Diploma thesis

Assessment of the intestinal microcirculatory disturbances during sepsis and obesity

submitted by

Georg Stephan Pirker

Mat.Nr.: 0434503

in order to obtain the degree of

Doktor der gesamten Heilkunde

(Dr. med. univ.) (MD)

at the

Medical University of Graz

performed at the

Department of Pediatric and Adolescent Surgery

supervised by

Ass. Prof. Priv.-Doz. Dr. med. univ. Georg Singer

Dr. med. univ. Elke Maria Ruttenstock

Ort, Datum …….. (Unterschrift)

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am ….. *Unterschrift*

Acknowledgments

Writing this diploma thesis I have been greatly supported by various people of whom I would like to mention just a few.

I want to thank Ass.Prof. Priv.-Doz. Dr.med.univ. Georg Singer and Dr.med.univ. Elke Maria Ruttenstock who have been supervising my work and always did provide support when I confronted a delicate problem during my work.

I also want to thank my lab mates Rebecca Ruedl, BSc. and Manfred Sagmeister who have been great colleagues for the last year. It was due to the great team-spirit and the contribution of everyone, that we were able to overcome certain obstacles that occurred during our experiments.

Also I want to thank my parents Walter and Christa and my three sisters Eva, Elisabeth and Sophia who did support me throughout my medical studies and all my friends who made the last six years of medical schooling a rich and very joyful experience for me.

This study was supported by the Austrian Science Fund (FWF) grant number P22567.

Abstract

In the last years the world is confronted with an ever increasing incidence of obesity. Obesity is associated with a decreased life expectancy, the development of several other diseases and high costs of care. Sepsis is a common disease that is associated with high mortality and high costs of care and is the leading cause of death in non-coronary intensive care units as well as the 10th leading cause of death in the United States. Alterations of the microcirculation have been shown to play a significant role in the development of sepsis in obesity models. The aim of the present diploma thesis was to describe the intestinal microcirculatory disturbances during sepsis in two strains of obese mice. Male C57BL/6J@Rj-ob (ob/ob) and male C57/KsJ@Rj-db (db/db) mice were used and compared to male C57BL/6J Rj (WT) mice. Sepsis was induced by cecal ligation and puncture. Weight, circulating blood cells and intra-peritoneal bacteria, wet-dry ratio of the lung and leukocyte rolling and adhesion of the small intestine was observed 6 hours following CLP and values were compared between septic obese mice and a group of WT mice following CLP and a sham operation respectively. Survival following surgery was observed for 168 hours. There was no significant difference between the four groups in the amount of circulating blood cells and the wet-dry ratio of the lung. The WT CLP group showed significantly more intra-peritoneal bacteria compared to the other groups. The amount of adherent leukocytes was significantly higher in groups experiencing CLP surgery when compared to the sham group. Leukocyte rolling did not significantly differ among the groups though there was a trend towards increased rolling in the CLP groups. A markedly decreased survival could be seen in both obese groups following induction of sepsis.

Zusammenfassung

Die Inzidenz von Adipositas nimmt weltweit stetig zu. Adipositas geht einher mit einer Verringerung der Lebenserwartung, dem Auftreten schwerer Folgeerkrankungen und hohen Kosten für das Gesundheitssystem. Sepsis ist eine häufige Erkrankung, die mit hoher Mortalität und hohen Kosten für das Gesundheitssystem einhergeht, und ist die führende Todesursache auf nicht kardiologischen Intensivstationen und die zehnt häufigste Todesurasche in den Vereinigten Staaten. Veränderungen der Mikrozirkulation spielen eine entscheidende Rolle bei Sepsis und Adipositas. Das Ziel dieser Studie war es die intestinale Mikrozirkulation während Sepsis in zwei Stämmen adipöser Mäuse zu beschreiben. Männliche C57BL/6J@Rj-ob (ob/ob) und männliche C57/KsJ@Rj-db (db/db) Mäuse wurden verwendet und Sepsis wurde durch zökale Ligatur und Punktion hervorgerufen. Das Blutbild, die Anzahl von Bakterien in der Peritonealhöhle, das feucht-trocken Verhältnis der Lunge sowie Leukozyten Rollen und Adhäsion in der Dünndarmmikrozirkulation wurden 6 Stunden nach Induktion von Sepsis untersucht und die Ergebnisse wurden mit männlichen C57BL/6J Rj (WT) Mäusen nach Induktion von Sepsis oder einer sham Operation verglichen.. Weiters wurde die Überlebensrate über 168 Stunden hinweg in allen 4 Gruppen beobachtet. Bei der Anzahl zirkulierender Zellen im Blut und dem feucht-trocken Verhältnis der Lunge konnte kein signifikanter Unterschied zwischen den Gruppen festgestellt werden. Die WT CLP Gruppe zeigte eine signifikante Erhöhung der intraperitonealen Bakterien im Vergleich zu den anderen Gruppen. Die Anzahl der adhärenten Leukozyten war signifikant höher in Gruppen, die mit CLP Operation behandelt wurden als in der sham Gruppe. Beim Leukozyten-Rollen unterschieden sich die vier Gruppen nicht signifikant, aber es konnte eine Tendenz zu erhöhtem Rollen in CLP-Gruppen beobachtet werden. Eine deutlich verminderte Überlebensrate wurde in septischen adipösen Mäusen beobachtet.

Contents

1 Introduction....1
1.1 Obesity....1
1.1.1 Definition....1
1.1.1.1 Body-Mass-Index....2
1.1.1.2 Waist Circumference and Waist to Hip Ratio....2
1.1.1.3 Value of anthropometric parameters....2
1.1.2 Adipose tissue....3
1.1.2.1 Leptin....3
1.1.2.2 Adiponectin....4
1.2 Sepsis....4
1.2.1 SIRS....5
1.2.2 MODS....6
1.2.3 Sepsis and the small intestine....7
1.2.4 Sepsis and Obesity....7
1.3 Microcirculation....8
2 Material and Methods....9
2.1 Animals....9
2.1.1 Wild type Mice....9
2.1.2 ob/ob Mice....9
2.1.3 db/db Mice....10
2.2 Experimental Protocols....10
2.3 Surgical Procedures....10
2.3.1 Anesthetization....10
2.3.1.1 Domitor....11
2.3.1.2 Ketanest....11
2.3.1.3 Butomidor....11
2.3.1.4 Antisedan....11
2.3.2 Induction of Sepsis....12
2.3.2.1 Cecal Ligation and Puncture (CLP)....12
2.3.2.2 Sham....13
2.3.3 Cannulation of the Carotid Artery....14
2.3.4 Cannulation of the Jugular Vein....16

2.4 Weight....17
2.5 Blood Cell Count....17
2.6 Bacterial Count....17
2.7 Wet-Dry Ratio....17
2.8 Survival Experiment....18
2.9 Intravital Microscopy....18
2.9.1 Microscope....18
2.9.2 Preparations....18
2.9.3 Recording of videos....19
2.9.4 Leukocyte Activity....19
2.10 Statistical Analysis....19
3 Results....21
3.1 Weight....21
3.2 Blood Cell Count....22
3.3 Bacterial Counts....23
3.4 Wet-Dry Ratio....23
3.5 Survival....24
3.6 Intravital Microscopy....25
3.6.1 Adherent Leukocytes....25
3.6.2 Rolling Leukocytes....26
4 Discussion....28
4.1.1 Weight....29
4.1.2 Blood Cell Count....29
4.1.3 Bacterial Count....30
4.1.4 Wet-Dry Ratio....30
4.1.5 Survival....31
4.1.6 IVM....31
4.2 Conclusion....33
5 Bibliography....34
6 Anesthetization Protocols....38
6.1 Anesthetization Protocol for Domitor and Ketamin....38
6.2 Anesthetization protocol for Butomidor and Antisedan....40
7 Curriculum Vitae....42

Glossary

BMI	Body-Mass-Index
BW	Body Weight
CBR	Center for Biomedical Research
CFU	Colony Forming Unit
CLP	Cecal Ligation and Puncture
CRP	C-reactive Protein
HDL	high density Lipoprotein
ICU	Intensive Care Unit
LDL	low density Lipoprotein
MODS	Multiple Organ Dysfunction Syndrome
MUG	Medical University of Graz
NaCl	Sodium Chloride
PBS	Phosphate Buffered Saline
PE	Polyethylene
RBCs	Red Blood Cells
SIRS	Systemic Inflammatory Response Syndrome
WC	Waist Circumference
WHO	World Health Organization
WHR	Waist to Hip Ratio

Figures

Figure 1: Fixation of the mouse....12
Figure 2: Cecal ligation....12
Figure 3: Cecal puncture....13
Figure 4: Squeezing out feces....13
Figure 6: Consecutive suture of the skin....14
Figure 5: Suture of the rectus abdominis....14
Figure 7: Ligation of the cranial part of the carotid artery....15
Figure 8: Fixation of the cannula in the carotid artery....15
Figure 9: Preparation of the jugular vein....16
Figure 10: Setup for IVM, including microscope, lighting and perfusor for the application of Rhodamine 6G....20
Figure 11: Weight [g] at 5 weeks of age....21
Figure 12: CFUs in peritoneal lavage fluid after 24h incubation....22
Figure 13: Survival rate after CLP or sham operation....24
Figure 14: Leukocyte adhesion [#/mm2 endothelial surface] observed in the small intestines....25
adhesion 25
Figure 15: Leukocyte rolling observed in the small intestines....27

Tables

Table 1: Circulating blood cells 6h after CLP or sham operation....22
Table 2: Wet-dry ratio of lung lobes (dry lobes were incubated for 24h at 60°C)....23

1 Introduction

1.1 Obesity

Due to a constant decrease in physical activity in combination with a diet consisting mainly of high caloric food, obesity has become a worldwide healthcare problem. Initially limited to countries with high income, obesity has nowadays become a problem also in countries with middle and low income, especially in urban settings (1). The incidence of obesity has risen constantly over the past few decades and nowadays about 34% of the adult population and 17% of children and adolescents, with an age of 19 years or below, in the United States are obese (2). While the Austrian population shows significantly lower rates of obese adults (12.8% in males and 13.4% in females) numbers have still been growing in the last decade and therefore should not be neglected (11). The metabolic state, caused by obesity, results in decreased life expectancy and is associated with the development of several other diseases including coronary heart syndrome, heart attack, stroke, atherosclerosis, hypertension, diabetes, arthritis, nonalcoholic steatohepatitis, thrombosis, pulmonary embolism, colo-rectal cancer, breast cancer, and endometrial cancer (1) (3) (4). Furthermore, obesity is associated with higher rates of mortality in patients admitted to intensive care units in correlation to longer need for mechanical ventilation and a prolonged weaning period as well as a higher number of comorbidities individually known to result in poorer prognosis (12). Also obesity is generally associated with high costs of care, consuming between 2.1% and 4.7% of the healthcare budget of western European countries and reduces life expectancy by about 4.5 years (13). Thus, obesity is a major topic of modern healthcare.

1.1.1 Definition

Obesity is most commonly described as an increased amount of body fat, compared to the average population, resulting in increased body weight as well as increased body circumference, caused by an imbalance between the uptake of calories via consumed food and the consumption of calories throughout the day

(3). This increased amount of body fat is defined by the World Health Organization (WHO) through the body-mass index (BMI). In addition to the BMI other anthropometric parameters such as waist to hip ratio (WHR) and waist circumference (WC) are commonly used for diagnostic and prognostic values.

1.1.1.1 Body-Mass-Index

The BMI is used to assume the amount of fat in total body mass and serves as a diagnostic criterion as well as a prognostic factor to determine the risk for the development of the abovementioned diseases. To calculate the BMI total body mass in kilograms is divided by the square of total body height in meters. In normally weighted individuals it ranges from 18.5 kg/m² to 24.9 kg/m². Patients with a BMI greater than 25 are considered over-weighted. A BMI of 30 or higher is considered obese and patients with a BMI of 40 or higher are classified as morbidly obese (1) (3).

1.1.1.2 Waist Circumference and Waist to Hip Ratio

WC is an important marker for obesity and is often used together with the BMI. It is measured between the lowest rib and the iliac crest on the standing patient and, in correlation to an elevated BMI, indicates excessive body fat at a circumference equaling or exceeding 40 inches in men and 35 inches in women (5) (14). The WHR is calculated dividing WC by hip circumference which is measured above the great trochanters (14). Together with the BMI, WC and WHR are especially useful to estimate intra-abdominal fat deposition (15). The major drawback of WC and WHR are possible variations between different operators (15).

1.1.1.3 Value of anthropometric parameters

In daily practice usefulness of diagnostic procedures is limited by the complexity of performance, time consumed, invasiveness, comparability of results and costs of performance. Anthropometric parameters are easy and cheap to perform, non-invasive, consume little time and show good comparability. Furthermore, recent publications have shown that BMI and WC elicit high correlation to visceral fat

measured by computed tomography, ultrasonography and bioelectrical impedance analysis (15). Also, in patients suffering from metabolic syndrome, BMI correlates with serum levels of C-reactive protein (CRP), WHR positively correlates to serum levels of triglycerides, total- and low density lipoprotein (LDL-) cholesterol and negatively to high density lipoprotein (HDL-) cholesterol and both have been shown to correlate to serum levels of insulin while fasting (16).

1.1.2 Adipose tissue

Adipose tissue is the main storage site for lipids in the human body. Though this role as a long term energy storage organ was considered the main purpose of adipocytes for a long time, several recent publications have shown that adipose tissue is a highly active endocrine organ that not only produces hormones associated to energy balance but also secretes pro-inflammatory proteins and thus influences systemic and vascular inflammation (17) (18). The major compartment of adipose tissue of the human body consists of white adipose tissue that is highly vascularized and innervated and shows a variety of interactions with the brain and other organs (17). The hormones produced by adipose tissue, usually referred to as adipokines, show increased serum levels in obese patients as well as a decrease in serum levels correlating to reduction of fat mass suggesting a pivotal role of the alterations in energy balance, metabolism and immune function caused by adipokines, in the deleterious effects of obesity (17) (18). Among these adipokines are leptin and adiponectin, both being subject to recent investigations.

1.1.2.1 Leptin

Leptin is produced by fat cells and is the key hormone in the regulation of food intake according to energy expenditure (19). In the presence of adequately filled fat reservoirs leptin circulates in the bloodstream and serum levels are directly proportional to the amount and size of the lipocytes whereas during fasting leptin falls in concert with insulin (17) (19). Furthermore, leptin has been identified to play a pivotal role in the inflammatory processes (17). Leptin receptors are expressed on circulating leukocytes and during inflammation leptin directly influences

phagocytic activity of macrophages and regulates proliferation, activation and cytokine production in T-lymphocytes (20-22). Additionally, leptin exerts its pro-inflammatory effects on endothelial cells, resulting in the expression of endothelial cell adhesion molecules that are necessary for the recruitment of leukocytes (21) Leptin also induces platelet aggregation leading to thrombosis (21). Thus, leptin is likely linked to the chronic state of low-grade inflammation observed in obesity (21).

1.1.2.2 Adiponectin

Adiponectin is produced by adult lipocytes and shows the highest plasma concentration of all adipokines (20) (23). In contrast to leptin adiponectin shows strong anti-inflammatory effects, enhances insulin sensitivity, inhibits expression of TNF-alpha and other pro-inflammatory cytokines and attenuates expression of endothelial cell adhesion molecules (20) (24). Furthermore, adiponectin suppresses the function of adult macrophages as well as the growth of macrophage precursors and has been shown to be equally diminished in septic and morbidly obese patients (23). High levels of adiponectin are associated with weight loss and elicit positive effects on atherosclerosis and endothelial dysfunction (24). In conclusion, the various effects of this adipokine further underscore the importance of adipose tissue as an endocrine organ and its lower serum level during obesity may be an important factor in the development of several co-morbidities associated with excessive weight gain.

1.2 Sepsis

Sepsis is a severe systemic inflammation characterized by an identified underlying infection in combination with the presence of at least two criteria of the systemic inflammatory response syndrome (SIRS) (6). It is a very common disease that is associated with high consumption of healthcare resources (7). 51.1% of patients suffering from sepsis need intensive care unit treatment (7). In 1995, the average costs of medical care per patient were $22,100, resulting in a total annual cost of $16.7 billion in the United States alone (7). While the average costs of care per

patient, with mean costs of $54,300 per case, are highest in infants, with a total of $13.8 billion, representing 83.2% of the total annual costs of care for patients suffering from sepsis, the largest portion of the annual costs is distributed among patients ranging from 65 years to 75 years of age, consuming $8.7 billion, and patients aged 75 years or older, consuming $5.1 billion (7). With an average mortality of 28.6%, sepsis shows high rates of mortality, especially in patients with preexisting diseases, medical conditions or multiple organ failures during its time course, and is the most common cause of death in non-coronary intensive care units as well as the 10th leading cause of death in the United States (6-8). If associated with organ dysfunction, hypoperfusion abnormalities or hypotension, mortality rates can easily exceed 40% and the systemic inflammation is classified as severe sepsis (6) (7). Furthermore, a correlation between an increasing number of organs failing and increasing rates of mortality has been shown, eliciting the importance of multiple organ failure in the time-course of sepsis (25). From 1979 to 2000 the incidence of sepsis in the United States constantly increased from 82.7 cases/100,000 population to 240.4 cases/100,000 population for a total increase of 8.7% each year (8). Also in the same period the mean age of the patients increased form 57.4 years to 60.8 years and incidence of organ failure as a marker for severity increased from 19.1% to 30.2% leading to 256,033 identified cases of severe sepsis in 2000 (8). These numbers highlight the deleterious role of sepsis in modern time medicine.

1.2.1 SIRS

The term systemic inflammatory response syndrome is used to describe systemic inflammation independent of its cause and was first introduced to clinical usage in 1992 by Bone et al. in their article “Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine.” (6).

It is characterized by the presence of at least two of the following symptoms:

- Body temperature > 38°C or < 36°C
- Heart rate > 90/min
- Respiratory rate > 20/min or $PaCO_2$ < 32 mmHg
- Leukocyte count > 12,000/ µL or < 4000/ µL

and may be triggered by infection, trauma, ischemia, thermal injury and non-infectious inflammatory processes (6) (26). If an infection can be identified to be the cause to the systemic inflammatory response the term sepsis is used (6).

1.2.2 MODS

The term multiple organ dysfunction syndrome (MODS) was introduced in 1992 by Bone et al. in their article “Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine.” to attend to the increasing incidence of morbidity and mortality caused by multiple organ failure, to provide terminology fit for clinical usage, to challenge a variety of inadequate conventional terms referring to similar or only slightly different entities and to improve epidemiological descriptions of this syndrome (6). The authors defined MODS to be characterized by the detection of altered organ function resulting in the inability to maintain homeostasis in an absolute or relative continuum of change over time clinically manifested as a pattern of multiple, progressive symptoms thought to be pathogenetically related (6). MODS is a feared and severe complication in the time-course of either sepsis and SIRS as mortality is gravely increased in the presence of this syndrome, leading to mortality rates between 35% and 65% in septic patients (27). Furthermore the multiple organ dysfunction syndrome is the number 1 cause of death in surgical intensive care units (ICU) and is responsible for 50% to 80% of deaths in these wards (27).

1.2.3 Sepsis and the small intestine

Besides its digestive function the bowel plays a very important protective role in defending the body against microorganisms colonizing the intestines and their endotoxins. In about 30% of patients dying from MODS a septic focus cannot be identified even though bacteremia and thus sepsis have been verified leading to the assumption that another site of infection needs to exist (27). Although in only 8.6% of the patients suffering from sepsis the bowel is identified as the primary site of infection, clinical observations have shown that often microorganisms associated to the normal enteric flora cause the bacteremia in patients dying from MODS without showing a primary septic focus (7) (28). Rotstein stated in his article “Pathogenesis of Multiple Organ Dysfunction Syndrome: Gut Origin, Protection, and Decontamination.” that bacteria and endotoxins are able to translocate from the bowel to mesenteric lymph nodes and distant organs in patients with compromised barrier function of the intestinal mucosa leading to either direct infection or acting as a priming stimulus resulting in amplified inflammatory response on a second insult (27). The most important cause for such loss of barrier function in the intestines is assumed to be bowel ischemia (28). Thus alterations in the intestinal microcirculation may play a pivotal role and may be a hallmark in the development of severs sepsis and MODS.

1.2.4 Sepsis and Obesity

The incidence of both, sepsis and obesity, have been rising constantly and have become two important diseases in modern healthcare systems of western countries (2) (8). Furthermore it has been shown that obesity has a great influence on the development as well as the outcome of sepsis (12) (29). Morbidly obese patients show prolonged mechanical ventilation time, longer weaning periods and higher ICU mortality than their lean counterparts (12). Also morbidly obese patients, defined by a BMI greater than 40 kg/m², show significantly higher rates of mortality and nursing home admission than less obese patients as defined by a BMI between 31 kg/m² and 40 kg/m² (29). Furthermore, when comparing obese patients to morbidly obese patients, the incidence of complications during ICU stay such as sepsis, catheter infection and renal failure as well as the need for and the

duration of mechanical ventilation is significantly elevated in the morbidly obese (29).

1.3 Microcirculation

The term microcirculation is used to describe the circulation in small vessels with a mean diameter smaller than 100 µm, including arterioles, capillaries and venules as well as the various functions of these vessels such as metabolic exchange and endothelial barrier function (9). Recent publications have shown that alterations in these vessels such as endothelial dysfunction or loss of endothelial barrier function play a key role in the development of sepsis (10). Also endothelial dysfunction has been identified to be an early process in obesity and together with capillary rarefaction results in reduced tissue perfusion in obese individuals (9). Furthermore during the time course of sepsis dysregulations in arteriolar tone, loss of endothelial cell integrity and elevated leukocyte adhesion and migration occur, resulting in tissue damage, multiple organ failure or death (30). Leukocytes as well as platelets are in an activated state during sepsis showing hyperadhesive behavior and accumulate in capillaries which, together with vascular cell activation, possibly leads to organ ischemia (31-33). Concluding that alterations in microcirculation result in organ ischemia and that bacterial translocation as a result of the loss of barrier function of the intestine, which is thought to be caused by bowel ischemia, is a very important factor in the time course of sepsis, further research on microcirculation may not only provide additional understanding of the pathogenesis of sepsis, SIRS and MODS but may also reveal cellular pathways that may be useful in a therapeutic approach (27) (28) (31-33).

2 Material and Methods

2.1 Animals

All groups of mice used in the experimental trials were bred from the C57BL strain and obtained from Janvier (St. Berthevin, Cedex, France). Upon usage mice were at least 5 weeks old and had experienced an acclimatization period of two weeks in the conventional animal keeping, according to guidelines of the Center for Biomedical Research (CBR) of the Medical University of Graz (MUG). Animal keeping and veterinary care was performed by CBR personnel. Mice were kept in type III standard open cages in groups of 5 and experienced a simulated environment including temperature between 20°C and 24°C, relative humidity between 45% and 65% and automated 12h day/night circles of lighting. Lighting periods were adapted according to the use of daylight saving time in Austria resulting in a lightened period from 6 am to 6 pm during summer and 7 am to 7 pm in winter. Standard Chow and Water was freely accessible at any given time.

2.1.1 Wild type Mice

Wild Type Mice used in the experiments were all males of the strain C57BL/6J Rj. They are similar to their naturally occurring relatives in terms of healthiness, weight, weight gain and response to infection and thus serve as non-obese counterparts to their littermates from the obese groups.

2.1.2 ob/ob Mice

Ob/ob mice used in the experiments were all males of the strain C57BL/6J@Rj-ob. This group of mice was genetically altered to lack expression of the adipokine leptin. As a result of the loss of leptin expression a constant state of hunger induces weight gain to an extend where the average weight of the mice equals three times the weight of their WT counterparts (19).

2.1.3 db/db Mice

Db/db mice used in the experiments were all males of the strain C57/KsJ@Rj-db. This group of mice was, similarly to the ob/ob group, genetically altered in order to become obese and included to provide another model of obesity. Db/db mice lack the expression of the leptin receptor also showing increased weight gain as a result of the disturbed leptin signaling pathway.

2.2 Experimental Protocols

In a first set of experiments blood samples were acquired six hours after performing cecal ligation and puncture (CLP) operation or sham operation in four groups of mice to perform analysis of the circulating blood cells. The groups included a WT group, an ob/ob group and a db/db group following CLP procedure and a WT group after sham surgery. After analyzing the circulating cells, lavage fluid of the peritoneal caves was obtained in the same mice and brushed onto agar plates to assess bacterial infection. Additionally the left lobe of the lung was explanted to observe wet-dry ratio. In a second set of experiments, intravital microscopy (IVM) of the small intestines was performed in a group of WT mice, ob/ob mice and db/db mice six hours after CLP and a group of WT mice six hours after sham operation to obtain leukocyte rolling and adhesion. Animal handling procedures were in accordance and approved by the Medical University of Graz and the Animal Care and Use Committee of the Austrian Federal Ministry of Science and Research.

2.3 Surgical Procedures

2.3.1 Anesthetization

A triple anesthesia consisting of Domitor, Ketanest and Butomidor was used for all operations. All Substances were applied subcutaneously; dosing was performed according to standard protocols and in accordance to CBR and MUG guidelines. After surgical procedures Antisedan was given as an antidote and 1mL of NaCl 0.9% was applied for fluid resuscitation.

2.3.1.1 Domitor

- Substance: Medetomidine
- Class: alpha2-adrenergic agonist
- Effect: sedation and analgesia
- Dosing: 0.25 mg / kg Body weight (BW)

2.3.1.2 Ketanest

- Substance: Ketamine
- Class: NMDA receptor antagonist
- Effect: analgesia and sedation
- Dosing: 75 mg / kg BW

2.3.1.3 Butomidor

- Substance: Butorphanol
- Class: opioid analgesic
- Effect: analgesia and sedation
- Dosing: 0.001 mg / g BW

2.3.1.4 Antisedan

- Substance: Atipamezole
- Mechanism: alpha2-adrenoceptor antagonist
- Effect: reversal of sedation induced by Medetomidine or Dexmedetomidine
- Dosing: 0.001 mg / g BW

2.3.2 Induction of Sepsis

2.3.2.1 Cecal Ligation and Puncture (CLP)

The anesthetized Mouse was brought to a supine position upon an acrylic glass sheet and all four extremities were fixed with regular tape. The upper limbs were fixed about 1 cm above the acrylic glass sheet. Thereafter the abdomen was thoroughly shaved and cleaned of any hair. Now a midline laparotomy along the linea alba was performed. The midline laparotomy was especially useful as, if performed correctly, this method of incision would not cause any bleeding and therefore there was no need for cautery. The next incision dissected the linea alba itself, again not causing any bleeding. Utilizing two cotton swabs moisturized with sodium chloride (NaCl) 0.9% the cecum was cautiously drawn forth and ligation was performed without causing an ileus. Then a 20G needle is used to puncture the cecum in it's upper, mid and lower part and two NaCl 0.9% moisturized cotton swabs were used to squeeze some feces out of each puncture to prevent wound closure. The cecum was then re-inserted into the abdominal cavity and the rectus abdominis and the skin were sealed using a continuous suture. After the procedure 1 mL of NaCl 0.9% was given subcutaneously for rehydration.

Figure 1: Fixation of the mouse

Figure 2: Cecal ligation

Figure 3: Cecal puncture

Figure 4: Squeezing out feces

2.3.2.2 Sham

The anesthetized Mouse was brought to a supine position upon a acrylic glass sheet and all four extremities were fixed with regular tape. The upper limbs were fixed about 1cm above the acrylic glass sheet. Thereafter the abdomen was thoroughly shaved and cleaned of any hair. Now a midline laparotomy along the linea alba was performed. The next incision dissected the linea alba itself. Utilizing two cotton swabs moisturized with NaCl 0.9% the cecum was cautiously drawn forth and squeezed in all three thirds to provide a similar stress on the tissue as with the CLP. The cecum was then re-inserted into the abdominal cavity and the rectus abdominis and the skin were sealed using a continuous suture. After the procedure 1 mL of NaCl 0.9% was given subcutaneously for fluid resuscitation.

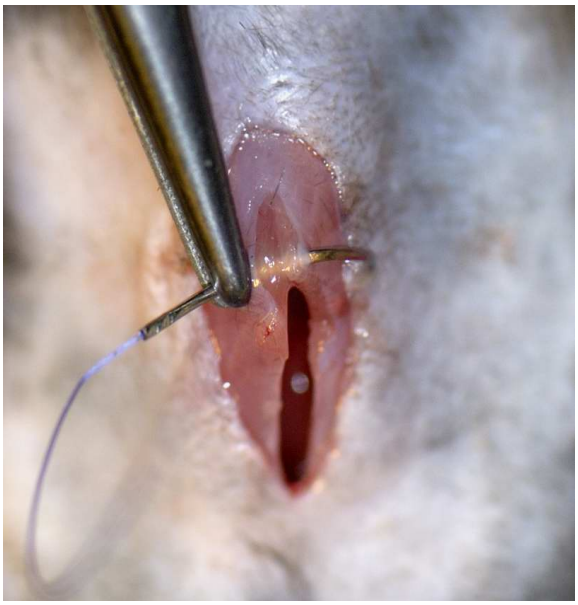

Figure 5: Suture of the rectus abdominis

Figure 6: Consecutive suture of the skin

2.3.3 Cannulation of the Carotid Artery

This method was used to obtain blood samples. After anesthetization the animals were shaved and a straight cut from the right to the left clavicle was performed with a pair of surgical scissors, followed by a second, caudal-cranial cut directly above the right mandible and a third cut parallel to the first one. This results in a rectangular shaped skin lobe that is flapped over to the left. The lobes of the submandibular gland are then separated from each other using two anatomical pincers and flapped to each side. Now the carotid artery is mobilized and separated from the vagus nerve that also runs in the carotid sheath. After this the cranial part of the carotid artery is ligated and the caudal part is clamped. The cannula is finally introduced into the carotid artery through a small incision, fixed with threads and the caudal clamp is removed. Cannulas were self-made using a 0.58mm/0.96mm polyethylene (PE) tube with a tip made of a 0.28mm/0.61mm PE tube and a 26G needle serving as connector.

Figure 7: Ligation of the cranial part of the carotid artery

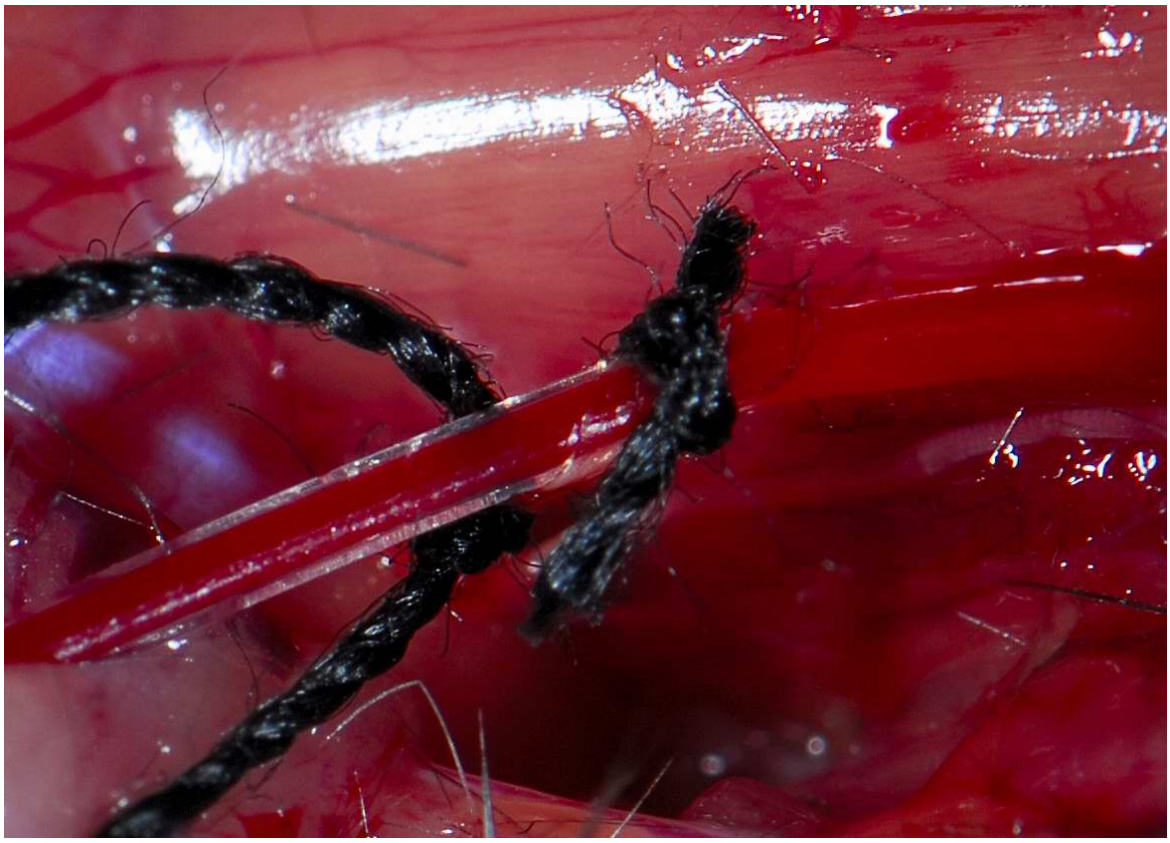

Figure 8: Fixation of the cannula in the carotid artery

2.3.4 Cannulation of the Jugular Vein

This method was used to infuse Rhodamine 6G. After anesthetization the animals were shaved and a straight cut from the right to the left clavicle was performed with a pair of surgical scissors, followed by a second, caudal-cranial cut directly above the right mandible and a third cut parallel to the first one. This results in a rectangular shaped skin lobe that is flapped over to the left. The lobes of the submandibular gland are then separated from each other using two anatomical pincers and flapped to each side. Now the jugular vein is mobilized and freed of any surrounding fatty tissue. After this the cranial part of the jugular vein is ligated the cannula is finally introduced via a small incision and fixed with threads. As the blood pressure in the jugular vein is very low, distal clamping is only performed in case of excessive bleeding. Cannulas were self-made using a 0.28mm/0.61mm polyethylene tube and a 26G needle.

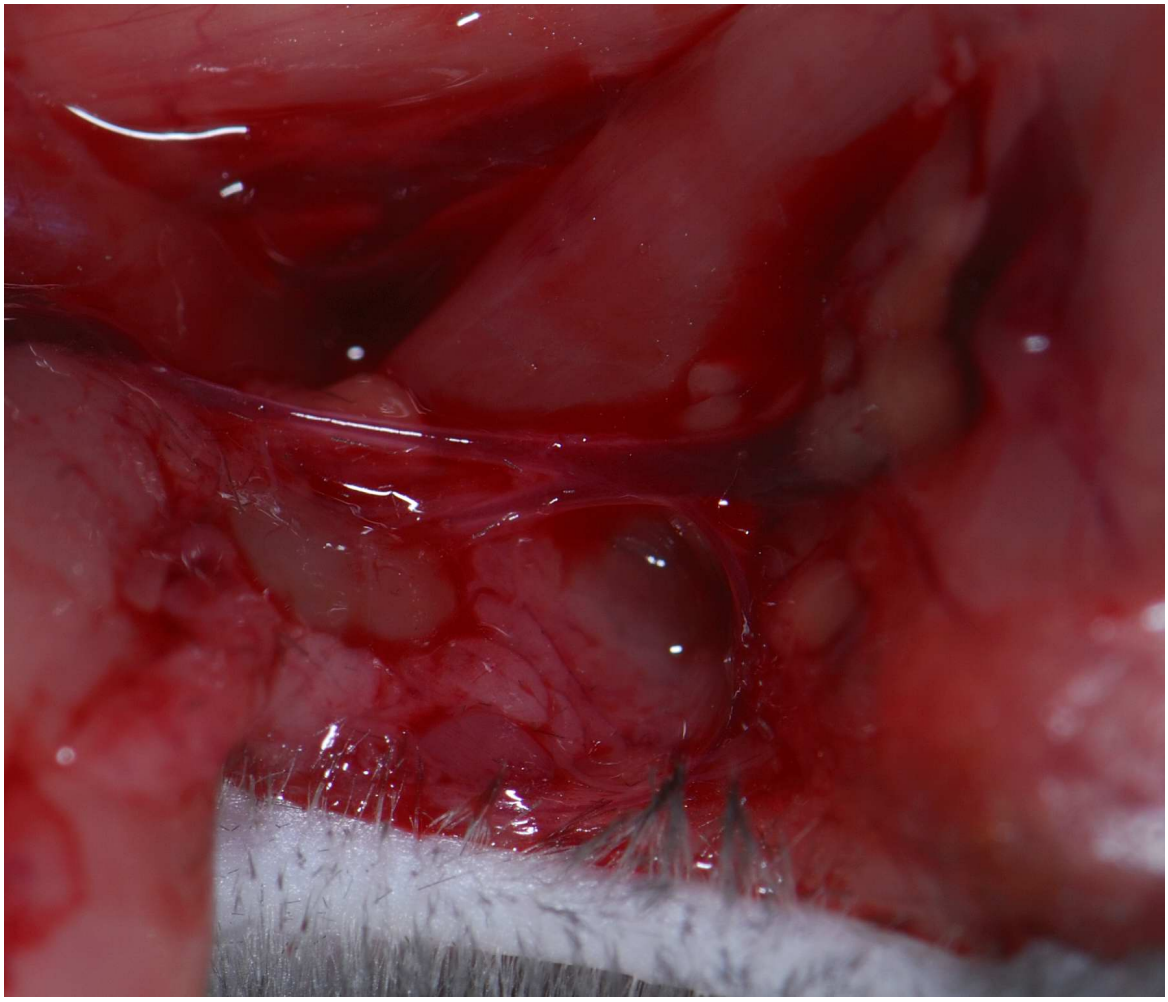

Figure 9: Preparation of the jugular vein

2.4 Weight

Four groups of mice (n=4-5/group) including a group of WT, ob/ob and db/db mice after CLP operation, and a control-group of WT mice following sham operation were weighted six hours after the surgical procedures.

2.5 Blood Cell Count

Blood samples of 100 µL were acquired from four groups of mice (n=4-5/group) including a group of WT, ob/ob and db/db mice following CLP operation, and a control-group of WT mice after sham operation. Analysis was performed using a MS9-5V Vet Hematology Analyzer.

2.6 Bacterial Count

Bacterial counts were performed in four groups of mice (n=4-5/group) including a group of WT, ob/ob and db/db mice post CLP, and a control-group of WT mice after sham operation. Six hours after surgery the peritoneal cavity was infused with 2 mL phosphate buffered saline (PBS) with a pH of 7.4 under sterile conditions. After 5 minutes the fluid was extracted from the peritoneal cave and diluted with PBS in a relation of 1:10. Then 10 µL of the solution were brushed onto petri dishes containing colombia-agar + 5% horse blood attained from bioMerieux (Vienna, Austria). After incubation at 37°C for 24 hours the total amount of colony forming units (CFU) was assessed. With each incubation period a control agar plate only containing 10µL of PBS was included.

2.7 Wet-Dry Ratio

Six hours after surgery the left pulmonary lobe of four groups of mice (n=4-5/group) including a group of WT, ob/ob and db/db mice after CLP operation, and a control-group of WT mice post sham operation was explanted in total and weighted. After incubation at 60°C for 24 hours weight was obtained again and the wet-dry ratio was calculated.

2.8 Survival Experiment

Anesthesia was performed according to protocol, followed by CLP in three groups of mice (n=5/group) including WT, ob/ob and db/db. A sham operated group of WT mice served as controls. After the surgical procedures mice received anti-anesthetization according to protocol and 1mL of NaCl 0.9% subcutaneously for rehydration. Each mouse was then transferred to an individual cage in the conventional animal keeping and survival was assessed every twelve hours. Mice were considered survivors if death did not occur in one week (168 h) after the surgery.

2.9 Intravital Microscopy

2.9.1 Microscope

An Olympus IX71S1F-3 inverted microscope was used with an X-Cite Series 120PC Q fluorescence light source.

2.9.2 Preparations

Six hours after either CLP or Sham operation the mice were re-anesthetized according to the protocol. Mice were then fixed on an acrylic glass sheet in a supine position using regular tape to pin down all four extremities and a thread of yarn was wrapped around the maxillary incisors and straightened in order to fix the head. Now the neck was shaved and cannulation of the jugular vein was performed as described above. After successful cannulation fixation was removed and a midline laparotomy was performed cutting the continuous sutures of the previous CLP or sham operation. Mice were now transferred to another acrylic glass plate bearing an embedded microscope slide. Utilizing two cotton swabs, moisturized with NaCl 0.9%, a loop of the small intestines was exteriorized and placed on the microscope slide. To prevent drying of the bowel loop a cotton pad was moisturized with NaCl 0.9% and used to cover the intestinal loop. Now the cannula was fixed on the acrylic glass plate to prevent accidental removal from the jugular vein, and the plate was brought upon the microscope.

2.9.3 Recording of videos

120 µL Rhodamine 6G was infused via the jugular vein and allowed to circulate for 5 minutes to stain leukocytes. Then three post-capillary venules with a diameter ranging from 60 µm to 175 µm were selected and digital recordings with a length of 60 seconds were performed. For recording Olympus Cell^P software was utilized. Videos were recorded uncompressed with a frame rate between 10 fps and 20 fps.

2.9.4 Leukocyte Activity

Leukocyte activity was assessed via interactions between cells and the vessel-wall and was classified as either rolling or adherent. Leukocytes were defined as rolling if they moved slower than RBCs for at least 5 seconds and were counted as stationary if they were adherent to the venular-wall for at least 30 seconds. Rolling leukocytes were expressed as number per vessel. Adherent cells were given as number per mm^2, calculated assuming a cylindrical vessel geometry.

2.10 Statistical Analysis

Data were entered in a SPSS 15.0 spreadsheet and analyzed using a one-way ANOVA, with Scheffe's post hoc test. The values are expressed as means ± SE and statistical significance was set at $p < 0.05$.

Figure 10: Setup for IVM, including microscope, lighting and perfusor for the application of Rhodamine 6G

3 Results

3.1 Weight

Both db/db (34.5g ± 3.7g) and ob/ob (44.6g ± 0.3g) mice showed significantly elevated weight at the age of 5 weeks when compared to the group of WT mice following CLP (22.0g ± 1.2g) operation and the group of WT mice after sham (21.7g ± 1.2g) operation. Furthermore a significant difference in weight between the db/db group and the ob/ob group could be observed as shown in *Figure 11.*

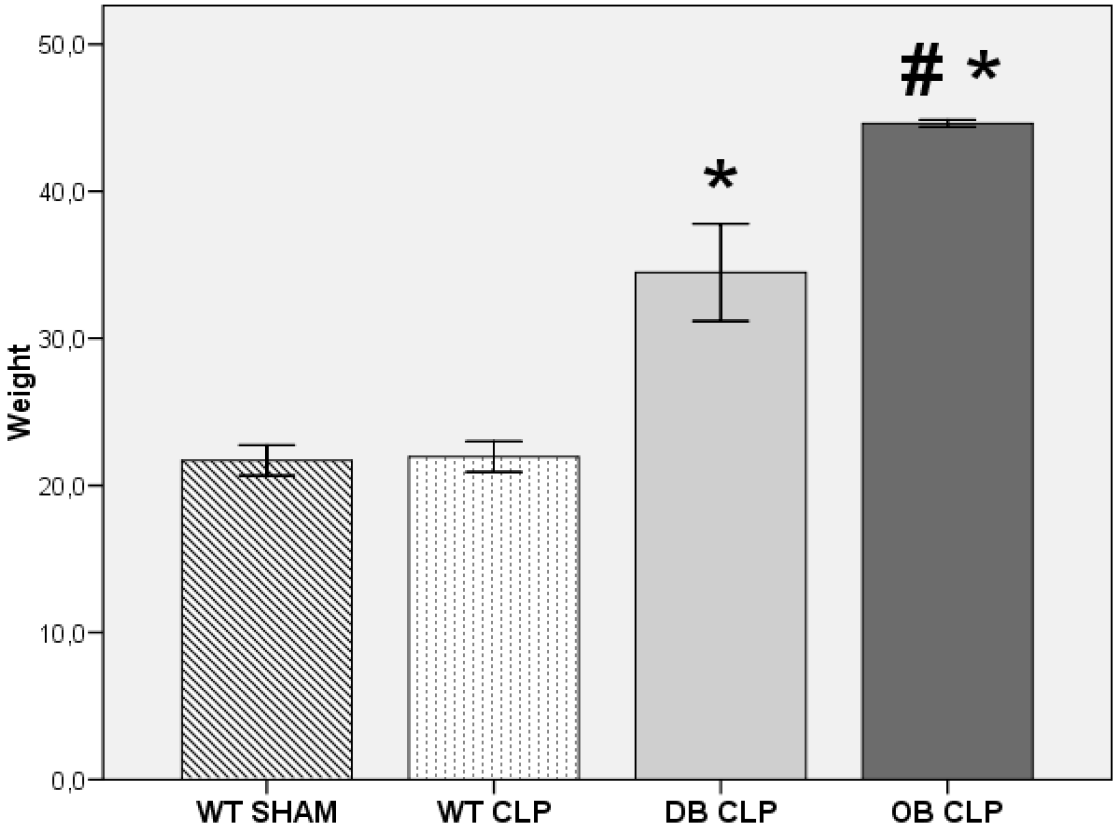

Figure 11: Weight [g] at 5 weeks of age

** indicates p < 0.05 vs the WT group following CLP surgery; # indicates p < 0.05 vs db/db group following CLP operation*

3.2 Blood Cell Count

Table 1 shows the mean amount of circulating blood cells for platelets, leukocytes and red blood cells (RBCs) per µL. In all four experimental groups no differences could be observed.

	platelets [10^3/µl]	leukocytes [10^3/µl]	red blood cells [10^6/µl]
WT Sham	893 ± 124	1.6 ± 0.2	8.3 ± 1.4
WT CLP	708 ± 319	1.5 ± 0.6	6.8 ± 2.6
db/db CLP	846 ± 28	0.9 ± 0.5	7.1 ± 0.7
ob/ob CLP	560 ± 230	1.5 ± 1.3	5.4 ± 2.2

Table 1: Circulating blood cells 6h after CLP or sham operation

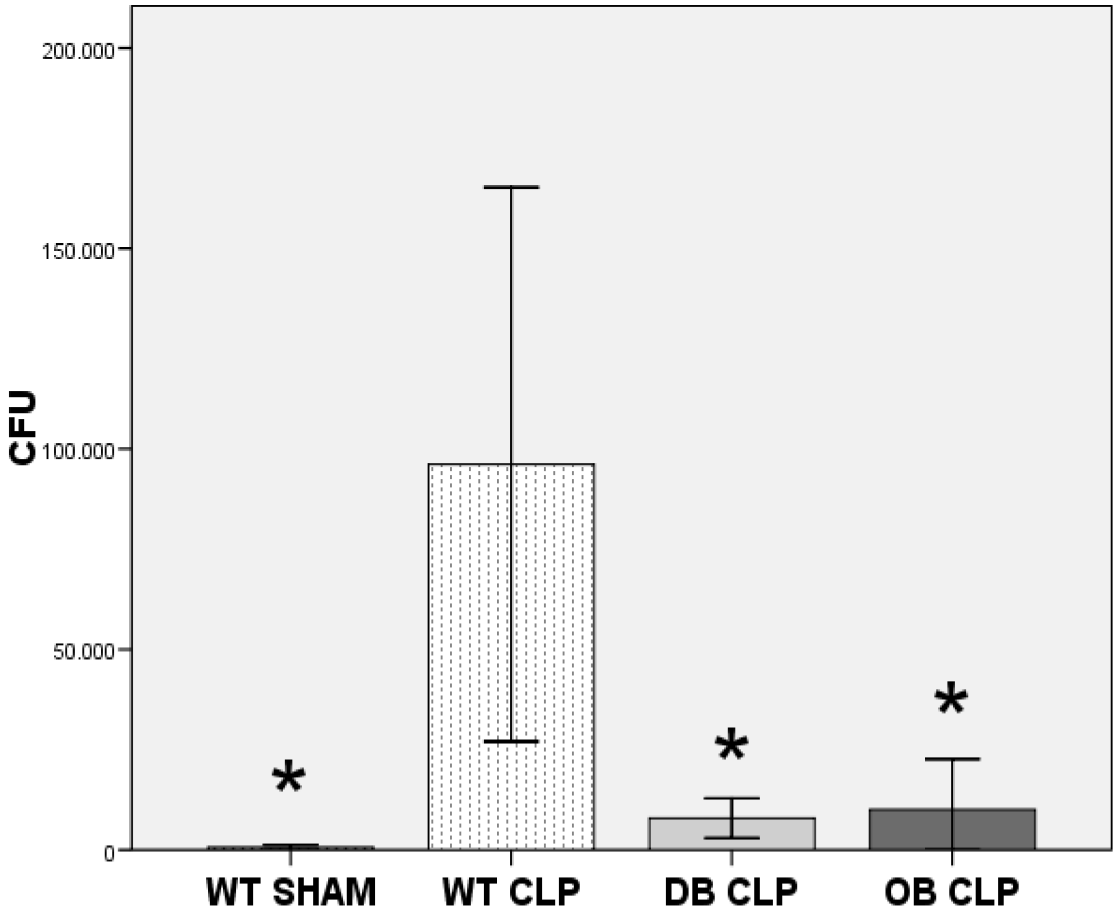

Figure 12: CFUs in peritoneal lavage fluid after 24h incubation

** indicates p < 0.05 vs the WT CLP group*

3.3 Bacterial Counts

Analysis of total CFUs per agar plate did show a significant difference between the group of WT mice following CLP operation (96,150 ± 69,141 CFUs) and the other three groups. While the sham group did not show any signs of bacterial infection in the peritoneal lavage fluid, mice following CLP procedure had significantly increased number of CFUs in the peritoneal fluid. However, both strains of mice, namely ob/ob and db/db, had decreased numbers of CFUs following induction of sepsis compared to the WT CLP group. Results are shown in *Figure 12.*

3.4 Wet-Dry Ratio

All four groups showed a very homogeneous ratio between the weight of the wet lung lobe and the dried weight. The two WT groups (the group following CLP operation and the group following sham operation) expressed the most similar results. The db/db group was close to the WT groups. In the ob/ob group a slightly lower ratio could be observed, but a significant difference could not be shown when compared to the other groups. Total weight at 0h incubation, 24h incubation and ratio are shown in *Table 2.*

	lung after 0h [mg]	**lung after 24h [mg]**	**ratio**
WT Sham	42.75 ± 4.99	9.25 ± 0.50	4.63 ± 0.62
WT CLP	56.40 ± 11.22	12.20 ± 2.17	4.61 ± 0.21
db/db CLP	43.00 ± 9.27	10.25 ± 2.22	4.41 ± 1.74
ob/ob CLP	39.00 ± 2.58	10.25 ± 0.96	3.81 ± 0.15

Table 2: Wet-dry ratio of lung lobes (dry lobes were incubated for 24h at 60°C)

3.5 Survival

During the observation period of one week all sham operated mice survived the full 168 hours. These mice recovered completely in two or three days following surgery. Only two mice of the WT CLP group died during the observation period, one 60 h after surgery, the other 72 h following operation. During these 72 h the other three mice of the WT CLP group showed a notable increase in vitality when compared to their littermates with fatal outcome. Mice of the ob/ob group (43.2 ± 18.2 days of survival) survived almost twice as long compared to mice from the db/db group (21.6 ± 10.0 days of survival). These results are shown in *Figure 13.*

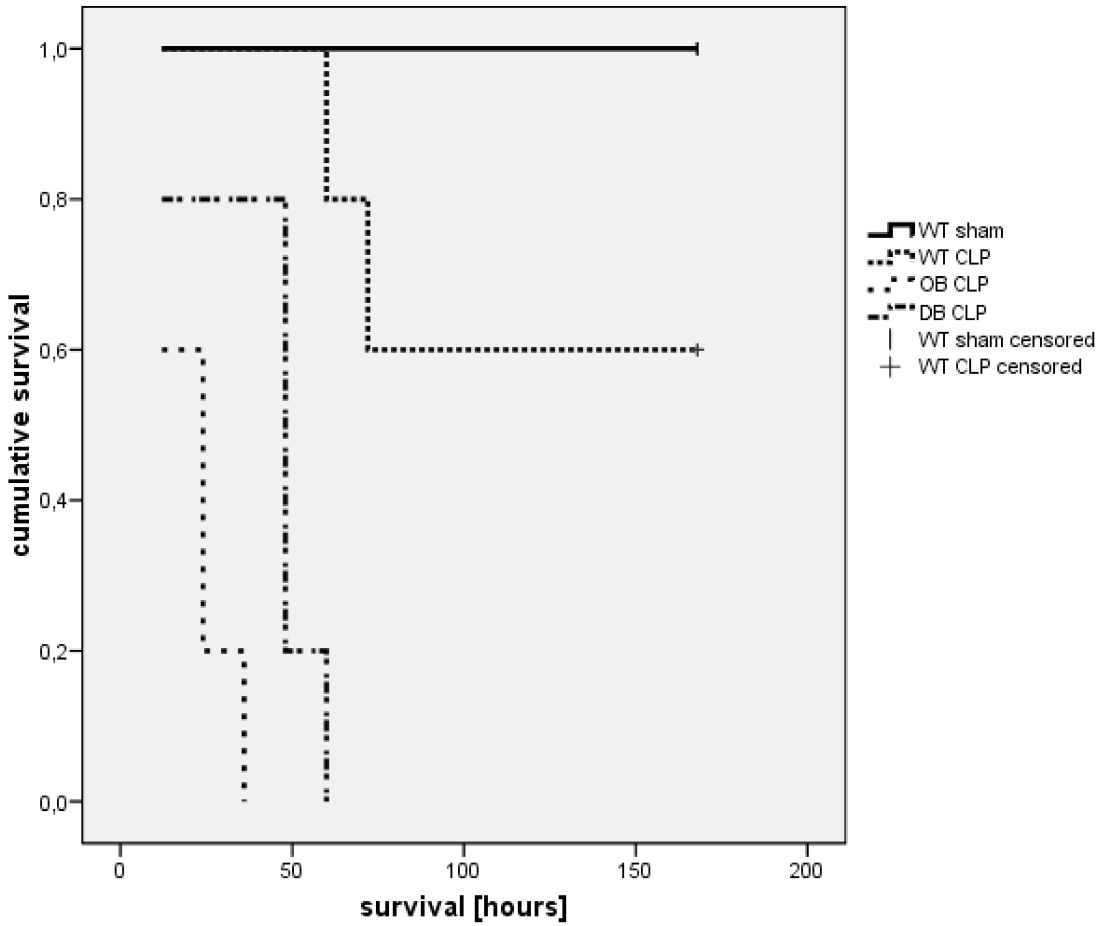

Figure 13: Survival rate after CLP or sham operation

3.6 Intravital Microscopy

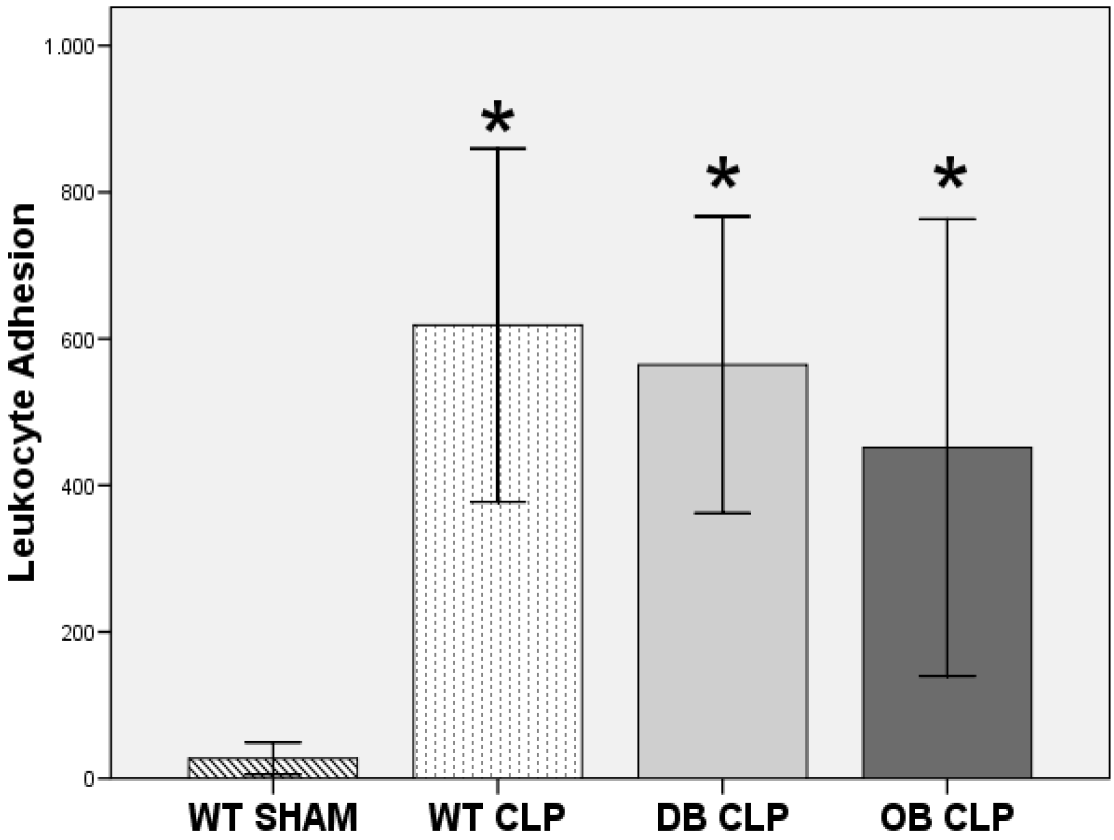

Figure 14: Leukocyte adhesion [#/mm^2 endothelial surface] observed in the small intestines

** indicates p < 0.05 vs the WT sham group*

3.6.1 Adherent Leukocytes

Mice after sham operation showed almost no leukocyte adhesion. The highest amount of stationary leukocytes was observed in the WT CLP group (618.5 ± 269.4 stationary leukocytes/mm^2) followed by the db/db group (564.6 ± 226.4 stationary leukocytes/mm^2). Leukocyte adhesion was lowest in the ob/ob group (451.6 ± 348.6 leukocytes/mm^2) among the three groups following CLP operation. The increase of leukocyte adhesion in the three groups experiencing CLP surgery was significant when compared to mice

after sham operation. However there was no significant difference between the three CLP groups, though there was a trend of less leukocyte adhesion in obese mice. (*Figure 14*)

3.6.2 Rolling Leukocytes

Leukocyte rolling was observed in all four groups. The db/db group (303 ± 154 rolling leukocytes) and the ob/ob group (304 ± 59 rolling leukocytes) showed a similar amount of rolling leukocytes. In the WT sham group (128 ± 16 rolling leukocytes) the amount of rolling leukocytes was smallest in all four groups. Mice of the WT CLP group (214 ± 95 rolling leukocytes) ranged between the other groups showing almost double the amount of rolling leukocytes when compared to the WT sham group and almost two thirds as many as their obese littermates elicited. These results underscore a notable trend of increased leukocyte rolling during inflammation and a further increase in murine obesity. However none of the observed differences were statistically significant. (*Figure 15*)

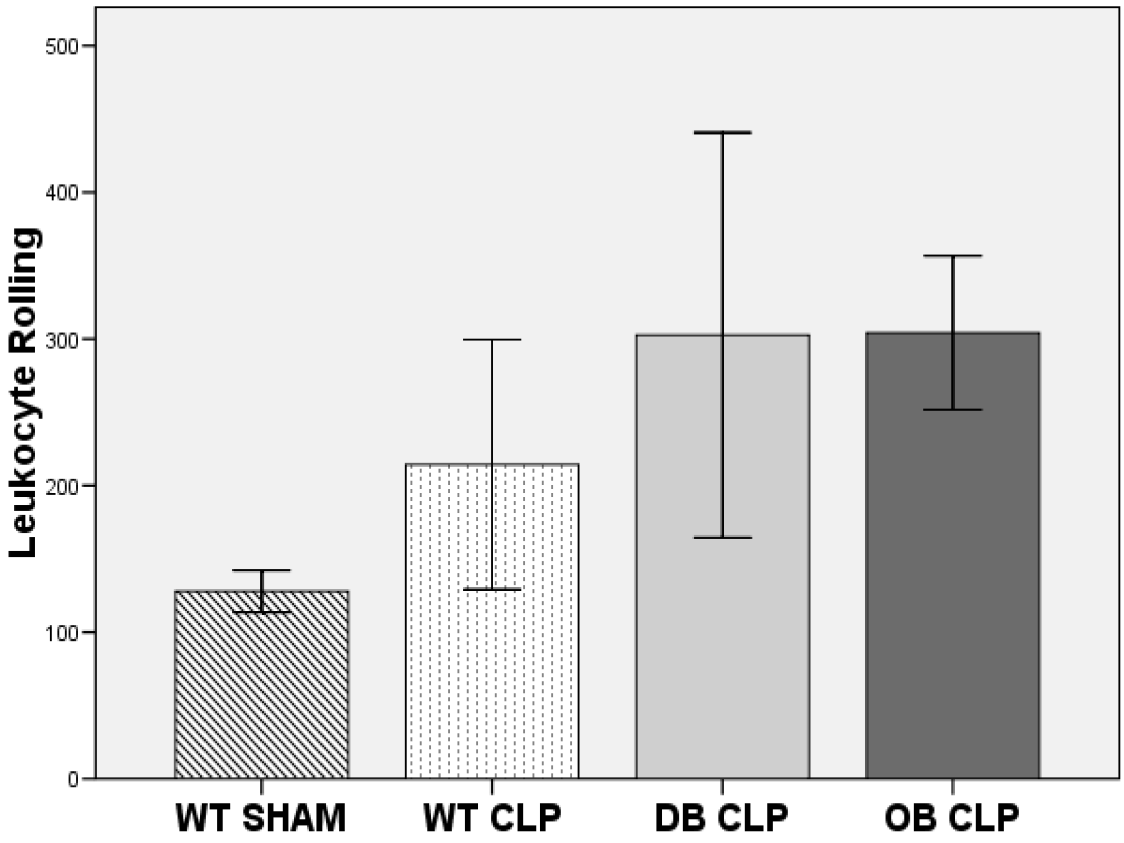

Figure 15: Leukocyte rolling observed in the small intestines

4 Discussion

In the United States more than 60% of adults are overweight and more than 30% are considered obese and among children more than 30% are overweight or at the risk for overweight (34). Obesity negatively influences the development and outcome of sepsis and both diseases are associated with high costs of care (7) (12) (13) (29). While the deleterious effects of obesity are well known, the pathophysiological causes for these effects are far from completely understood. Lately, the idea that a pro-inflammatory setting induced by increased serum levels of adipokines in obesity, deriving from the increase of adipose tissue, contributes to the higher mortality and morbidity of obese patients has become a widely accepted theory (12) (17) (18) (29). In a study conducted by Engstrom et al. mice on a high fat, high cholesterol diet showed elevated serum levels of leptin and insulin after 6 weeks and significantly increased serum levels of CCL2 and IL-6 after 16 weeks (35). Furthermore Aygun et al. showed that serum levels of leptin, IL-1β, IL-6 and TNF-α are significantly elevated in obese children (36). Several other studies have shown that morbidly obese patients admitted to ICUs show prolonged mechanical ventilation time, longer weaning periods and a significantly higher mortality (12) (29). El-Solh et al. compared obese patients admitted to the medical ICUs to non-obese patients and found that in-hospital mortality was 30% in morbidly obese patients compared to 17% in non-obese patients (12). It has been shown that alterations in the microcirculation play a significant role in the time course of sepsis and obesity (9) (10). These alterations include changes in arteriolar tone, increased leukocyte adhesion and extravasation and capillary leakage and result in tissue damage, organ failure and death (30). However, there is still the need to shed more light on the pathophysiological changes of the microcirculation during sepsis and obesity. The aims of this thesis were, therefore, to (a) describe the intestinal microcirculatory disturbances during sepsis and obesity, (b) to assess lung edema using the wet-dry ratio as a marker for endothelial dysfunction and capillary leakage, (c) to observe the effects of sepsis and obesity on circulating blood cells and (d) to assess the effects of sepsis and obesity on mortality comparing survival following CLP or sham surgery.

4.1.1 Weight

The significantly increased weight that was found in the two obese groups of mice was according to the expectations. In “Molecular Biology of the cell” ob/ob mice are described to have three times the weight of normal mice (19). When compared to WT mice neither ob/ob mice nor db/db mice showed an increase in body weight to this extend at the age of 5 weeks. These findings correlate to the observations of Singer et al. who also used ob/ob, db/db and WT mice that were 6 weeks old (25). However, a further weight gain could be observed after 5 weeks and obese mice reached three times the weight of WT mice after 15 weeks (results not shown). This massive weight gain over time suggests that differences of the observed parameters between the four groups might have been greater in older mice, since it has been shown that the pro-inflammatory state induced by sepsis correlates to the amount of total body fat (17) (18) (21).

4.1.2 Blood Cell Count

Although the amount of circulating platelets, leukocytes and RBCs was not significantly different between the groups, a slight trend towards decreased numbers of RBCs and platelets in groups experiencing CLP operation could be observed. These findings do not correlate with the literature. Singer et al. described a significantly lower number of circulating leukocytes in WT mice six hours following CLP operation when compared to WT mice after sham procedure. (37). Furthermore, their data shows that this decrease in circulating leukocytes was not significant two and four hours after surgery, respectively (37). Additionally in another report the same group of authors showed a significant decrease in circulating leukocytes and platelets when comparing WT, ob/ob and db/db mice six hours after CLP surgery to corresponding mice six hours after sham surgery (25). The findings in these papers suggest that the time between surgery and acquisition of the blood samples may have been too short in the present experiments. Another possible explanation for the discrepancies might be the different methods of data acquisition. While an automated system was used in the present report, Singer et al utilized a standard hemocytometer in order to assess blood cell counts. Interestingly, this lack of decrease in circulating blood cells

correlates to the lack of increase in leukocyte rolling and adhesion in the small intestines observed during IVM in the present set of data.

4.1.3 Bacterial Count

The amount of bacteria observed in the peritoneal cavities showed significantly higher numbers in mice from the WT CLP group. While there was a trend to increased CFUs in both septic obese groups of mice, a significant difference between the ob/ob group and db/db group and the WT sham group could not be shown. This indicates the development of a less severe sepsis in these two groups when compared to the WT CLP group. Possible reasons for this difference in severity of sepsis that may derive from the surgical procedure including insufficient diameter of puncture and subsequent coverage of the perforation after re-insertion of the cecum. Regarding the diameter of the puncture a 20G needle was used in the present experiments. This size should provide a sufficient puncture since other groups used 18G to 30G needles and observed significant differences between mice experiencing CLP operation and the corresponding control groups (25) (37) (38). Our data suggest that either coverage of the perforation occurred or that the time between surgery and acquisition of the samples was not sufficient for the development of a severe sepsis with live bacteria being present in the peritoneal cavity. Also several studies indicated significant differences in gut microbiota in obese subjects compared to lean subjects (39) (40). DiBaise et al. stated that ob/ob mice had 50% less Bacteroidetes and correspondingly more Firmicutes in their cecum when compared to their lean counterparts and that Bacteroidetes are also decreased in obese humans while Firmicutes are increased (39).

4.1.4 Wet-Dry Ratio

Sepsis results in a generalized activation of the host immune system often leading to lung injury and increased capillary permeability characterized by pulmonary edema (41). However, no significant difference between the four observed groups could be shown. Rojas et al. described a significant development of pulmonary edema in mice two hours after the intra-peritoneal administration of

lipopolysaccharide, an endotoxin produced by gram-negative bacteria (42). They assessed weight prior to and after desiccation under a vacuum at 25°C (42). In conclusion the different observations may result from the differences of the methodology as well as the method to induce sepsis (42). Asaduzzaman et al. have shown a significant increase in the wet-dry ratio in mice experiencing CLP operation when compared to mice experiencing Sham operation (41). The CLP procedure performed by this group only slightly differed from the operation performed in the present report. While three punctures were performed in the present set of experiments, Asaduzzaman only punctured the cecum twice (41). However, there were grave differences in the assessment of the weight of the lung. Most importantly, lungs were explanted 24 hours following CLP or sham operation and cannot be compared to the six hours explantation time of the present setting and secondly lungs were incubated for 72 hours as compared to 24 hours (41). Thus, it is a valid assumption that the different observations may result from the fact that sepsis induced by CLP surgery does not induce a significant formation of lung edema in the first 6 hours after the operation.

4.1.5 Survival

All WT mice following sham procedure survived for 168 hours and had fully recovered in the days following the operation. This supports the assumption that the Sham surgery does not induce inflammation. While 60% of the WT mice survived the CLP procedure, all obese mice, i.e. ob/ob and db/db, died within 68 hours following induction of sepsis. These results indicate that obesity causes the same increase of mortality during sepsis seen in humans (12).

4.1.6 IVM

Assessment of adherent leukocytes showed a significant difference between the groups experiencing CLP operation and the sham group. This was expected as leukocytes and platelets have been shown to circulate in an activated state during sepsis eliciting hyper-adhesive behavior (31-33). However, there was no significant difference between the three groups experiencing CLP operation and

there was a trend towards less adhesion in obese mice. Regarding leukocyte rolling no significant difference between the four groups could be observed. There was a moderate amount of rolling leukocytes in all animals of the sham group. This indicates leukocyte rolling to be a naturally occurring phenomenon in the small intestines that may be linked to the endothelial barrier function. The amount of rolling leukocytes was notably higher in WT CLP group when compared to the sham group and further increased in the two groups of obese mice. Significance may have been reached with a higher number of specimen. Singer et al. performed IVM 6h after CLP or sham operation and found a significant increase in leukocyte adherence to post-capillary venules in the small intestines (25). Secco et al. observed intestinal leukocyte rolling two hours and adhesion four hours after intra-peritoneal administration of low doses of caraggeenan to induce sepsis and found a significant increase in leukocyte rolling and adherence in septic mice compared to a control group that was treated with an intra-peritoneal injection of PBS (43). Torres-Duenas et al. assessed leukocyte rolling two hours and leukocyte adhesion four hours after either CLP with an 18G needle to induce moderate sepsis or a 30G needle to induce severe sepsis (38). They found a significant increase in leukocyte rolling and adhesion in WT mice with moderate sepsis when compared to WT mice following sham operation (38). However neither rolling nor adhesion of leukocytes was increased in WT mice with severe sepsis when compared to a sham group (38). The lack of increased adhesion observed in the present series of experiment correlated to the lack of decrease in circulating blood cells. This suggests that the decrease in circulating blood cells observed by other groups is causally linked to the increased numbers of adherent leukocytes. The findings of Torres-Duanes et al. are in accordance to the present findings. Torrres-Duanes and coworkers have described a reduction of neutrophil/endothelium interaction and a consequent failure of neutrophil migration into infection foci and hence a susceptibility to severe sepsis. Likewise, survival rates in the present set of experiments were worse in obese mice compared to WT mice showing that an increase in intestinal leukocyte adhesion is not a requisite for an worse survival rate in obese mice. However, subsequent experiments and additional quantification of severity of sepsis in mice used for IVM have to clarify

whether severity of sepsis plays a pivotal role in the disturbances seen in the intestinal microcirculation.

4.2 Conclusion

Endothelial dysfunction and capillary leakage could not be quantified using the wet-dry ratio of the lung as a marker for pulmonary edema. However the findings of other groups suggest that six hours of sepsis may not be enough time for the development of a lung edema. Therefore, another set of experiments with a longer period of sepsis would be interesting to perform. Assessment of leukocyte rolling and adhesion in post-capillary venules did not provide proof that leukocyte activity is increased in obese subjects suffering from sepsis when compared to lean subjects. This contradicts the findings of other groups and could be explained by an increased severity of sepsis as shown by decreased survival rates in obese mice following induction of sepsis . Hence, in another set of experiments a method of quantification of sepsis may clarify whether the observed results are influenced by the severity of sepsis. Nevertheless, additional investigations of the deleterious effects of sepsis and obesity and the underlying pathophysiological mechanisms are mandatory as further knowledge of the causes of these effects may lead to the development of new treatment options.

5 Bibliography

1. WHO | Obesity and overweight [Internet]. [cited 2011 Mai 8];Available from: http://www.who.int/mediacentre/factsheets/fs311/en/index.html

2. Freedman DS, Centers for Disease Control and Prevention (CDC). Obesity --- United States, 1988--2008. Morbidity and Mortality Weekly Report. 2011 Jan 14;(Vol. 60):73–7.

3. Böcker W. Pathologie : mit 164 Tabellen [Online-Zugang interaktive Extras]. 3. Aufl. München [u.a.]: Elsevier Urban und Fischer; 2006.

4. Haslam DW, James WPT. Obesity. The Lancet. 2005 Okt;366(9492):1197–209.

5. Bickley L. Bates' guide to physical examination and history taking. 10. Edition Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2009.

6. Bone R, Balk R, Cerra F, Dellinger R, Fein A, Knaus W, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992 June;101(6):1644–55.

7. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: Analysis of incidence, outcome, and associated costs of care. Critical Care Medicine. 2001 July;29(7):1303–10.

8. Martin GS, Mannino DM, Eaton S, Moss M. The Epidemiology of Sepsis in the United States from 1979 through 2000. N Engl J Med. 2003 Apr;348(16):1546–54.

9. Villela NR, Kramer-Aguiar LG, Bottino DA, Wiernsperger N, Bouskela E. Metabolic disturbances linked to obesity: the role of impaired tissue perfusion. Arquivos Brasileiros de Endocrinologia; Metabologia. 2009;53(2):238–45.

10. Lee WL, Liles WC. Endothelial activation, dysfunction and permeability during severe infections. Current opinion in hematology. 2011;18(3):191.

11. STATISTIK AUSTRIA - BMI (Body Mass Index) [Internet]. [cited 2011 Mai 8];Available from: http://www.statistik.at/web_de/statistiken/gesundheit/gesundheitsdeterminanten/bmi_body_mass_index/025420.html

12. El-Solh A. Morbid Obesity in the Medical ICU. Chest. 2001 Dez;120(6):1989–97.

13. von Lengerke T, Krauth C. Economic costs of adult obesity: A review of recent European studies with a focus on subgroup-specific costs. Maturitas. 2011 July;69(3):220–9.

14. Han TS, van Leer EM, Seidell JC, Lean MEJ. Waist circumference action levels in the identification of cardiovascular risk factors: prevalence study in a random sample. BMJ. 1995 Nov 25;311(7017):1401–5.

15. Berker D, Koparal S, Isik S, Pasaoglu L, Aydin Y, Erol K, et al. Compatibility of different methods for the measurement of visceral fat in different body mass index strata. Diagn Interv Radiol [Internet]. 2009 [cited 2011 Aug 27];Available from: http://dirjournal.org/summary_en_doi.php3?doi=10.4261/1305-3825.DIR.2749-09.1

16. Ledoux S, Coupaye M, Essig M, Msika S, Roy C, Queguiner I, u. a. Traditional Anthropometric Parameters Still Predict Metabolic Disorders in Women With Severe Obesity. Obesity. 2009 Okt;18(5):1026–32.

17. Ahima RS. Adipose Tissue as an Endocrine Organ. Obesity. 2006 Aug;14:242S-249S.

18. Lyon CJ. Minireview: Adiposity, Inflammation, and Atherogenesis. Endocrinology. 2003 June;144(6):2195–200.

19. Alberts B. Molecular biology of the cell. 5. Aufl. New York: Garland Science; 2008.

20. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González Á, Esquivel-Chirino C, et al. Inflammation, Oxidative Stress, and Obesity. IJMS. 2011 May;12(5):3117–32.

21. Vachharajani V, Granger DN. Adipose tissue: A motor for the inflammation associated with obesity. IUBMB Life. 2009 Apr;61(4):424–30.

22. Vachharajani V. Obesity and Sepsis. Journal of Intensive Care Medicine. 2006 Sep;21(5):287–95.

23. Hillenbrand A, Knippschild U, Weiss M, Schrezenmeier H, Henne-Bruns D, Huber-Lang M, et al. Sepsis induced changes of adipokines and cytokines-septic patients compared to morbidly obese patients. BMC surgery. 2010;10(1):26.

24. Wang Z, Nakayama T. Inflammation, a link between obesity and cardiovascular disease. Mediators Inflamm. 2010;535918.

25. Singer G, Stokes KY, Terao S, Granger DN. Sepsis-induced intestinal microvascular and inflammatory responses in obese mice. Shock. 2009 March;31(3):275–9.

26. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive care medicine. 2003;29(4):530–8.

27. Rotstein OD. Pathogenesis of Multiple Organ Dysfunction Syndrome: Gut Origin, Protection, and Decontamination. Surgical Infections. 2000 Sep;1(3):217–25.

28. Magnotti LJ, Deitch EA. Burns, Bacterial Translocation, Gut Barrier Function, and Failure. Journal of Burn Care & Rehabilitation. 2005 Sep;26(5):383–91.

29. Yaegashi M. Outcome of Morbid Obesity in the Intensive Care Unit. Journal of Intensive Care Medicine. 2005 May;20(3):147–54.

30. McGown CC, Brookes ZLS. Beneficial effects of statins on the microcirculation during sepsis: the role of nitric oxide. British journal of anaesthesia. 2007;98(2):163.

31. Russwurm S, Vickers J, Meier-Hellmann A, Spangenberg P, Bredle D, Reinhart K, et al. Platelet and Leukocyte Activation Correlate with the Severity of Septic Organ Dysfunction. Shock. 2002 Apr;17(4):263–8.

32. Mazzoni MC, Schmid-Schönbein GW. Mechanisms and consequences of cell activation in the microcirculation. Cardiovascular research. 1996;32(4):709.

33. Gawaz M, Dickfeld T, Bogner C, Fateh-Moghadam S, Neumann FJ. Platelet function in septic multiple organ dysfunction syndrome. Intensive care medicine. 1997;23(4):379–85.

34. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. JAMA: the journal of the American Medical Association. 2004;291(23):2847.

35. Engstrom LW, Bober L, Chen SC, Fine JS, Li Y, Stanton MC, u. a. Kinetic Assessment and Therapeutic Modulation of Metabolic and Inflammatory Profiles in Mice on a High-Fat and Cholesterol Diet. PPAR research. 2010;2010.

36. Aygun AD, Gungor S, Ustundag B, Gurgoze MK, Sen Y. Proinflammatory Cytokines and Leptin Are Increased in Serum of Prepubertal Obese Children. Mediators of Inflammation. 2005;2005:180–3.

37. Singer G, Urakami H, Specian RD, Stokes KY, Granger DN. Platelet Recruitment in the Murine Hepatic Microvasculature During Experimental Sepsis: Role of Neutrophils. Microcirculation. 2006 Jan;13(2):89–97.

38. Torres-Duenas D, Celes MRN, Freitas A, Alves-Filho JC, Spiller F, Dal-Secco D, et al. Peroxynitrite mediates the failure of neutrophil migration in severe polymicrobial sepsis in mice. British journal of pharmacology. 2007;152(3):341–52.

39. DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE. Gut microbiota and its possible relationship with obesity. In: Mayo Clinic Proceedings. 2008. p. 460.

40. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring Bacterial Community of Human Gut Microbiota Reveals an Increase in Lactobacillus in Obese Patients and Methanogens in Anorexic Patients. PLoS ONE. 2009 Sep 23;4:e7125.

41. Asaduzzaman M, Rahman M, Jeppsson B, Thorlacius H. P-selectin glycoprotein-ligand-1 regulates pulmonary recruitment of neutrophils in a platelet-independent manner in abdominal sepsis. British journal of pharmacology. 2009;156(2):307–15.

42. Rojas M, Woods CR, Mora AL, Xu J, Brigham KL. Endotoxin-induced lung injury in mice: structural, functional, and biochemical responses. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2005;288(2):L333.

43. Secco DD, Paron JA, de Oliveira SH., Ferreira SH, Silva JS, Cunha FQ. Neutrophil migration in inflammation: nitric oxide inhibits rolling, adhesion and induces apoptosis. Nitric Oxide. 2003;9(3):153–64.

6 Anesthetization Protocols

6.1 Anesthetization Protocol for Domitor and Ketamin

	Domitor			Ketamin		
KG Maus [g]	**mg**	**ml**	**1:10**	**mg**	**ml**	**1:10**
20	0.005	0.005	**0.05**	1.5	0.015	**0.15**
20.5	0.005125	0.005125	**0.05**	1.5375	0.015375	**0.15**
21	0.00525	0.00525	**0.05**	1.575	0.01575	**0.16**
21.5	0.005375	0.005375	**0.05**	1.6125	0.016125	**0.16**
22	0.0055	0.0055	**0.06**	1.65	0.0165	**0.17**
22.5	0.005625	0.005625	**0.06**	1.6875	0.016875	**0.17**
23	0.00575	0.00575	**0.06**	1.725	0.01725	**0.17**
23.5	0.005875	0.005875	**0.06**	1.7625	0.017625	**0.18**
24	0.006	0.006	**0.06**	1.8	0.018	**0.18**
24.5	0.006125	0.006125	**0.06**	1.8375	0.018375	**0.18**
25	0.00625	0.00625	**0.06**	1.875	0.01875	**0.19**
25.5	0.006375	0.006375	**0.06**	1.9125	0.019125	**0.19**
26	0.0065	0.0065	**0.07**	1.95	0.0195	**0.20**
26.5	0.006625	0.006625	**0.07**	1.9875	0.019875	**0.20**
27	0.00675	0.00675	**0.07**	2.025	0.02025	**0.20**
27.5	0.006875	0.006875	**0.07**	2.0625	0.020625	**0.21**
28	0.007	0.007	**0.07**	2.1	0.021	**0.21**
28.5	0.007125	0.007125	**0.07**	2.1375	0.021375	**0.21**
29	0.00725	0.00725	**0.07**	2.175	0.02175	**0.22**
29.5	0.007375	0.007375	**0.07**	2.2125	0.022125	**0.22**
30	0.0075	0.0075	**0.08**	2.25	0.0225	**0.23**
30.5	0.007625	0.007625	**0.08**	2.2875	0.022875	**0.23**
31	0.00775	0.00775	**0.08**	2.325	0.02325	**0.23**
31.5	0.007875	0.007875	**0.08**	2.3625	0.023625	**0.24**
32	0.008	0.008	**0.08**	2.4	0.024	**0.24**
32.5	0.008125	0.008125	**0.08**	2.4375	0.024375	**0.24**
33	0.00825	0.00825	**0.08**	2.475	0.02475	**0.25**
33.5	0.008375	0.008375	**0.08**	2.5125	0.025125	**0.25**
34	0.0085	0.0085	**0.09**	2.55	0.0255	**0.26**
34.5	0.008625	0.008625	**0.09**	2.5875	0.025875	**0.26**
35	0.00875	0.00875	**0.09**	2.625	0.02625	**0.26**
35.5	0.008875	0.008875	**0.09**	2.6625	0.026625	**0.27**
36	0.009	0.009	**0.09**	2.7	0.027	**0.27**
36.5	0.009125	0.009125	**0.09**	2.7375	0.027375	**0.27**
37	0.00925	0.00925	**0.09**	2.775	0.02775	**0.28**
37.5	0.009375	0.009375	**0.09**	2.8125	0.028125	**0.28**
38	0.0095	0.0095	**0.10**	2.85	0.0285	**0.29**
38.5	0.009625	0.009625	**0.10**	2.8875	0.028875	**0.29**
39	0.00975	0.00975	**0.10**	2.925	0.02925	**0.29**
39.5	0.009875	0.009875	**0.10**	2.9625	0.029625	**0.30**
40	0.01	0.01	**0.10**	3	0.03	**0.30**
40.5	0.010125	0.010125	**0.10**	3.0375	0.030375	**0.30**
41	0.01025	0.01025	**0.10**	3.075	0.03075	**0.31**

41.5	0.010375	0.010375	**0.10**	3.1125	0.031125	**0.31**
42	0.0105	0.0105	**0.11**	3.15	0.0315	**0.32**
42.5	0.010625	0.010625	**0.11**	3.1875	0.031875	**0.32**
43	0.01075	0.01075	**0.11**	3.225	0.03225	**0.32**
43.5	0.010875	0.010875	**0.11**	3.2625	0.032625	**0.33**
44	0.011	0.011	**0.11**	3.3	0.033	**0.33**
44.5	0.011125	0.011125	**0.11**	3.3375	0.033375	**0.33**
45	0.01125	0.01125	**0.11**	3.375	0.03375	**0.34**
45.5	0.011375	0.011375	**0.11**	3.4125	0.034125	**0.34**
46	0.0115	0.0115	**0.12**	3.45	0.0345	**0.35**
46.5	0.011625	0.011625	**0.12**	3.4875	0.034875	**0.35**
47	0.01175	0.01175	**0.12**	3.525	0.03525	**0.35**
47.5	0.011875	0.011875	**0.12**	3.5625	0.035625	**0.36**
48	0.012	0.012	**0.12**	3.6	0.036	**0.36**
48.5	0.012125	0.012125	**0.12**	3.6375	0.036375	**0.36**
49	0.01225	0.01225	**0.12**	3.675	0.03675	**0.37**
49.5	0.012375	0.012375	**0.12**	3.7125	0.037125	**0.37**
50	0.0125	0.0125	**0.13**	3.75	0.0375	**0.38**
50.5	0.012625	0.012625	**0.13**	3.7875	0.037875	**0.38**
51	0.01275	0.01275	**0.13**	3.825	0.03825	**0.38**
51.5	0.012875	0.012875	**0.13**	3.8625	0.038625	**0.39**
52	0.013	0.013	**0.13**	3.9	0.039	**0.39**
52.5	0.013125	0.013125	**0.13**	3.9375	0.039375	**0.39**
53	0.01325	0.01325	**0.13**	3.975	0.03975	**0.40**
53.5	0.013375	0.013375	**0.13**	4.0125	0.040125	**0.40**
54	0.0135	0.0135	**0.14**	4.05	0.0405	**0.41**
54.5	0.013625	0.013625	**0.14**	4.0875	0.040875	**0.41**
55	0.01375	0.01375	**0.14**	4.125	0.04125	**0.41**

6.2 Anesthetization protocol for Butomidor and Antisedan

	Butomidor			Antisedan		
KG Maus [g]	**mg**	**ml**	**1:10**	**mg**	**ml**	**1:10**
20	0.02	0.002	**0.02**	0.02	0.004	**0.04**
20.5	0.0205	0.00205	**0.02**	0.0205	0.0041	**0.04**
21	0.021	0.0021	**0.02**	0.021	0.0042	**0.04**
21.5	0.0215	0.00215	**0.02**	0.0215	0.0043	**0.04**
22	0.022	0.0022	**0.02**	0.022	0.0044	**0.04**
22.5	0.0225	0.00225	**0.02**	0.0225	0.0045	**0.05**
23	0.023	0.0023	**0.02**	0.023	0.0046	**0.05**
23.5	0.0235	0.00235	**0.02**	0.0235	0.0047	**0.05**
24	0.024	0.0024	**0.02**	0.024	0.0048	**0.05**
24.5	0.0245	0.00245	**0.02**	0.0245	0.0049	**0.05**
25	0.025	0.0025	**0.03**	0.025	0.005	**0.05**
25.5	0.0255	0.00255	**0.03**	0.0255	0.0051	**0.05**
26	0.026	0.0026	**0.03**	0.026	0.0052	**0.05**
26.5	0.0265	0.00265	**0.03**	0.0265	0.0053	**0.05**
27	0.027	0.0027	**0.03**	0.027	0.0054	**0.05**
27.5	0.0275	0.00275	**0.03**	0.0275	0.0055	**0.06**
28	0.028	0.0028	**0.03**	0.028	0.0056	**0.06**
28.5	0.0285	0.00285	**0.03**	0.0285	0.0057	**0.06**
29	0.029	0.0029	**0.03**	0.029	0.0058	**0.06**
29.5	0.0295	0.00295	**0.03**	0.0295	0.0059	**0.06**
30	0.03	0.003	**0.03**	0.03	0.006	**0.06**
30.5	0.0305	0.00305	**0.03**	0.0305	0.0061	**0.06**
31	0.031	0.0031	**0.03**	0.031	0.0062	**0.06**
31.5	0.0315	0.00315	**0.03**	0.0315	0.0063	**0.06**
32	0.032	0.0032	**0.03**	0.032	0.0064	**0.06**
32.5	0.0325	0.00325	**0.03**	0.0325	0.0065	**0.07**
33	0.033	0.0033	**0.03**	0.033	0.0066	**0.07**
33.5	0.0335	0.00335	**0.03**	0.0335	0.0067	**0.07**
34	0.034	0.0034	**0.03**	0.034	0.0068	**0.07**
34.5	0.0345	0.00345	**0.03**	0.0345	0.0069	**0.07**
35	0.035	0.0035	**0.04**	0.035	0.007	**0.07**
35.5	0.0355	0.00355	**0.04**	0.0355	0.0071	**0.07**
36	0.036	0.0036	**0.04**	0.036	0.0072	**0.07**
36.5	0.0365	0.00365	**0.04**	0.0365	0.0073	**0.07**
37	0.037	0.0037	**0.04**	0.037	0.0074	**0.07**
37.5	0.0375	0.00375	**0.04**	0.0375	0.0075	**0.08**
38	0.038	0.0038	**0.04**	0.038	0.0076	**0.08**
38.5	0.0385	0.00385	**0.04**	0.0385	0.0077	**0.08**
39	0.039	0.0039	**0.04**	0.039	0.0078	**0.08**
39.5	0.0395	0.00395	**0.04**	0.0395	0.0079	**0.08**
40	0.04	0.004	**0.04**	0.04	0.008	**0.08**
40.5	0.0405	0.00405	**0.04**	0.0405	0.0081	**0.08**
41	0.041	0.0041	**0.04**	0.041	0.0082	**0.08**
41.5	0.0415	0.00415	**0.04**	0.0415	0.0083	**0.08**
42	0.042	0.0042	**0.04**	0.042	0.0084	**0.08**

42.5	0.0425	0.00425	**0.04**	0.0425	0.0085	**0.09**
43	0.043	0.0043	**0.04**	0.043	0.0086	**0.09**
43.5	0.0435	0.00435	**0.04**	0.0435	0.0087	**0.09**
44	0.044	0.0044	**0.04**	0.044	0.0088	**0.09**
44.5	0.0445	0.00445	**0.04**	0.0445	0.0089	**0.09**
45	0.045	0.0045	**0.05**	0.045	0.009	**0.09**
45.5	0.0455	0.00455	**0.05**	0.0455	0.0091	**0.09**
46	0.046	0.0046	**0.05**	0.046	0.0092	**0.09**
46.5	0.0465	0.00465	**0.05**	0.0465	0.0093	**0.09**
47	0.047	0.0047	**0.05**	0.047	0.0094	**0.09**
47.5	0.0475	0.00475	**0.05**	0.0475	0.0095	**0.10**
48	0.048	0.0048	**0.05**	0.048	0.0096	**0.10**
48.5	0.0485	0.00485	**0.05**	0.0485	0.0097	**0.10**
49	0.049	0.0049	**0.05**	0.049	0.0098	**0.10**
49.5	0.0495	0.00495	**0.05**	0.0495	0.0099	**0.10**
50	0.05	0.005	**0.05**	0.05	0.01	**0.10**
50.5	0.0505	0.00505	**0.05**	0.0505	0.0101	**0.10**
51	0.051	0.0051	**0.05**	0.051	0.0102	**0.10**
51.5	0.0515	0.00515	**0.05**	0.0515	0.0103	**0.10**
52	0.052	0.0052	**0.05**	0.052	0.0104	**0.10**
52.5	0.0525	0.00525	**0.05**	0.0525	0.0105	**0.11**
53	0.053	0.0053	**0.05**	0.053	0.0106	**0.11**
53.5	0.0535	0.00535	**0.05**	0.0535	0.0107	**0.11**
54	0.054	0.0054	**0.05**	0.054	0.0108	**0.11**
54.5	0.0545	0.00545	**0.05**	0.0545	0.0109	**0.11**
55	0.055	0.0055	**0.06**	0.055	0.011	**0.11**

7 Curriculum Vitae

Personal Data

Name:	Georg Stephan Pirker
Date of birth:	02.06.1986
Place of birth:	Graz
Nationality:	Austria

Scientific Curriculum Vitae

University:	Medical University of Graz	since 02/2005
Grammar School:	Akademisches Gymnasium Graz	02/1999 – 06/2004
	B(R)G Carnerigasse Graz	09/1996 – 02/1999
Elementary School:	VS Graben Graz	09/1994 – 06/1996
	VS Muchargasse Graz	09/1992 – 06/1994