tartu, Estonia. Incidence, prevalence and medical treatment. Tartu, 2005.
107. **Piibe Muda.** Homocysteine and hypertension: associations between homocysteine and essential hypertension in treated and untreated hypertensive patients with and without coronary artery disease. Tartu, 2005.
108. **Külli Kingo.** The interleukin-10 family cytokines gene polymorphisms in plaque psoriasis. Tartu, 2005.
109. **Mati Merila.** Anatomy and clinical relevance of the glenohumeral joint capsule and ligaments. Tartu, 2005.
110. **Epp Songisepp**. Evaluation of technological and functional properties of the new probiotic *Lactobacillus fermentum* ME-3. Tartu, 2005.
111. **Tiia Ainla.** Acute myocardial infarction in Estonia: clinical characteristics, management and outcome. Tartu, 2005.
112. **Andres Sell.** Determining the minimum local anaesthetic requirements for hip replacement surgery under spinal anaesthesia – a study employing a spinal catheter. Tartu, 2005.
113. **Tiia Tamme.** Epidemiology of odontogenic tumours in Estonia. Pathogenesis and clinical behaviour of ameloblastoma. Tartu, 2005.
114. **Triine Annus**. Allergy in Estonian schoolchildren: time trends and characteristics. Tartu, 2005.
115. **Tiia Voor.** Microorganisms in infancy and development of allergy: comparison of Estonian and Swedish children. Tartu, 2005.
116. **Priit Kasenõmm.** Indicators for tonsillectomy in adults with recurrent tonsillitis – clinical, microbiological and pathomorphological investigations. Tartu, 2005.
117. **Eva Zusinaite.** Hepatitis C virus: genotype identification and interactions between viral proteases. Tartu, 2005.
118. **Piret Kõll.** Oral lactoflora in chronic periodontitis and periodontal health. Tartu, 2006.
119. **Tiina Stelmach.** Epidemiology of cerebral palsy and unfavourable neurodevelopmental outcome in child population of Tartu city and county, Estonia Prevalence, clinical features and risk factors. Tartu, 2006.
120. **Katrin Pudersell.** Tropane alkaloid production and riboflavine excretion in the field and tissue cultures of henbane (*Hyoscyamus niger* L.). Tartu, 2006.
121. **Külli Jaako.** Studies on the role of neurogenesis in brain plasticity. Tartu, 2006.

122. **Aare Märtson.** Lower limb lengthening: experimental studies of bone regeneration and long-term clinical results. Tartu, 2006.
123. **Heli Tähepõld.** Patient consultation in family medicine. Tartu, 2006.
124. **Stanislav Liskmann.** Peri-implant disease: pathogenesis, diagnosis and treatment in view of both inflammation and oxidative stress profiling. Tartu, 2006.
125. **Ruth Rudissaar.** Neuropharmacology of atypical antipsychotics and an animal model of psychosis. Tartu, 2006.
126. **Helena Andreson.** Diversity of *Helicobacter pylori* genotypes in Estonian patients with chronic inflammatory gastric diseases. Tartu, 2006.
127. **Katrin Pruus.** Mechanism of action of antidepressants: aspects of serotoninergic system and its interaction with glutamate. Tartu, 2006.
128. **Priit Põder.** Clinical and experimental investigation: relationship of ischaemia/reperfusion injury with oxidative stress in abdominal aortic aneurysm repair and in extracranial brain artery endarterectomy and possibilities of protection against ischaemia using a glutathione analogue in a rat model of global brain ischaemia. Tartu, 2006.
129. **Marika Tammaru.** Patient-reported outcome measurement in rheumatoid arthritis. Tartu, 2006.
130. **Tiia Reimand.** Down syndrome in Estonia. Tartu, 2006.
131. **Diva Eensoo.** Risk-taking in traffic and Markers of Risk-Taking Behaviour in Schoolchildren and Car Drivers. Tartu, 2007.
132. **Riina Vibo.** The third stroke registry in Tartu, Estonia from 2001 to 2003: incidence, case-fatality, risk factors and long-term outcome. Tartu, 2007.
133. **Chris Pruunsild.** Juvenile idiopathic arthritis in children in Estonia. Tartu, 2007.
134. **Eve Õiglane-Šlik.** Angelman and Prader-Willi syndromes in Estonia. Tartu, 2007.
135. **Kadri Haller.** Antibodies to follicle stimulating hormone. Significance in female infertility. Tartu, 2007.
136. **Pille Ööpik.** Management of depression in family medicine. Tartu, 2007.
137. **Jaak Kals.** Endothelial function and arterial stiffness in patients with atherosclerosis and in healthy subjects. Tartu, 2007.
138. **Priit Kampus.** Impact of inflammation, oxidative stress and age on arterial stiffness and carotid artery intima-media thickness. Tartu, 2007.
139. **Margus Punab.** Male fertility and its risk factors in Estonia. Tartu, 2007.
140. **Alar Toom**. Heterotopic ossification after total hip arthroplasty: clinical and pathogenetic investigation. Tartu, 2007.
141. **Lea Pehme.** Epidemiology of tuberculosis in Estonia 1991–2003 with special regard to extrapulmonary tuberculosis and delay in diagnosis of pulmonary tuberculosis. Tartu, 2007.
142. **Juri Karjagin.** The pharmacokinetics of metronidazole and meropenem in septic shock. Tartu, 2007.
143. **Inga Talvik.** Inflicted traumatic brain injury shaken baby syndrome in Estonia – epidemiology and outcome. Tartu, 2007.

144. **Tarvo Rajasalu.** Autoimmune diabetes: an immunological study of type 1 diabetes in humans and in a model of experimental diabetes (in RIP-B7.1 mice). Tartu, 2007.
145. **Inga Karu.** Ischaemia-reperfusion injury of the heart during coronary surgery: a clinical study investigating the effect of hyperoxia. Tartu, 2007.
146. **Peeter Padrik.** Renal cell carcinoma: Changes in natural history and treatment of metastatic disease. Tartu, 2007.
147. **Neve Vendt**. Iron deficiency and iron deficiency anaemia in infants aged 9 to 12 months in Estonia. Tartu, 2008.
148. **Lenne-Triin Heidmets.** The effects of neurotoxins on brain plasticity: focus on neural Cell Adhesion Molecule. Tartu, 2008.
149. **Paul Korrovits.** Asymptomatic inflammatory prostatitis: prevalence, etiological factors, diagnostic tools. Tartu, 2008.
150. **Annika Reintam.** Gastrointestinal failure in intensive care patients. Tartu, 2008.
151. **Kristiina Roots.** Cationic regulation of Na-pump in the normal, Alzheimer's and CCK_2 receptor-deficient brain. Tartu, 2008.
152. **Helen Puusepp.** The genetic causes of mental retardation in Estonia: fragile X syndrome and creatine transporter defect. Tartu, 2009.
153. **Kristiina Rull.** Human chorionic gonadotropin beta genes and recurrent miscarriage: expression and variation study. Tartu, 2009.
154. **Margus Eimre.** Organization of energy transfer and feedback regulation in oxidative muscle cells. Tartu, 2009.
155. **Maire Link.** Transcription factors FoxP3 and AIRE: autoantibody associations. Tartu, 2009.
156. **Kai Haldre.** Sexual health and behaviour of young women in Estonia. Tartu, 2009.
157. **Kaur Liivak.** Classical form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency in Estonia: incidence, genotype and phenotype with special attention to short-term growth and 24-hour blood pressure. Tartu, 2009.
158. **Kersti Ehrlich.** Antioxidative glutathione analogues (UPF peptides) – molecular design, structure-activity relationships and testing the protective properties. Tartu, 2009.
159. **Anneli Rätsep.** Type 2 diabetes care in family medicine. Tartu, 2009.
160. **Silver Türk.** Etiopathogenetic aspects of chronic prostatitis: role of mycoplasmas, coryneform bacteria and oxidative stress. Tartu, 2009.
161. **Kaire Heilman.** Risk markers for cardiovascular disease and low bone mineral density in children with type 1 diabetes. Tartu, 2009.
162. **Kristi Rüütel.** HIV-epidemic in Estonia: injecting drug use and quality of life of people living with HIV. Tartu, 2009.
163. **Triin Eller.** Immune markers in major depression and in antidepressive treatment. Tartu, 2009.

164. **Siim Suutre.** The role of TGF-β isoforms and osteoprogenitor cells in the pathogenesis of heterotopic ossification. An experimental and clinical study of hip arthroplasty. Tartu, 2010.
165. **Kai Kliiman.** Highly drug-resistant tuberculosis in Estonia: Risk factors and predictors of poor treatment outcome. Tartu, 2010.
166. **Inga Villa.** Cardiovascular health-related nutrition, physical activity and fitness in Estonia. Tartu, 2010.
167. **Tõnis Org.** Molecular function of the first PHD finger domain of Autoimmune Regulator protein. Tartu, 2010.
168. **Tuuli Metsvaht.** Optimal antibacterial therapy of neonates at risk of early onset sepsis. Tartu, 2010.
169. **Jaanus Kahu.** Kidney transplantation: Studies on donor risk factors and mycophenolate mofetil. Tartu, 2010.
170. **Koit Reimand.** Autoimmunity in reproductive failure: A study on associated autoantibodies and autoantigens. Tartu, 2010.
171. **Mart Kull.** Impact of vitamin D and hypolactasia on bone mineral density: a population based study in Estonia. Tartu, 2010.
172. **Rael Laugesaar.** Stroke in children – epidemiology and risk factors. Tartu, 2010.
173. **Mark Braschinsky.** Epidemiology and quality of life issues of hereditary spastic paraplegia in Estonia and implemention of genetic analysis in everyday neurologic practice. Tartu, 2010.
174. **Kadri Suija.** Major depression in family medicine: associated factors, recurrence and possible intervention. Tartu, 2010.
175. **Jarno Habicht.** Health care utilisation in Estonia: socioeconomic determinants and financial burden of out-of-pocket payments. Tartu, 2010.
176. **Kristi Abram.** The prevalence and risk factors of rosacea. Subjective disease perception of rosacea patients. Tartu, 2010.
177. **Malle Kuum.** Mitochondrial and endoplasmic reticulum cation fluxes: Novel roles in cellular physiology. Tartu, 2010.
178. **Rita Teek.** The genetic causes of early onset hearing loss in Estonian children. Tartu, 2010.
179. **Daisy Volmer.** The development of community pharmacy services in Estonia – public and professional perceptions 1993–2006. Tartu, 2010.
180. **Jelena Lissitsina.** Cytogenetic causes in male infertility. Tartu, 2011.
181. **Delia Lepik.** Comparison of gunshot injuries caused from Tokarev, Makarov and Glock 19 pistols at different firing distances. Tartu, 2011.
182. **Ene-Renate Pähkla**. Factors related to the efficiency of treatment of advanced periodontitis. Tartu, 2011.
183. **Maarja Krass.** L-Arginine pathways and antidepressant action. Tartu, 2011.
184. **Taavi Lai.** Population health measures to support evidence-based health policy in Estonia. Tartu, 2011.

185. **Tiit Salum.** Similarity and difference of temperature-dependence of the brain sodium pump in normal, different neuropathological, and aberrant conditions and its possible reasons. Tartu, 2011.
186. **Tõnu Vooder**. Molecular differences and similarities between histological subtypes of non-small cell lung cancer. Tartu, 2011.
187. **Jelena Štšepetova.** The characterisation of intestinal lactic acid bacteria using bacteriological, biochemical and molecular approaches. Tartu, 2011.
188. **Radko Avi.** Natural polymorphisms and transmitted drug resistance in Estonian HIV-1 CRF06_cpx and its recombinant viruses. Tartu, 2011, 116 p.
189. **Edward Laane**. Multiparameter flow cytometry in haematological malignancies. Tartu, 2011, 152 p.
190. **Triin Jagomägi.** A study of the genetic etiology of nonsyndromic cleft lip and palate. Tartu, 2011, 158 p.
191. **Ivo Laidmäe.** Fibrin glue of fish (*Salmo salar*) origin: immunological study and development of new pharmaceutical preparation. Tartu, 2012, 150 p.
192. **Ülle Parm.** Early mucosal colonisation and its role in prediction of invasive infection in neonates at risk of early onset sepsis. Tartu, 2012, 168 p.
193. **Kaupo Teesalu.** Autoantibodies against desmin and transglutaminase 2 in celiac disease: diagnostic and functional significance. Tartu, 2012, 142 p.
194. **Maksim Zagura**. Biochemical, functional and structural profiling of arterial damage in atherosclerosis. Tartu, 2012, 162 p.
195. **Vivian Kont.** Autoimmune regulator: characterization of thymic gene regulation and promoter methylation. Tartu, 2012, 134 p.
196. **Pirje Hütt.** Functional properties, persistence, safety and efficacy of potential probiotic lactobacilli. Tartu, 2012, 246 p.
197. **Innar Tõru.** Serotonergic modulation of CCK-4- induced panic. Tartu, 2012, 132 p.
198. **Sigrid Vorobjov.** Drug use, related risk behaviour and harm reduction interventions utilization among injecting drug users in Estonia: implications for drug policy. Tartu, 2012, 120 p.
199. **Martin Serg.** Therapeutic aspects of central haemodynamics, arterial stiffness and oxidative stress in hypertension. Tartu, 2012, 156 p.
200. **Jaanika Kumm.** Molecular markers of articular tissues in early knee osteoarthritis: a population-based longitudinal study in middle-aged subjects. Tartu, 2012, 159 p.
201. **Kertu Rünkorg.** Functional changes of dopamine, endopioid and endocannabinoid systems in CCK2 receptor deficient mice. Tartu, 2012, 125 p.
202. **Mai Blöndal.** Changes in the baseline characteristics, management and outcomes of acute myocardial infarction in Estonia. Tartu, 2012, 127 p.
203. **Jana Lass.** Epidemiological and clinical aspects of medicines use in children in Estonia. Tartu, 2012, 170 p.
204. **Kai Truusalu.** Probiotic lactobacilli in experimental persistent *Salmonella* infection. Tartu, 2013, 139 p.

205. **Oksana Jagur.** Temporomandibular joint diagnostic imaging in relation to pain and bone characteristics. Long-term results of arthroscopic treatment. Tartu, 2013, 126 p.
206. **Katrin Sikk.** Manganese-ephedrone intoxication – pathogenesis of neurological damage and clinical symptomatology. Tartu, 2013, 125 p.
207. **Kai Blöndal.** Tuberculosis in Estonia with special emphasis on drug-resistant tuberculosis: Notification rate, disease recurrence and mortality. Tartu, 2013, 151 p.
208. **Marju Puurand.** Oxidative phosphorylation in different diseases of gastric mucosa. Tartu, 2013, 123 p.
209. **Aili Tagoma.** Immune activation in female infertility: Significance of autoantibodies and inflammatory mediators. Tartu, 2013, 135 p.
210. **Liis Sabre.** Epidemiology of traumatic spinal cord injury in Estonia. Brain activation in the acute phase of traumatic spinal cord injury. Tartu, 2013, 135 p.
211. **Merit Lamp.** Genetic susceptibility factors in endometriosis. Tartu, 2013, 125 p.
212. **Erik Salum.** Beneficial effects of vitamin D and angiotensin II receptor blocker on arterial damage. Tartu, 2013, 167 p.
213. **Maire Karelson.** Vitiligo: clinical aspects, quality of life and the role of melanocortin system in pathogenesis. Tartu, 2013, 153 p.
214. **Kuldar Kaljurand.** Prevalence of exfoliation syndrome in Estonia and its clinical significance. Tartu, 2013, 113 p.
215. **Raido Paasma.** Clinical study of methanol poisoning: handling large outbreaks, treatment with antidotes, and long-term outcomes. Tartu, 2013, 96 p.
216. **Anne Kleinberg.** Major depression in Estonia: prevalence, associated factors, and use of health services. Tartu, 2013, 129 p.
217. **Triin Eglit.** Obesity, impaired glucose regulation, metabolic syndrome and their associations with high-molecular-weight adiponectin levels. Tartu, 2014, 115 p.
218. **Kristo Ausmees.** Reproductive function in middle-aged males: Associations with prostate, lifestyle and couple infertility status. Tartu, 2014, 125 p.
219. **Kristi Huik.** The influence of host genetic factors on the susceptibility to HIV and HCV infections among intravenous drug users. Tartu, 2014, 144 p.
220. **Liina Tserel.** Epigenetic profiles of monocytes, monocyte-derived macrophages and dendritic cells. Tartu, 2014, 143 p.
221. **Irina Kerna.** The contribution of *ADAM12* and *CILP* genes to the development of knee osteoarthritis. Tartu, 2014, 152 p.
222. **Ingrit Liiv.** Autoimmune regulator protein interaction with DNA-dependent protein kinase and its role in apoptosis. Tartu, 2014, 143 p.