Diploma Thesis

on the subject of

Murine Hepatic Microcirculatory Changes during Sepsis and Obesity – The Role of Leptin

submitted by

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Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Graz, March 03, 2015 Manfred Gerald Sagmeister eh.

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Glossary

ACD	**A**cid–**C**itrate–**D**extrose
ALT	**AL**anine **T**ransaminase
aPTT	**a**ctivated **P**artial **T**hromboplastin **T**ime
ARDS	**A**cute **R**epiratory **D**istress **S**yndrome
BAT	**B**rown **A**dipose **T**issue
Bc	**B**rady**C**ardia
BMI	**B**ody - **M**ass - **I**ndex
BP	**B**lood **P**ressure
CARS	**C**ompensatory **A**nti-inflammatory **R**esponse **S**yndrome
CFSE	**C**arboxyfluorescein **F**iacetate **S**uccinimidyl **E**ster
CLP	**C**ecal **L**igation and **P**uncture
CO	**C**arbon **M**onoxide
CRP	**C** - **R**eactive **P**roteine
CT	**C**omputed **T**omography
DIC	**D**isseminated **I**ntravascular **C**oagulation
DIO	**D**iet **I**nduced **O**besity
ECM	**E**xtra**C**ellular **M**atrix
GCS	**G**lasgow **C**oma **S**core
H_2S	**H**ydrogen **S**ulfide
HA	**H**epatic **A**rtery
HMWK	**H**igh-**M**olecular-**W**eight **K**ininogen
i.p.	**i**ntra**p**eritoneal
ICAM-1	**I**nter**C**ellular **A**dhesion **M**olecule-**1**

ICD-10	**I**nternational **C**lassification of **D**iseases **-10**th revision
ICU	**I**ntensive **C**are **U**nit
IFN	**I**nter**F**ero**N**
IL	**I**nter**L**eukin
INR	**I**nternational **N**ormalized **R**atio
IVM	**I**ntra**V**ital **M**icroscopy
JAM	**J**unctional **A**dhesion **M**olecules
LSD	**L**east **S**ignificant **D**ifference
MAP	**M**ean **A**rterial **P**ressure
MIMIC	**M**ultiparameter **I**ntelligent **M**onitoring in **I**ntensive **C**are
MODS	**M**ultiple **O**rgan **D**ysfunction **S**yndrome
Mpx	**M**ega**p**i**x**el
MRI	**M**agnetic **R**esonance **I**maging
NaCl	Sodium (ger: **Na**trium) **C**h**l**oride
NK cells	**N**atural **K**iller cells
NO	**N**itric **O**xide
OECD	**O**rganisation **E**conomic **C**o-operation and **D**evelopment
PaCO$_2$	**P**artial pressure of **a**rterial **C**arbon **D**ioxide
PaO$_2$	**P**artial pressure of **a**rterial **O**xygen
PaO$_2$/FiO$_2$	Ratio of **P**artial pressure of **a**rterial **O**xygen to **F**ractional **i**nspired **O**xygen
PBS	**P**hosphate **B**uffered **S**aline
PECAM-1	**P**latelet/**E**ndothelial **C**ell **A**dhesion **M**olecule-**1**
PELOD	**P**ediatric **L**ogistic **O**rgan **D**ysfunction
PMODS	**P**ediatric **M**ultiple **O**rgan **D**ysfunction **S**yndrome
PV	**P**ortal **V**ein
px	**p**i**x**el

rpm	**r**ounds **p**er **m**inute
SBP	**S**ystolic **B**lood **P**ressure
SD	**S**tandard **D**eviation
SIRS	**S**ystemic **I**nflammatory **R**esponse **S**yndrome
SOAP	**S**epsis **O**ccurrence in **A**cutely ill **P**atients
SPF	**S**pecific **P**athogen–**F**ree
SvO_2	**S**aturation of mixed **v**enous **O**xygen
TAC	**T**otal **A**ntioxidant **C**apacity
TBARS	**T**hio**B**arbituric **A**cid-**R**eactive **S**ubstances
Tc	**T**achy**C**ardia
TEM	**T**rans**E**ndothelial **M**igration
TF	**T**issue **F**actor
TFPI	**T**issue **F**actor **P**athway **I**nhibitor
TGF	**T**ransforming **G**rowth **F**actor
THV	**T**erminal **H**epatic **V**enule
TNF	**T**umor **N**ecrosis **F**actor
U.S.	**U**nited **S**tates
USA	**U**nited **S**tates of **A**merica
VCAM-1	**V**ascular **C**ell **A**dhesion **M**olecule-**1**
vWF	**v**on **W**illebrand **F**actor
WAT	**W**hite **A**dipose **T**issue
WBCs	**W**hite **B**lood **C**ell**s**
WC	**W**aist **C**ircumference
WHO	**W**orld **H**ealth **O**rganization
WHR	**W**aist to **H**ip **R**atio
WT	**W**ild **T**ype

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1 | Abstract

1.1 Abstract

Background: Both, clinical obesity, as a predisposition for several diseases, and sepsis, as a leading cause of death in critically ill patients, are global major health care problems at all ages. There is lack of knowledge regarding the microcirculatory changes of the liver in sepsis under the influence of obesity.

Methods: We used intravital microscopy *(IVM)* to quantify leukocyte as well as platelet rolling and adhesion in hepatic postcapillary venules in either sham operated or septic mice. Polymicrobial sepsis was induced by cecal ligation and puncture *(CLP)* in lean wild type *(WT)* mice, two genetically altered models of obesity, namely, db/db and ob/ob mice, as well as a diet induced obesity *(DIO)* group. To determine the role of the adipocytokine leptin, which is, inter alia, responsible for the sense of satiety, we exogenously supplied leptin subcutaneously in a leptin deficient mouse group *(ob/ob + leptin)*.

Results: Increased leukocyte rolling was seen in sham operated leptin deficient obese mice (ob/ob and ob/ob + leptin), whereas leukocyte adhesion was highest in the high caloric fed group *(DIO)*, with nearly no changes in platelet recruitment in the non-septic sham groups. CLP induced sepsis increased the number of rolling leukocytes in DIO and ob/ob groups associated with a reversibility by leptin treatment. Leukocyte adherence was highest in septic WT mice, followed by DIO and ob/ob + leptin group, whereby the most heavy db/db mice had lowest sticking leukocyte counts. Platelet rolling was significantly increased in septic ob/ob mice, whereas a reduction of about 50% was demonstrated when treated with leptin. The ob/ob group had significantly elevated platelet adhesion and leptin treatment had no protective effect.

Conclusions: The findings of this study indicate that the environment of diet induced obesity compared to genetically altered models of obesity reacts differently in an inflammatory state such as sepsis, as demonstrated in our murine hepatic microcirculation model. Leptin plays likely a key role in the mediation of inflammatory responses and is probably receptor– and blood-level depended.

1.2 Zusammenfassung

Hintergrund: Sowohl die Fettleibigkeit, als Prädisposition für verschiedene Erkrankungen, als auch Sepsis, als führende Todesursache bei kritisch erkrankten Menschen, sorgen im Gesundheitswesen weltweit und in allen Altersgruppen für Probleme. Es besteht eine Wissenslücke bezüglich Veränderungen der Mikrozirkulation in der Leber während Sepsis unter dem Einfluss von Fettleibigkeit.

Methoden: Wir nutzten Intravitalmikroskopie *(IVM)* zur Quantifizierung der Adhäsion und dem Rollen von Leuko– und Thrombozyten in postkapillären Venulen der Leber in sham-operierten oder septischen Mäusen. Polymikrobielle Sepsis wurde durch zökale Ligation und Punktion *(CLP)* in normalgewichtigen 'Wildtyp' *(WT)* Mäusen, zwei genetisch veränderten Modellen von Fettleibigkeit, namentlich, db/db und ob/ob Mäusen, und weiters in einer Gruppe von diät-induzierter Adipositas *(DIO)*, hervorgerufen. Um die Rolle des Adipozytokines Leptin zu ermitteln, welches unter anderem verantwortlich für das Sättigungsgefühl ist, führten wir subkutan Leptin, in einer Leptin-defizienten Gruppe von Mäusen (ob/ob + leptin), zu.

Ergebnisse: Erhöhtes Leukozyten-Rollen wurde in sham-operierten Leptin-defizienten Mäusen (ob/ob and ob/ob + leptin) beobachtet, wobei Leukozyten-Adhäsion in der hochkalorisch gefütterten Mausgruppe am höchsten war und nahezu keinen Veränderungen in der Thrombozytenrekrutierung in den nicht-septischen sham Gruppen festgestellt werden konnte. CLP induzierte Sepsis erhöhte das Leukozyten-Rollen in DIO und ob/ob Gruppen mit einer Umkehrbarkeit nach einer Leptin Behandlung. Die Leukozytenhaftung war am höchsten in den septischen WT-Mäusen, gefolgt von DIO und ob/ob + Leptin Gruppen, wobei die schwersten db/db Mäuse die niedrigsten Leukozytenzahlen erreichten. Thrombozyten-Rollen war signifikant erhöht in septischen ob/ob Mäusen, wobei eine Reduktion von ungefähr 50% durch Leptingabe erreicht werden konnte. Die ob/ob Gruppe wies signifikant erhöhte Thrombozytenadhäsionen auf, wobei die Gabe von Leptin keinen schützenden Effekt erzielen konnte.

Fazit: Die Erkenntnisse unseres Mausmodells der Mikrozirkulation der Leber weisen darauf hin, dass die von diät-induzierter Fettleibigkeit, verglichen mit genetisch veränderten Mausmodellen von Fettleibigkeit, anders auf entzündliche Prozesse wie Sepsis reagieren. Leptin spielt möglicherweise eine Schlüsselrolle als Vermittler der Immunantwort und ist wahrscheinlich Rezeptor– und Blutspiegelabhängig.

2 | Introduction

2.1 SIRS | Sepsis

A severe systemic reaction of the organism to a suspected or proven infection relating to a 'Systemic Inflammatory Response Syndrome' *(SIRS)* is called sepsis. The phrase SIRS was first introduced at the 'ACCP/SCCM Consensus Conference' held in Chicago, Illinois in 1991. SIRS can be triggered of course by localized or generalized infection but also from noninfectious insults such as trauma, thermal injury or sterile inflammatory processes like pancreatitis or ischemia as summarized in **F**igure **1**.[1] At this conference other terms like infection, bacteremia, severe sepsis, and septic shock were discussed and newly defined. Since the usefulness of this definitions for clinical usage and further research remained controversial a resistance in the researchers-community was established.[2,3] In a revision at the '2001 SCCM/ESICM/ACCP/ATS/SIS[1] International Sepsis Definitions Conference' several variables of possible signs of systemic inflammation in response to infection were provided to the SIRS–criteria.[4] According to these guidelines the diagnostic criteria for sepsis in adults are summarized in **T**able **1**.

Because of massive physiological differences in the developmental stages of children compared to adults a modification regarding to the SIRS–criteria and sepsis had to be done. This happened at the 'International Pediatric Sepsis Consensus Conference' held in San Antonio, Texas in 2002.[5] Firstly, six clinically and physiologically meaningful age groups[2] due to age-specific norms of vital signs and laboratory data (**T**able **2**) were defined and secondly, a consensus definition for infection, SIRS, sepsis, severe sepsis, and septic shock was developed. The main difference between adults and children in the definition of SIRS is that temperature or leukocyte abnormalities must be present. **T**able **3** depicts the definitions for infection, SIRS and sepsis modified to account for the different physiology of children.

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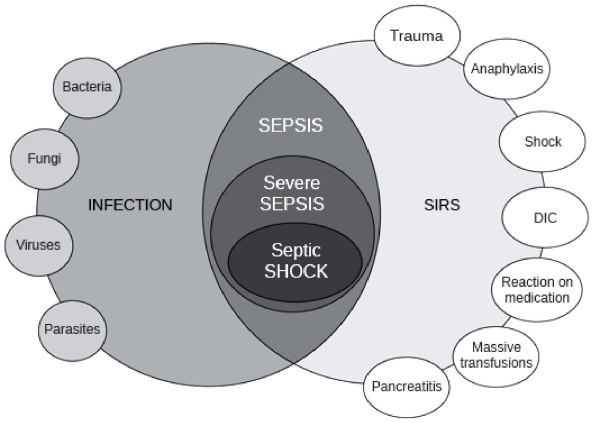
[1] ACCP... '**A**merican **C**ollege of **C**hest **P**hysicians' | ATS... '**A**merican **T**horacic **S**ociety' | ESICM... '**E**uropean **S**ociety of **I**ntensive **C**are **M**edicine' | SCCM... '**S**ociety of **C**ritical **C**are **M**edicine' | SIS... '**S**urgical **I**nfection **S**ociety'

[2] Below all these age groups are termed 'children'

Figure 1 | Relations between infection, SIRS and sepsis



DIC... '**D**isseminated **I**ntravascular **C**oagulation'; SIRS... '**S**ystemic **I**nflammatory **R**esponse **S**yndrome' | Modified from ref. [6]

2.1.1 Epidemiology

Sepsis in adults was the 11th-leading cause of death in the United States of America *(USA)* in 2012.[7] The synonymous but old and unsuitable term septicemia[1] is used in this yearly report however, in accordance with the official 'World Health Organization' *(WHO)* 'International Classification of Diseases and Related Health Problems,10th revision' *(ICD-10)* codes[2] A40 – A41 which is about sepsis from different triggers. The age-adjusted mortality rate[3] due to sepsis was at its peak in the year 2002 with a total of 11.9 and decreased over the past years to its low of 10.3 in 2012.[7] Martin and Sorvillo have shown in their study[8] that in the United States *(U.S.)* in a 6-year period 1,017,616 sepsis-associated deaths occurred (6% of all deaths). They also confirmed that men were at increased risk for sepsis-associated deaths persisting in every age group and among all races.[8] It is also a fact that the elderly and young children experience the greatest burden of sepsis-related death.[7,8]

There is much less literature regarding to the epidemiology of *pediatric sepsis* than severe sepsis in children. One prospective cohort study from Montreal, Canada with 1,058 critically ill children aged between 0 and 18 years has shown that SIRS occurred in 82% (n=869) of hospital admissions and the cumulated incidence[4] rate for sepsis is 23% (n=245). In conclusion the authors found that children admitted to the pediatric intensive care unit *(ICU)* have a distinct risk of mortality in the presence of SIRS, sepsis or septic shock.[9]

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[1] Elimination of this term was proposed at the 'ACCP/SCCM Consensus Conference' in 1992! [2] 2nd edition 2004 [3] Age-adjusted mortality rates are per 100,000 U.S. standard population [4] The rate of occurrence of new cases of a particular disease in a population being studied

Table 1 | Diagnostic criteria for sepsis in adults[a]

Parameters	**Sepsis definition** Suspected or proven infection and at least two of the following
General variables	
Hyperthermia *(Fever)**	Core temperature $>38.3^{\circ}$C
Hypothermia*	Core temperature $<36.0^{\circ}$C
Heart rate*	$>90\ \mathrm{min}^{-1}$
Tachypnea*	Mean respiratory rate $\geq 20\ \mathrm{min}^{-1}$ or $PaCO_2 \leq 32$ mm Hg
Significant edema or positive fluid balance	>20 ml/kg over 24 hrs
Hyperglycemia	Plasma glucose >140 or >7.7 mmol/L in the absence of diabetes
Altered mental status	
Inflammatory variables	
Leukocytosis*	WBC count $>12{,}000\ \mu\mathrm{L}^{-1}$
Leukopenia*	WBC count $<4{,}000\ \mu\mathrm{L}^{-1}$
Normal WBC count*	With $>10\%$ immature forms
Plasma CRP	>2 SD above the normal value
Plasma procalcitonin	>2 SD above the normal value
Hemodynamic variables	
Arterial hypotension	SBP <90 mm HG, MAP <70, or an SBP decrease >40 mm HG
SvO_2 saturation	$>70\%$
Cardiac index	$>3.5\ \mathrm{L{\cdot}min^{-1}{\cdot}M^{-23}}$
Organ dysfunction variables	
Acute oliguria	Urine output $<0.5\ \mathrm{mL{\cdot}kg^{-1}{\cdot}hr^{-1}}$ or 45 mmol/L for at least 2 hrs
Hyperbilirubinemia	Plasma total bilirubin >4 mg/dL or 70 mmol/L
Arterial hypoxemia	$PaO_2/FiO_2 < 300$
Coagulopathy	INR >1.5 or aPTT >60 secs
Creatinine increase	>0.5 mg/dL or 44.2 µmol/L
Ileus	Absent bowel sounds
Thrombocytopenia	Platelet count $<100{,}000\ \mu\mathrm{L}^{-1}$
Tissue perfusion variables	
Hyperlactatemia	>1mmol/L
Capillary refill	Decreased or mottling

$PaCO_2$... '**P**artial pressure of **a**rterial **C**arbon **D**ioxide'; WBC... '**W**hite **B**lood **C**ell'; CRP... '**C**–**R**eactive **P**rotein'; SBP... '**S**ystolic **B**lood **P**ressure'; MAP... '**M**ean **A**rterial **P**ressure'; SvO_2... '**S**aturation of mixed **v**enous **O**xygen'; PaO_2... '**P**artial pressure of **a**rterial **O**xygen'; INR... '**I**nternational **N**ormalized **R**atio'; aPTT... '**a**ctivated **P**artial **T**hromboplastin **T**ime'

[a] Modified from ref. [10]

* Part of the SIRS–criteria

Table 2 | Age-specific vital signs and laboratory variables[a]

Age group for severe sepsis	**Heart rate** Beats/min	**Respiratory rate** Breaths/min	**Systolic blood pressure** mm HG	**Leukocyte count** Leukocytes x 10^3/µL
Newborn 0 days to 1 wk	Tc >180 Bc <100	>50	<65	>34.0
Neonate 1 wk to 1 mo	Tc >180 Bc <100	>40	<75	>19.5 or <5.0
Infant 1 mo to 1 yr	Tc >180 Bc <90	>34	<75	>17.5 or <5.0
Toddler and preschool 2 – 5 yrs	Tc >140 Bc – NA	>22	<94	>15.5 or <6.0
School age child 6 – 12 yrs	Tc >130 Bc – NA	>18	<105	>13.5 or <4.5
Adolescent and young adult 13 to <18 yrs	Tc >110 Bc – NA	>14	<117	>11.0 or <4.5

Tc... '**T**achy**c**ardia'; Bc... '**B**rady**c**ardia'; NA... '**N**ot **A**pplicable'

[a] Lower values for heart rate, leukocyte count, and systolic blood pressure are for the 5th and upper values for heart rate, respiration rate, or leukocyte count for the 95th percentile | Modified from ref. [5]

Table 3 | Diagnostic criteria for sepsis in children[a]

SIRS–criteria	**Sepsis definition** Signs and symptoms of inflammation plus suspected or proven infection[b] and at least two of the following SIRS–criteria[c]
Hyperthermia *(Fever)*	Core temperature >38.5°C
Hypothermia	Core temperature <36.0°C
Heart rate	Tachycardia[d] or for children <1 year of age: bradycardia[e]
Tachypnea	Mean respiratory rate >2 SD above normal for age
Leukocyte count	Elevated or depressed for age[f] or >10% immature neutrophils

SD... '**S**tandard **D**eviation'

[a] Modified from ref. [5]

[b] Caused by any pathogen OR a clinical syndrome associated with a high probability of infection. Evidence of infection includes positive findings on clinical exam, imaging, or laboratory test

[c] One of which must be abnormal temperature or leukocyte count

[d] Defined as a mean heart rate >2 SD above normal for age in the absence of external stimulus, chronic drugs, or painful stimuli OR otherwise unexplained persistent elevation over a 0.5 – to 4-hr time period | May be absent in hypothermic patients

[e] Defined as a mean heart rate <10th percentile for age in the absence of external vagal stimulus, β-blocker drugs, or congenital heart disease OR unexplained persistent depression over a 0.5-hr time period

[f] Not secondary to chemotherapy-induced leukopenia

2.2 Severe Sepsis | Septic Shock

The terms and definitions for severe sepsis, septic shock and 'Multiple Organ Dysfunction Syndrome' *(MODS)* originally also stem from the 'ACCP/SCCM Consensus Conference' in 1992[1] and were modified two times since.[10,11] *Severe sepsis in adults* is currently defined as sepsis plus sepsis-induced tissue hypoperfusion[1] or organ dysfunction and any of the listed variables thought to be due to the infection depicted in **T**able **4**. If a sepsis-induced hypotension persists despite adequate fluid resuscitation it is termed septic shock.[10] As a frequent complication of SIRS a development of an organ system dysfunction can occur which the authors named *MODS*. By definition MODS is the presence of altered organ function in a critically ill patient and the inability to maintain homeostasis without intervention.[1]

Table 4 | Diagnostic criteria for severe sepsis in adults[a]

Organ dysfunctions	**Severe sepsis definition** Sepsis plus sepsis-induced organ dysfunction (any of the following thought caused by the infection) OR tissue hypoperfusion
Hyperlactatemia	Above upper limits of laboratory normal
Acute lung injury	With PaO_2/FiO_2 <250 in the absence of pneumonia as infection source
Acute lung injury	With PaO_2/FiO_2 <200 in the presence of pneumonia as infection source
Creatinine increase	>2.0 mg/dL or 176.8 µmol/L
Bilirubinemia	>2.0 mg/dL or 34.2 µmol/L
Sepsis–induced changes[b]	Arterial hypotension Acute oliguria Thrombocytopenia Coagulopathy

PaO_2/FiO_2... 'Ratio of **P**artial pressure of **a**rterial **O**xygen to **F**ractional **i**nspired **O**xygen'

[a] Modified from ref. [10]

[b] For details see table 1

In children the above mentioned definitions were also discussed and agreed at the 'International Pediatric Sepsis Consensus Conference' in 2002. A *severe pediatric sepsis* persists if the critically ill child has a confirmed sepsis with either a cardiovascular organ

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[1] Defined as infection-induced hypotension, elevated lactate, or oliguria (Defined in **T**able **1**)

dysfunction or an ‘Acute Respiratory Distress Syndrome’ *(ARDS)* or two or more other organ dysfunctions as listed in **T**able **5**.[5] Because of a variety of classifications of shock[1] and also distinct clinical presentations in young acutely ill patients a definition for *pediatric septic shock* remains problematic. The authors defined it as sepsis in the presence of cardiovascular dysfunction (**T**able **5**) which is the same as severe sepsis with cardiovascular dysfunction. To detect and classify pediatric multiple organ problems several scoring systems for measuring pediatric *MODS* have been described in literature. At this conference the authors developed the organ dysfunction criteria summarized in **T**able **5** based on the criteria used in the ‘open-label recombinant human activated protein C study’[12,13] and MODS scores used in the ‘Pediatric Logistic Organ Dysfunction’ *(PELOD)*, ‘Pediatric-MODS’ *(PMODS)* and ‘Multiple Organ System Failure scores’.[5]

2.2.1 Epidemiology

A trend analysis for the period from 1993 to 2003 has shown that both, the rate of hospitalization due to sepsis in adults has exceeded the expected rate as well as the overall mortality has increased.[14] In 2001, an observational cohort study from Angus et al.[15] made in seven large states in the United States in accordance with the ICD-9-CM[2] criteria has shown that *severe sepsis in adults* is a leading cause of death and the most common cause of death among critically ill patients in non-coronary ICU’s. The authors estimated the national age– and gender adjusted incidence of severe sepsis with about 300 cases per 100,000 U.S. population and an overall hospital mortality rate of 28.6%. Furthermore, the authors displayed the dependency of age to the number of cases (58.3% | aged ≥65 yrs) and the incidence. In infants the incidence was high (530/100,000 | aged <1 yr), decreased quickly in older children (20/100,000 | aged 5-14 yrs), increased slowly through most of adult population (530/100,000 | aged 60-64 yrs), and increased sharply in the elderly (2,620/100,000 | aged ≥85 yrs). A similar study with population data from seven states in accordance with the ICD-9-CM based *pediatric severe sepsis* criteria was published in 2003 from almost the same authors as mentioned above.[16] The incidence for severe sepsis is about 56 cases per 100,000 U.S. population per year nationally whereby the incidence for infants was the highest (516/100,000 | aged ≤1 yr) and decreased dramatically in older children (20/100,000 | aged 10-14 yrs).

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[1] e.g.: Warm and cold shock | Catecholamine resistant shock | Fluid refractory resistant shock

[2] ‘**I**nternational **C**lassification of **D**iseases, **9**th Revision, **C**linical **M**odification’

Table 5 | Diagnostic criteria for severe sepsis in children[a]

Organ dysfunctions	**Severe sepsis definition** Sepsis plus one of the following: cardiovascular organ dysfunction OR 'Acute Respiratory Distress Syndrome' *(ARDS)* OR two or more other organ dysfunctions
__C__ardiovascular dysfunction[b]	
Arterial hypotension	<5th percentile for age or <2 SD below normal for age
OR	
Vasoactive drugs required to maintain BP in normal range	Dopamine >5 µg/kg/min or dobutamine, epinephrine, or norepinephrine at any dose
OR	
Two of the following:	
Unexplained metabolic acidosis	Base deficit >5.0mEq/L
Core to peripheral temperature	Gap >3°C
Increased arterial lactate	>2 times upper limit of normal
Acute oliguria	Urine output <0.5 mL/kg/hr
Prolonged capillary refill	>5 secs
__R__espiratory[c]	
PaO_2/FiO_2	<300 mm Hg in absence of cyanotic heart disease or preexisting lung disease
OR	
PaCO_2	>20 mm Hg over baseline $PaCO_2$
OR	
$\mathbf{O_2}$–saturation	Proven need[d] or >50% FiO_2 to maintain saturation ≥92%
OR	
Mechanical Ventilation	Need for non–elective invasive or non–invasive mechanical ventilation
__N__eurologic	
Glasgow Coma Score	≤11
OR	
Altered mental status	Acute change in mental status with decrease in GCS ≥3 points from abnormal baseline
__H__ematologic	
Thrombocytopenia	Platelet count <80,000 μL^{-1} or a decline of 50% in platelet count from highest value recorded over the past 3 days[e]
OR	
Coagulopathy	International normalized ratio >2
__R__enal	
Creatinine increase	Serum creatinine ≥2 times upper limit of normal for age or 2-fold increase in baseline creatinine
__H__epatic	
Bilirubinemia	Total bilirubin ≥4.0 mg/dL or 68.4 µmol/L[f]
OR	
Alanin transaminase	ALT 2 times upper limit of normal for age

BP... '**B**lood **P**ressure'; GCS... '**G**lasgow **C**oma **S**core'; ALT... '**AL**anine **T**ransaminase'; PaO_2/FiO_2... 'Ratio of **P**artial pressure of **a**rterial **O**xygen *(PaO_2)* to **F**ractional **i**nspired **O**xygen *(FiO_2)*'

[a] Modified from ref. [5]

[b] Despite administration of isotonic intravenous fluid bolus ≥40 mL/kg in 1 hr

[c] Acute respiratory distress syndrome must include a PaO_2/ FiO_2 ratio ≤200 mm Hg, bilateral infiltrates, acute onset, and no evidence of left heart failure. Acute lung injury is defined identically except PaO_2/FiO_2 ratio must be ≤300 mm Hg

[d] Proven need assumes oxygen requirement was tested by decreasing flow with subsequent increase in flow if required

[e] For chronic hematology or oncology patients

[f] Not applicable for newborn

Boys were 15% more often affected than girls and the hospital mortality rate was 10.3% (6.2/100,000). The same study found that 49.0% of the cases had underlying diseases and 22.9% were low-birth-weight newborns[1]. A French study published 2003 analyzed little over 100,000 intensive care unit admissions from the years 1993 – 2000 to update the epidemiology of *septic shock in adults*.[17] The authors found an increase in the annual frequency of septic shock in that period from 7.0 (in 1993) to 9.7 (in 2000) per 100 admissions (overall frequency was 8.2), an increase in the rate of pulmonary infection and of multiresistant bacteria-related septic shock. Its crude mortality rate was 60.1% but declining from 62.1% (in 1993) to 55.9% (in 2000), however, the excess risk of death due to septic shock was 25.7%. One recent French large-scale epidemiological study[18] investigated the mortality of adult ICU patients over a 3-month period after an initial episode of septic shock which was defined based on the PROWESS-SHOCK study.[19] From all patients admitted between October 2009 and September 2011 in total almost 1,500 patients had septic shock (13.7% from all patients), the median age was 68 years (variation from 58-78 yrs) and the majority of admissions were medical (84%). After 3 months 776 patients or 52.2% had died, but patients in an ICU had a better outcome than in normal wards, the mortality rates were 39.4% and 48.6% respectively.[18]

In the early 60's of the last century *pediatric septic shock* was a certain death sentence with a 97% mortality rate in infants with gram-negative sepsis and septic shock.[20] The mortality rate dropped drastically since then from 57% in 1985[21] to 12% in 1991[22] when aggressive volume resuscitation was used and, finally, since this century to rates under 10% in previously healthy children as shown in many studies around the globe and summarized from Joseph A. Carcillo in the journal 'Critical Care Clinics' in 2003.[23]

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[1] Newborns less than 2,500 g birth weight

2.3 Sepsis | Pathophysiology

As previously described sepsis and its varieties are common causes of a serious medical condition triggered by an exaggerated immune response to a localized or body-wide infection or even in noninfectious patients. Repairing the affected tissue as well as localizing and controlling pathogen invasion is a complex process, but describes the normal host response. This process involves the activation of fixed tissue dependent and circulating phagocytic cells, as well as the production of anti– and pro-inflammatory mediators like 'Transforming Growth Factor' *(TGF)*-β1, 'InterLeukin' *(IL)*-4, IL-10, IL-13 or 'Tumor Necrosis Factor' *(TNF)*, 'InterFeroN-gamma' *(IFN-*γ*)*, IL-6, IL-12, IL-18.[24–26] The anti-inflammatory response is activated to dampen the pro-inflammatory response in sepsis and is termed as 'Compensatory Anti-inflammatory Response Syndrome' *(CARS)*. Tissue damage occurs by an uncoupling of the two phases, SIRS and CARS, or an excessive inflammatory response.[27] There are many different microbes such as bacteria, fungi, viruses or even parasites that can cause sepsis, however, bacteria are the most frequent agent. The so called 'innate immune system' initially reacts to an invading organism and might play a major role in the initiation of sepsis pathophysiology. Its response is not specific to any antigen and it reacts similarly to a variety of organisms. On the other hand the delayed but more specific 'adaptive immune response' attacks only the pathogen that triggers the response. Normally the inflammatory reaction during the innate immune response remains localized. A variety of cell types such as monocytes and endothelial cells release inflammatory mediators and cytokines. Further the pathogens lead to an activation of the complement cascade. After activation of the body's own immune system and the release of above mentioned soluble mediators into the bloodstream a combat against the infection begins, which triggering widespread inflammation, leads to leaky vessels, and in addition to activation of the coagulation cascade. This, on the other hand, leads to an worsened blood flow and microcirculation, which in turn damage organs *(MODS)* by depriving them from oxygen and nutrition.[24,26–28] How likely a local infection will progress to sepsis varies among other things to its source tissue. The most common tissues related to sepsis are the lungs with pneumonia, the liver, the kidneys and the bloodstream infection.[29]

2.3.1 Hemodynamic Changes

Many studies have shown, using ‘IntraVital (fluorescent) Microscopy’ *(IVM)* in experimental models of sepsis, that blood flow changes including impaired microcirculatory flow velocity, low density of perfused capillaries and increased heterogeneity of regional perfusion or even ‘stopped-flow’ microvessels occur.[30–32] Derangement of microcirculatory perfusion in sepsis appears to be one of the critical pathogenic events and can occur independent of arterial blood pressure. Actually the accurate relationship between global macrocirculatory– and microcirculatory hemodynamic changes in sepsis is considered unclear but one of the key pathophysiologic questions.[33] To increase local circulation and allowing immune cells to migrate and congregate, the blood vessels dilate. If the innate immune response becomes dysregulated and amplified this ‘first line of cellular defense’ results in an excessive release of cytokines and other inflammatory regulators. As a consequence an imbalance between pro-inflammatory and anti-inflammatory responses arises that leads to the presence of sepsis.[26] Circulating cells and macrophages express ‘Tissue Factor’ *(TF)* on their surface which is responsible for the initiation of the extrinsic pathway of the coagulation cascade in sepsis. Through a crosstalk and feedback mechanism between the extrinsic[1] and intrinsic[2] pathways of coagulation, this process is amplified which leads to generation of fibrin and thrombin. There is also the fact that there is a downregulation of anti-coagulant responses like the ‘Tissue Factor Pathway Inhibitor’ *(TFPI)* which is one the main factors that controls the extrinsic pathway.[27] As the endothelium is involved in the control of vascular tone, platelet reactivity, coagulation, and permeability, and endothelial cells are the gatekeepers between the bloodstream and the body’s tissues, as such, a healthy endothelium protects against excessive or abnormal inflammation and coagulation.[34] The endothelium can be activated by a wide variety of host-derived factors such as chemokines, cytokines, fibrin, serine proteases and activated circulating blood cells,[27] otherwise, synergistic effects of TNF-α and thrombin can increase the endothelial permeability.[35] This dysfunctional epithelial barriers are associated with the development of a pro-coagulant surface, abnormal vasomotor activity, and because of allowing pathogens and their products to further invade the host, an acceleration of the inflammation process.[34]

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[1] Considered to be the initiator [2] Considered to be the amplifier by contact activation, starting with ‘factor XII’, ‘**H**igh-**M**olecular-**W**eight **K**ininogen’ *(HMWK)* and ‘prekallikrein’ of the coagulation cascade

2.4 Obesity

The terms ‘overweight’ and ‘obesity’ are defined based on the WHO-guidelines as abnormal or excessive fat accumulation that poses a risk to health. For adults, the ranges are determined by using a simple calculation based on the ratio of someone’s height and weight to calculate a ratio called the ‘Body Mass Index’ *(BMI)*[1]. Decades of research have shown that BMI for most people correlates with their amount of body fat and also correlates well with important health outcomes like diabetes, heart disease, cancer, and overall mortality, so it is used by default. A BMI of greater than or equal to 25 to less than 30 (≥25 – <30) is considered overweight, a BMI of 30 to less than 35 (≥30 – <35) is considered grade 1 obesity, grade 2 obesity is defined as a BMI of 35 to less than 40 (≥35 – <40) and finally grade 3 obesity[2] is a BMI greater than or equal to 40 (≥40 and above)[3]. Other measurements and comparisons of estimating body fat and body fat distribution like calculation of ‘Waist-to-Hip Ratios’ *(WHR)*, measurements of skinfold thickness[4] and ‘Waist Circumference’ *(WC)*, as well as techniques such as ultrasound, ‘Magnetic Resonance Imaging’ *(MRI)* and ‘Computed Tomography’ *(CT)* can also provide some information regarding risk factors associated with weight. As BMI does not directly measure body fat, some people like athletes, may have a higher BMI even though they do not have excess body fat. However, as BMI is the same for all ages and for both sexes of adults, it provides the most useful population-level measure of overweight and obesity.[36–39]

In children and adolescents obesity is one of the most serious health challenges of the 21st century. This global problem is constantly affecting many low– and middle-income countries, especially in urban settings. The definitions of overweight and obesity differ from those in adults, because children’s body composition varies as they age and varies between boys and girls. As distinguished from the adults, there are no risk-based fixed values of BMI used to determine overweight in children. As a result, it is suggested using growth charts with percentiles of sex-specific BMI-for-age in a specified reference population. For children of the same age and sex overweight is defined as a BMI at or

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[1] BMI = weight by height^2 metric: [kg/m^2] [2] Also termed ‘morbidly obese’ or ‘very obese’ | BMI ≥60 is termed ‘extremely obese’ or ‘extreme obesity’ [3] National Heart, Lung, and Blood Institute’s terminology – not yet systematically reviewed [4] Calipers used too determine whether tissue is muscle (lean) or adipose tissue (fat)

above the 85th percentile and lower than the 95th percentile (≥85th – <95th percentile) and obesity is defined as a BMI at or above the 95th percentile (≥95th percentile or above).[40,41] For international comparisons the WHO created charts from healthy, breast-fed children from around the world intended to present a standard of physiologic growth instead of a descriptive reference. So they used cut-off values based on SD scores (z-scores), with obesity defined as a BMI-for-age value greater than or equal to a z-score of +2[1].[41,42] Overweight and obesity are largely preventable, however, overweight and obese children are likely to stay obese into adulthood. As a result it is more likely to develop cardiovascular and metabolic disorders at younger age.[40]

2.4.1 Epidemiology

Across the 'Organisation for Economic Co-operation and Development' *(OECD)* area[2] overweight and obese people are a majority and the obesity epidemic continues to spread. Since the 80's of the last century less than 10% of women and men were obese in OECD countries. The last data of the 'OECD obesity update' from 2014 have shown an increase in 2012 to an average of more than 18% of the adult population with sizeable differences across the Member States with a nearly tenfold variation in rates of overweight and obesity observed across OECD countries like Japan ∼3.7% compared with the U.S. ∼35%. Even though the obesity epidemic was further growing in the past years, the rates proceeded less quickly than before.[43,44]

As mentioned above a periodical recent study named 'Prevalence of Childhood and Adult Obesity in the United States' has demonstrated that the current prevalence[3] of obesity in adults is high, with over one-third. However, trends among children and adolescents (2 – 19 yrs) were similar to those among adults and with 17% alarmingly high too. The authors found, apart from the average increase of overweight and obesity, a significant decrease of obesity among 2- to 5-year-old children (from 13.9% to 8.4%).[45] In measurable terms estimated over 42 million children under the age of five were globally overweight or obese in 2013 and about 31 million of these are living in developing countries.[40]

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[1] Two '**S**tandard **D**eviations' *(SD)* body mass index for age and sex [2] Currently 34 Member States inclusive USA, Canada, Australia, Japan and many European countries | Founded in 1961 [3] The percentage of a population that is affected with a particular disease at a given time

2.4.2 Adipose Tissue | Adipocytokines

Adipose tissue is an anatomical term for loose connective tissue consisting mainly of adipocytes. ‘White Adipose Tissue’ *(WAT)* is the most common type and its main role is to synthesize and contain large globules of fat in form of lipids for energy storage for times of starvation or great exertion, within a structural network of fibres, vascular endothelial cells and a variety of immune cells. A further functionality is to insulate and cushion the body and its organs because of its global distribution. Further a specialized form of adipose tissue in humans is ‘Brown Adipose Tissue’ *(BAT)* which is found mainly in newborns and decreases with age.[46,47] It is specialized to convert chemical energy to heat as a defense against excessive feeding and cold, which may be vital in neonates as they are unable to shiver. Many researchers see a therapeutic potential of BAT to work against obesity and obesity-related diseases, including insulin resistance.[48]

2.4.2.1 White Adipose Tissue *(WAT)*

Besides its above mentioned functions, white adipose tissue is a highly dynamic endocrine organ secreting numerous bioactive substances including adipocytokines[1], classical cytokines and others into the surrounding environment and the bloodstream. This mediators which act locally and distally through autocrine, paracrine and endocrine effects, regulate energy metabolism, insulin sensitivity and vascular homeostasis. As adipose tissue is the largest organ of the human body and each adipocyte produces a small quantity of bioactive substances, their total amount strongly impacts body functions.[49–51] WAT in obesity is characterized by hypertrophic adipocytes and infiltration of inflammatory lymphocytes and macrophages, leading to enhanced generation of pro-inflammatory adipokines and vasoconstrictors which induce endothelial dysfunction and vascular inflammation. This dysfunctional adipose tissue in obesity and its related pathologies has a decreased secretion of adiponectin, an adipokine with anti-inflammatory and insulin sensitizing activities, and links obesity with insulin resistance, hypertension and cardiovascular disease.[50] Furthermore, an augmented activity of TNF-α and IL-6 are involved in the development of obesity-related insulin resistance. Weight loss in association with exercise reduces fat mass which can lower TNF-α and IL-6 levels and also increase adiponectin concentrations.[51]

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[1] Or adipokines

2.4.2.2 Leptin

Identification and characterization of the ‘ob gene’ product leptin in 1994 was a breakthrough for obesity research due to its prominent effects on energy expenditure, fat metabolism and its contribution to satiety signaling in the hypothalamus.[52,53] Consistent with previous studies, and our results, a continuous weight loss during leptin treatment in ob/ob mice, to a weight comparable to a WT mouse of the same age, can be observed.[54,55] It is also associated with a dramatically reduced fat pad weight and accompanying increase in adipose tissue apoptosis.[56] Aside from regulation of adipose tissue mass, reproduction and angiogenesis, leptin likely plays a role in regulating immune homeostasis. In obese patients elevated baseline levels of leptin were observed. An increased fat deposition, with its abnormal changes in various physiologic processes such as hyperglycemia, hyperlipidemia, abnormal angiogenesis and increased insulin secretion, results from deficiency of leptin or its long-form receptor gene (OB-Rb[1]). The leptin receptor OB-Rb mediates leptin actions, it is expressed in endothelial cells, as well as lymphocytes, neutrophils (short-form OB-Ra expressed predominantly) and macrophages (and their precursors, monocytes), and deficiency of this receptor affects innate and adaptive immune responses.[53] Leptin, on the one hand, has pro-inflammatory characteristics, such as reactive oxygen species generation, up-regulation of monocytes pro-inflammatory cytokine production and phagocytosis, induction of polymorphonuclear neutrophils chemotaxis and improved proliferation and cytotoxicity of ‘Natural Killer’ *(NK)* cells. On the other hand, leptin induction plays a key role in lympho– and myelopoiesis and representing a source of prosurvival signals to thymocytes during the maturation of T cells which seems to be a protective component of the immune response.[57] The prospective ‘West of Scotland Coronary Prevention Study’ *(WOSCOPS)* has shown that leptin may affect vascular structure and could be an independent risk factor for coronary heart disease. In 377 cases, men who experienced a coronary event during the 5-year follow-up period, the plasma leptin levels were significantly higher at baseline than in 783 male controls. These controls did not suffer a coronary event, were matched for age and smoking history and were representative of the entire WOSCOPS cohort.[58] Furthermore, in vitro and in vivo assays reinforce that leptin has angiogenic[2] activity and is involved in arterial thrombosis through the platelet leptin receptor.[51]

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[1] The leptin receptor OB-R belongs to class I cytokine receptor family and exists in at least six isoforms

[2] Physiological process through which new blood vessels form from pre-existing vessels

2.5 Obesity and Risk of Sepsis

Inconsistent data from both, laboratory and clinical settings regarding the influence of adult obesity on sepsis, created an impetus for many recent studies on this subject. Regarding to the outcome of obese critical ill patients these studies were published with similar but still differing criteria either with hospital care in normal wards or intensive care in ICU's, or either trauma or medical issues and with or without mechanical ventilation. Mostly they evaluated the length of stay in hospital as well as the morbidity and mortality rates for the individual patient groups.

2.5.1 Obesity – A Protective Impact?

Aside from many different opinions referring to protective or worrying effects of obesity in critically ill patients it appears to be clear that in some circumstances obesity per se may be protective in some contexts, besides the increased risks of all-cause and cancer mortality.[59] This often observed phenomenon called 'obesity paradox' describes that obese patients, although obesity predisposes individuals to a variety of risk factors for cardiovascular diseases[27] such as hypertension, hypercholesterolemia and type 2 diabetes, when acute cardiovascular decompensation occurs, like in congestive heart failure or myocardial infarction, may have a survival benefit. Moreover, some studies have shown that obese patients tend to fare better after certain operations, such as coronary artery bypass surgery.[60] A prospective, multicentre, observational study in 198 ICU's in 24 European countries published in 2008, divided adult patients in the above mentioned BMI-groups and followed them up until death, hospital discharge, or for 60 days. From the 2,878 included patients (mean age 60.8 ± 17.3 years, 62.3% male), 1,047 (36.4%) were overweight, 424 (14.7%) obese, and 81 (2.8%) very obese. As obese and very obese patients more often developed ICU-acquired infections, obesity was significantly associated with increased morbidity. There was also a significantly increase in the overall incidence in occurrence of respiratory failure and mechanical ventilation at some point during the ICU stay in higher BMI groups. Very obese patients had a trend for a stay of longer duration in hospital or ICU versus those of normal BMI. However, the overall incidence of sepsis syndromes during the ICU stay was similar among the groups and obesity was not associated with increased ICU or hospital mortality rates.[61] A meta-analysis of 23 eligible publications with 88,051 patients from PubMed and EMBASE databases as of March 2008 showed minor variations in the results as in the European observational ‘Sepsis Occurrence in Acutely ill Patients’ *(SOAP)* study as described immediately before. There was no difference in ICU mortality, but a lower hospital mortality rate for obese and morbidly obese compared to normal weight patients. Longer hospitalization was seen in morbidly obese subjects, however there was no association between obesity and duration of mechanical ventilation or ICU stay.[62] A cohort analysis of 16,812 adult patients at a tertiary care hospital in Boston, Massachusetts used the ‘Multiparameter Intelligent Monitoring in Intensive Care’ *(MIMIC-II)* database to examine the relation between BMI and mortality 30 days and 1 year after ICU admission.

This work published in 2012 demonstrated a lower mortality risk at 30 days and 1 year after ICU admission for overweight patients with nearly 20% and 30% and for obese patients with 26% and 43%, respectively, compared with normal-weight patients. Morbidly obese patients had a 30% lower mortality risk at 1 year, but did not have a significant survival advantage at 30 days. No significant difference in the length of stay in hospital and ICU was found across BMI categories.[63]

Although there are indications for a protective impact of obesity regarding to sepsis in adults, there is not much literature about clinical trials or laboratory data which confirm this for young obese subjects.

2.5.2 Obesity – A Deteriorating Condition?

Several studies provide an indication that obesity per se and, still rather morbidly obese people, have increased risks of morbidity and mortality through its multiple effects on nearly every human system.[64–66] They are more likely to develop infections of several organs and systems including postoperative– and other nosocomial infections, as well as more serious complications of common infections than those of normal weight.[67] Obesity results in increased admissions into hospitals and ICU’s with a higher mean length of stay and mechanical ventilation.[64,68] Postoperatively, especially after abdominal surgery, obese patients have an exceptionally risk of developing a surgical wound infection and therefore an increased risk of postoperative infections.[68] A french risk-adjusted matched cohort study compared the mortality rate between exposed and unexposed patients in an adult ICU according to eight criteria. Obesity was significantly associated with intensive care unit mortality (odds ratio: 2.1) especially in the youngest patients[1] and for the patients

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[1] Ages not indicated in the abstract and no free access to the paper

with a probability of intensive care unit death of 11-50%, whereas the age (±7 yrs) and probability of ICU-death (±5%) were two of the eight criteria. The authors conclude that these findings point out that obesity is an independent risk factor for intensive care unit death, but this might be explained by the higher risk of ICU-acquired complications among obese patients than among normal weight patients.[66] A retrospective study with 117 morbidly obese adult patients in an ICU in Buffalo, New York, has shown, besides a longer stay and mechanical ventilation, that the overall mortality rate was 13% higher in morbidly obese patients compared to nonobese controls. In conclusion, the authors considered that critically ill morbidly obese patients have higher ICU mortality and increased risk of morbidity associated with prolonged mechanical ventilation and extended 'weaning' period compared to the nonobese patients.[64] A more recent American multicenter international observational study[1] of ICU nutrition practices that took place in 355 ICU's sought to determine the association between extreme obesity and ICU outcomes. Patients older or equal than 18 years who remained longer than 72 hours in the ICU and were mechanically ventilated, were included in this analysis. Besides 3,490 normal weight controls the authors analyzed 524 extremely obese patients divided into 348 morbidly obese patients, 118 patients with a BMI from 50 to 59.9 kg/m^2 and 58 patients with a BMI greater than 60 kg/m^2 with regard to duration of mechanical ventilation, hospital and ICU length of stay, and 60-day mortality. The unadjusted analyses suggested an apparent trend of reduced 60-day mortality with increasing BMI, however, this association was not significant after adjustment for confounders. Although survival for extremely obese patients is at least as good as normal-weight patients, there is a higher morbidity with a significant longer duration of mechanical ventilation and ICU length of stay. Patients with a BMI greater than 60 kg/m^2 also had longer hospital lenght of stay.[69] A recent prospective study from a Greece University Hospital investigated how obesity influences the immune response of septic patients, by determination of serum and adipose tissue TNF-α levels, plasma oxidative stress markers and the number and activation state of subcutaneous and visceral adipose tissue macrophages[2]. A total of 106 patients were divided into four groups (control n=26, obesity n=27, sepsis n=27 and sepsis and obesity n=26) and tissue as well as blood-samples were obtained during a 5-year period (October 2008 to May 2013). The results showed an increased concentration of macrophages and their subtype M2 as well as TNF-α mRNA levels in visceral adipose tissue in sepsis

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[1] 33 countries from 2007 to 2009 [2] Inclusive their subtypes M1 and M2

and obesity but not in obesity alone. Obesity also increased the plasma oxidative stress marker 'ThioBarbituric Acid-Reactive Substances' *(TBARS)* and the protein carbonyls in septic patients. The plasma levels of the biomarker 'Total Antioxidant Capacity' *(TAC)* were decreased while TNF-α levels were elevated in sepsis unaffected by obesity. The authors concluded that obesity is associated with increased oxidative stress biomarkers and TNF-α adipose tissue production, which supports the pro-inflammatory response in septic patients.[70]

Obesity in children is in fact a major problem. This population is at risk for developing complications such as cardiovascular, respiratory, metabolic, endocrine, gastroenterological, dermatological, orthopedic, and even neurological, and psychological disorders. Genoni et al.[57] searched and collected current knowledge about obesity and infection in both, adults and children, published as a review in the 'European Journal of Pediatrics' in 2014. The authors found that just some studies investigated the impact of obesity on the immune response to infections as well as the risk of infections in an overweight population. The higher incidence of respiratory tract infections is, among other things, triggered by 'Obstructive Sleep Apnea Syndrome' *(OSAS)* in obese children and obesity per se. Via oxidative stress and neuroendocrine changes an enhanced inflammation and cytokine production as well as reduced nitric oxide production occurs. Furthermore, hypercapnia seems to be more likely in leptin resistance, and this stimulus suppresses the respiratory response in ob/ob mice. All these mechanisms may force the inflammatory response of the host to infectious agents which leads to serious respiratory infections in an altered susceptibility of obese children. Moreover, there is a higher risk for developing severe skin infections such as plantar hyperkeratosis, dermatoses as skin tags, striae distensae and highly important Staphylococcus aureus infections. In conclusion the authors found that there is a need for more studies on the association between obesity and infection in children. However, the current data suggest that obese pediatric subjects have an increased risk for at least respiratory, odontogenic, and cutaneous infections.[57] Available laboratory data suggest an increased inflammatory response and enhanced inflammation in the microvasculature of multiple organs in obese septic individuals.[27] Many mechanisms including increased activation of leukocytes, platelets, and endothelial cells leading into microcirculatory disturbances, as well as secretion of pro-inflammatory adipokines and various cytokines, activation of macrophages and oxidative stress along with increased insulin and leptin resistance are indications that obesity per se is a chronic inflammatory state.[51,53,71]

2.6 Hepatic Microcirculation

The liver is a functionally complex and the largest visceral organ in the body and receives approximately 25% of the cardiac output via two inflows, the portal vein[1] *(PV)* and the hepatic artery[2] *(HA)*. An important role in maintenance of liver function is the hepatic microcirculation because it regulates nutrition and function of the hepatic parenchyma and its supporting tissues. It is also responsible for the clearance of foreign bodies and toxic materials coming from the bloodstream and it serves as a gate for leukocyte entrance in hepatic inflammation. The microcirculation, which includes all of the intrahepatic vessels having an internal diameter less than 300 µm, generally refers to the hepatic circulatory system which in turn drains into the hepatic venous system. It consists of the terminal branches of the HA, the PV, and the portal bile ducts which distributes blood into the sinusoids[3] that compose the capillary bed in the hepatic parenchyma.

These sinusoids are characterized by the presence of fenestrae[4] and by the absence of a basement membrane which both facilitate transvascular exchange between the blood and liver cells. The sinusoidal endothelial cells are highly responsible to various vasoactive substances such as ‘Nitric Oxide’ *(NO)*, ‘Carbon Monoxide’ *(CO)*, ‘Hydrogen Sulfide’ (H_2S) and adenosine leading to a variation of the sinusoidal lumen by sphincteric mechanisms at the inlet and outlet of the sinusoids. The subsequent venous drainage, after perfusing the liver parenchyma, goes through ‘central or centrolobular veins’ into the postcapillary ‘Terminal Hepatic Venules’ *(THV)* followed by the collecting venules and the muscular venules and finally reaching the sublobular vein and hepatic veins, which leave the liver on the dorsal surface and have their outflows in the extrahepatic inferior vena cava.[72–74]

Upon invasion by a pathogen, there is local activation of inflammatory mediators and the neighboring endothelial cells. The endothelium responds by expressing adhesion molecules like E-selectin and ICAM-1 on the cell surface which results in increased leukocyte and platelet rolling or adhesion to contain the process of infection. If the pro-inflammatory process dominates and the infection spreads, a general recruitment of leukocytes and platelets in several organs can occur. Especially in the liver, acting as a first-line defense, these induced hemodynamic alterations may lead to liver damage urged

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[1] 70 to 80% of its blood | Rich in nutrients [2] 20 to 30% of its blood | Rich in oxygen [3] In different and complex ways | Described variously [4] Sieve-like pores on the endothelium

by direct and indirect assault on the hepatocytes through infectious agents, their products and debris in the microcirculation. Thus, an adequate microcirculation is necessary for the complex functions of the liver in metabolism, biosynthesis as well as host defense and clearance of the infectious agents and their products in case of an inflammatory process such as sepsis. [75]

2.6.1 Leukocytes | Platelets

Leukocytes, synonym for 'White Blood Cells' *(WBCs)*, are nucleated immune cells produced and derived from a hematopoietic, multipotent stem cell (hemocytoblast) from the bone marrow. This hemocytoblast can develop in two major lineages and become a 'common myeloid progenitor cell' or a 'common lymphoid progenitor cell'. Lymphocytes from the lymphoid lineage are much more common in the lymphatic system and lymphocytic tissue[1] than in blood and include T cells, B cells and natural killer cells. There are small lymphocytes with 7 – 8 µm and large lymphocytes with 9 – 15 µm in diameter.
White cells of the myeloid lineage are granulocytes, which in turn can be divided in neutrophils, eosinophils and basophils, as wells as monocytes. Granulocytes have a round shape and are 10 – 15 µm and monocytes 12 – 24 µm in average diameter. [76,77] Platelets, synonym for thrombocytes, are anucleate circulating blood cells, produced and released from megakaryocytes, which themselves derive from common myeloid progenitor cells. Unactivated platelets are biconvex discoid structures and only 1 – 3 µm in diameter.[77]

2.6.1.1 Leukocyte Diapedesis | Platelet Aggregation

All leukocytes are a functional part of the body's own immune defense system and have also an important disposal function for cell residues, fragments and infectious agents. To execute these functions it is necessary for the leukocytes to migrate from the bloodstream into the underlying tissue. This cell migration has to be tightly regulated because of possible destructive consequences for the surrounding tissue if the cell infiltration happens into healthy areas. The process of extravasation of leukocytes across the vascular wall needs interactions of adhesion receptors between leukocytes and endothelial cells. The expressed endothelial E– and P-selectins[2] touching the corresponding leukocyte ligands to slow down the velocity of the circulating immune cells, which leads ti leukocyte rolling

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[1] Only about 1% circulates in blood | Thus, they are more likely tissue cells

[2] E... Endothelial | P... Platelet

movement on the vessel wall. Emitted chemokines from endothelial cells can be sensed by the decelerated rolling leukocytes and as a result they will be stimulated to an integrin-mediated firm adhesion. Leukocyte integrins bind to members of the immunoglobulin superfamily, like ‘Intercellular Adhesion Molecule-1’ *(ICAM-1)*, ‘Vascular Cell Adhesion Molecule-1’ *(VCAM-1)*, ‘Platelet/Endothelial Cell Adhesion Molecule-1’ *(PECAM-1)*, and ‘Junctional Adhesion Molecules’ *(JAMs)*. The firmly attached leukocytes actively migrate, in amoeboid fashion, across the endothelial monolayer into the interstitium which is called diapedesis or ‘TransEndothelial Migration’ *(TEM)*.[78,79]

Platelets circulate, under physiological conditions, in close contact with microvascular endothelial cells but without adhering to their surface. The process by which platelets adhere to each other at sites of vascular injury or endothelial dysfunction is described as platelet cohesion although more commonly referred to as platelet aggregation and is important for hemostatic plug formation. On the whole, just few core element factors for mediating platelet aggregation are required, namely a platelet stimulus such as exposed ‘ExtraCellular Matrix’ *(ECM)* proteins after vascular lesions like ‘von Willebrand Factor’ *(vWF)* or collagen, a soluble adhesive protein like fibrinogen, and a membrane-bound platelet receptor such as integrin receptor $\alpha_{\text{IIb}}\beta 3$[1] and collagen receptor $\alpha_2\beta_1$. In fact this process is much more complex and it greatly depends on hemodynamic conditions like the vessel diameter and type (venules, large veins, arterioles,...) as well as the blood flow resulting in different shear conditions.[80–82]

2.6.1.2 Fluorophores

The vital dye ‘rhodamine 6G’ is used for contrast enhancement in intravital fluorescence microscopy. This dye accumulates in the mitochondria of WBCs, whereby an intravascular injection of rhodamine 6G results in adequate staining of monocytes and granulocytes, but only some lymphoid cells are stained[2].[83]

For labeling of platelets the reactive dye 5-(and 6)-Carboxyfluorescein diacetate succinimidyl ester *(CFSE or CFDA-SE)*, a nonfluorescent precursor taken up into platelets and partly into leukocytes, is used. This diacetate form is able to cross intact cell membranes and binds covalently to cytosolic and membrane proteins and intracellular esterases cleaving the acetate groups to yield the fluorescent carboxyfluorescein molecule.[80]

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[1] Also labeled GPIIb-IIIa [2] Probably because of too few circulating cells, as mentioned above

2.7 Hypothesis

We hypothesize that obesity results in a chronic state of low-grade inflammation and this pro-inflammatory environment leads to exaggerated leukocyte and platelet accumulation in the liver following a septic stimulus due to a dysregulation of endothelial cells with augmented expression of adhesion molecules. In previous laboratory studies it has been shown in several organs that WT mice exhibit an increased adhesion of both leukocytes and platelets following a septic stimulus.[32,84–86] However, there is lack of data about the microcirculatory changes and the underlying mechanisms in obese mice during sepsis. To determine whether obese mice exhibit an increase of hepatic microcirculatory adhesion of leukocytes and platelets we applied the ‘Cecal Ligation and Puncture’ *(CLP)* method of inducing a polymicrobial sepsis in three different murine models of obesity, namely ob/ob mice, db/db mice and mice on a high fat diet *(DIO)*[1] and a lean group of WT mice. Six hours after the induction of sepsis we compared the alterations of the hepatic microvasculature using intravital fluorescence microscopy in those groups of septic obese mice and compared the obtained values for leukocyte and platelet rolling and adhesion to the respective sham groups as well as a group of WT mice following either sham or CLP operation. To determine whether it is indeed the leptin pathway that modulates the immune responses to sepsis we additionally performed these experiments in a group of ob/ob mice repleted with leptin via an osmotic minipump and compared the obtained values to all other groups.

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[1] DIO... ‘**D**iet **I**nduced **O**besity’

3 | Material and Methods

3.1 Animals

For the experiments all mice were obtained from Janvier[1] with an age of 5 weeks followed by a two weeks period of acclimatization. Upon usage the male mice were between 7 and 11 weeks old. The following strains were used:

- C57BL/6 *(WT)*
  wild type, non-obese control group
  high susceptibility to diet-induced obesity *(DIO)*
- B6.BKS(D)-Lepr^{db} *(db/db)*
  homozygous for the *diabetes* spontaneous mutation
  lack of expression of the leptin receptor
- B6.Cg-Lep^{ob} *(ob/ob)*
  homozygous for the *obese* spontaneous mutation
  lack of expression of the adipocytokine leptin

Both, the db/db and ob/ob mice were on C57BL background. The mice were housed in a 'Specific Pathogen-Free' *(SPF)* environment at the 'Institute for Biomedical Research of the Medical University of Graz' and kept in groups of 5 in a simulated environment[2] with free access to food and water. Animal handling procedures were approved by the Medical University of Graz and the Government of Austria ('Bundesministerium für Wissenschaft und Forschung' *(BMWF*-66.010/0092-II/3b/2010)).

3.2 Experimental Protocol

The hepatic microcirculatory responses were assessed by mean values of either rolling or adhesive leukocytes and platelets along the endothelium of the vessel wall obtained from 'Intravital Microscopy' *(IVM)* 6 hours following either 'Cecal Ligation and Puncture' *(CLP)* or sham operation in the above mentioned three strains of mice.

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[1] Obtained from St. Berthevin, Cedex, France [2] 12 hours light/dark cycle | Temperature between 20°C and 24°C | Relative humidity between 45% and 65%

In an additional set of experiments the microcirculatory disturbances of the liver were measured in a group of WT mice fed with high fat diet *(DIO)* and a group of ob/ob mice following repletion with leptin *(ob/ob + Lep)* using mini-osmotic pumps. The obtained data were compared to a group of WT mice following either CLP or sham procedure or compared to corresponding sham animals.

3.3 Diet Induced Obesity

One group of WT mice was fed with a high fat 'Western Type Diet'[1] (**T**able **6**) for at least four weeks. All animals were weighed regularly to follow the weight gain.

Table 6 | Ssniff 'Western Type Diet' (TD88137)

Nutrition contents	**Metabolizable energy**[a]
Carbohydrates	43 kJ%
Fat	42 kJ%
Protein	15 kJ%

Modified from ref. [87]

[a] Metabolizable energy calculated with the Atwater factors

3.4 Leptin Repletion

In one group of ob/ob mice leptin repletion experiments were performed. Mice were weighed and Alzet osmotic pumps model 2002[2] (**F**igure **2**) were filled free of air bubbles with a leptin[3] and distilled water mix for an infusion rate of 0.3 mg/kg per day calculated using the formulation calculator provided by Charles River. Animals were anesthetized with sevoflurane (1.5 l O_2/min, 3% sevoflurane[4]). In a sterile setting under a laminar flow cabinet a midline interscapular incision was performed followed by a subcutaneous blunt dissection using a forceps and implantation of the prefilled osmotic pump[5]. The wound was closed with Histoacryl[6] and the area cleaned with povidone iodine (Betaisodona) solution (**F**igure **3**). 1 ml sodium chloride *(NaCl)* solution 0.9% was injected subcutaneously for rehydration. Pumps were changed every two weeks using the same protocol.

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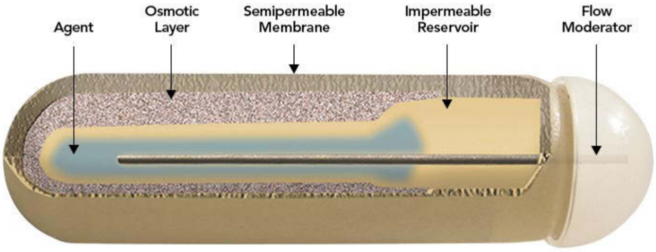
[1] Obtained from Ssniff, Soest, Germany [2] Obtained from Charles River, Sulzfeld, Germany [3] Obtained from Protein Laboratories, Rehovot, Israel [4] Maintenance dose between 1.5 – 2% [5] With the opening first [6] A tissue adhesive used to close fresh wounds

3.5 Cecal Ligation and Puncture *(CLP)*

Animals were anesthetized with ketamine hydrochloride (150 mg/kg) and xylazine (7.5 mg/kg) injected intraperitoneal *(i.p.)*. Thereafter, the mouse was brought to a supine position upon an acrylic glass part *(Plexiglas)*, all four extremities were fixed with adhesive tape and any hair from the abdomen was thoroughly trimmed and removed. Subsequently, an approximately 1.5 cm midline laparotomy was performed, the cecum was gently exteriorized and ligated distal of the ileocecal valve without causing intestinal obstruction. The cecum was then perforated three times (top, middle, and bottom third) using a 21-gauge needle and squeezed to extrude fecal contents that were spread around the cecum using a cotton swab. The intestine were then carefully re-inserted in situ and the rectus abdominis muscle as well as the skin were closed using a continuous suture (**F**igure **4**). After the surgery each mouse received 0.01 mg/kg Butomidor® filled up with isotonic sodium chloride solution to 1 ml subcutaneously for analgesia and fluid resuscitation. The animals were kept separately until full recovery of the anesthesia and had free access to standard chow and water after induction of sepsis. In sham animals, the cecum was exteriorized without ligation and perforation.

Figure 2 | Example ALZET® osmotic pump

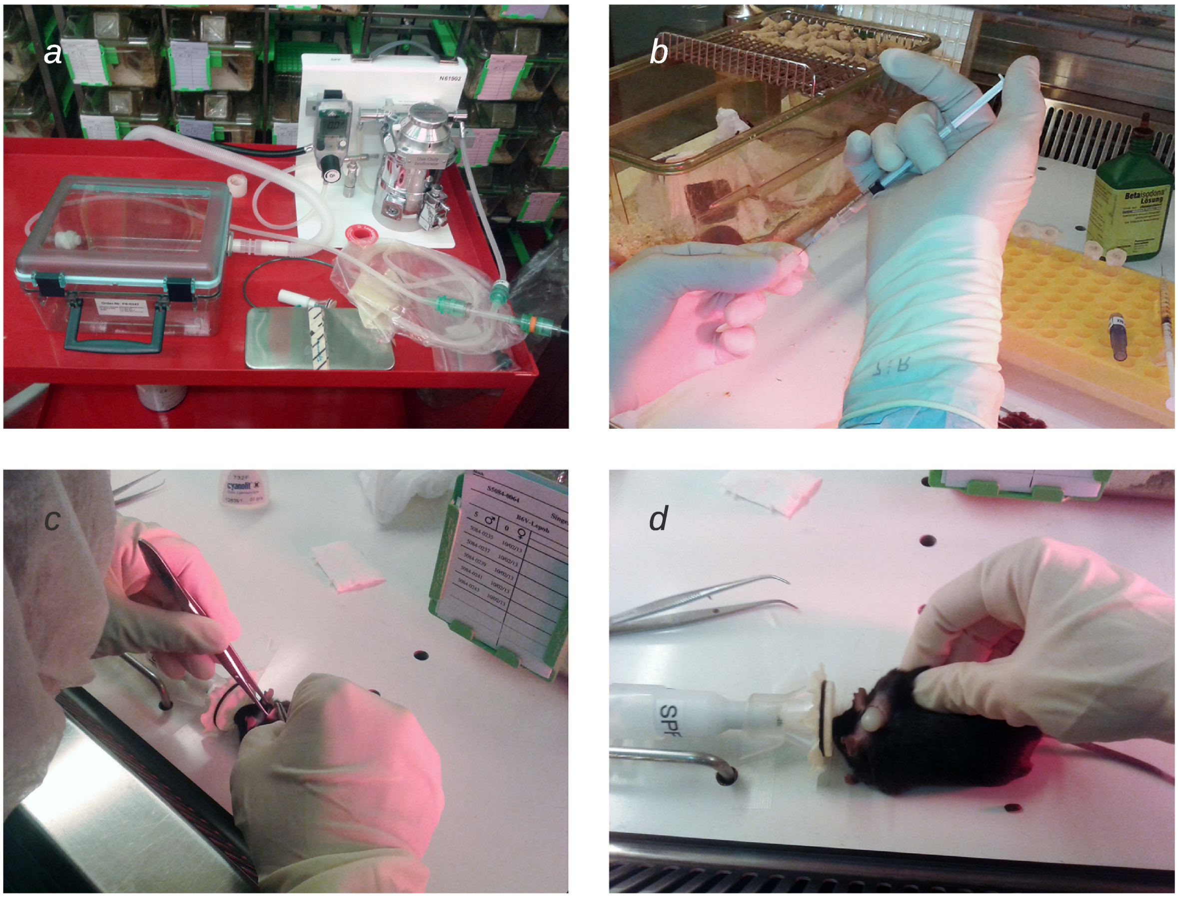


Model 2002 | Reservoir volume: 200 µl | Duration 2 weeks

3.6 Isolation of Platelets

Platelets were isolated from corresponding donor mice as described in the literature [32] according to the following protocol. Animals were anesthetized with ketamine hydrochloride (150 mg/kg) and xylazine (7.5 mg/kg) injected intraperitoneally. The right carotid artery was cannulated as explained in **T**able **7** and shown in **F**igure **5**.

Figure 3 | Minipump exchange under laminar flow cabinet



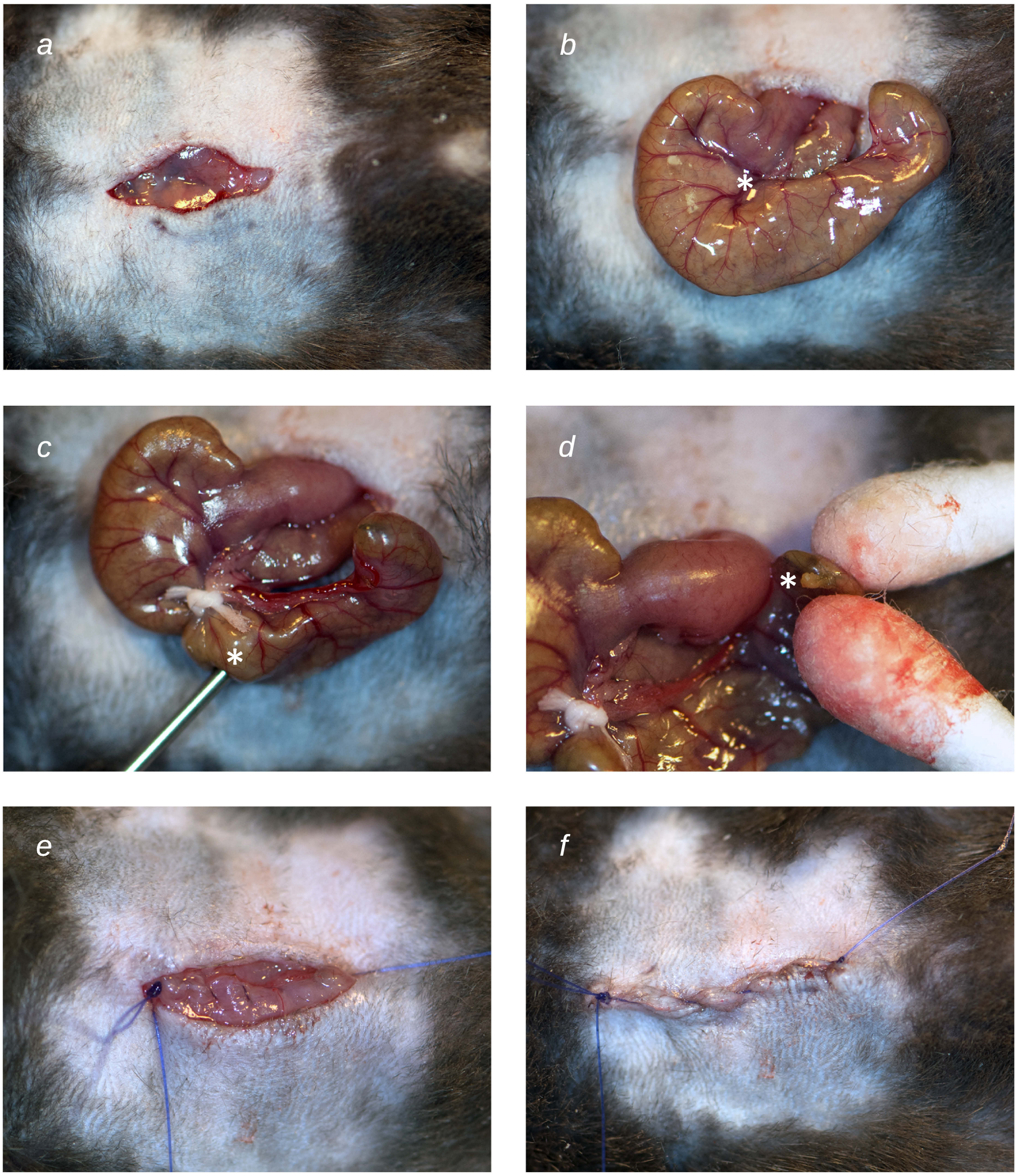
panel a | Inhalational anesthesia unit with isoflurane vaporizer and anesthesia box **panel b** | ALZET® osmotic pump filling with the provided syringe top part **panel c** | Blunt dissection with forceps for the subcutaneous pump-pocket **panel d** | Pump explantation with gently pressure through the skin on the pump

Table 7 | Short description of the cannulation of the right carotid artery

#	Description
1	Fixing the mouse with adhesive tape on all four extremities in a supine position
2	Easy tensioning the neck with a yarn and trim and remove hair from the neck area
3	Cut the skin from the mandible over both sides until the beginning of the thorax
4	Blunt dissection and shift to the sides of both salivary glands with a forceps
5	Gentle blunt dissection along the right side of the trachea until the carotid artery appears*
6	Ligature of its cranial end and easy tensioning with a yarn*
7	Caudal positioning of a clamp and preparation for fixation of the catheter with another yarn*
8	Carefully cut into upper third of the exposed carotid artery*
9	Insert the obliquely cut catheter (prefilled with ACD-buffer) and fix it with the prepared yarn*
10	Gently open the clamp and draw up slowly the blood into the syringe

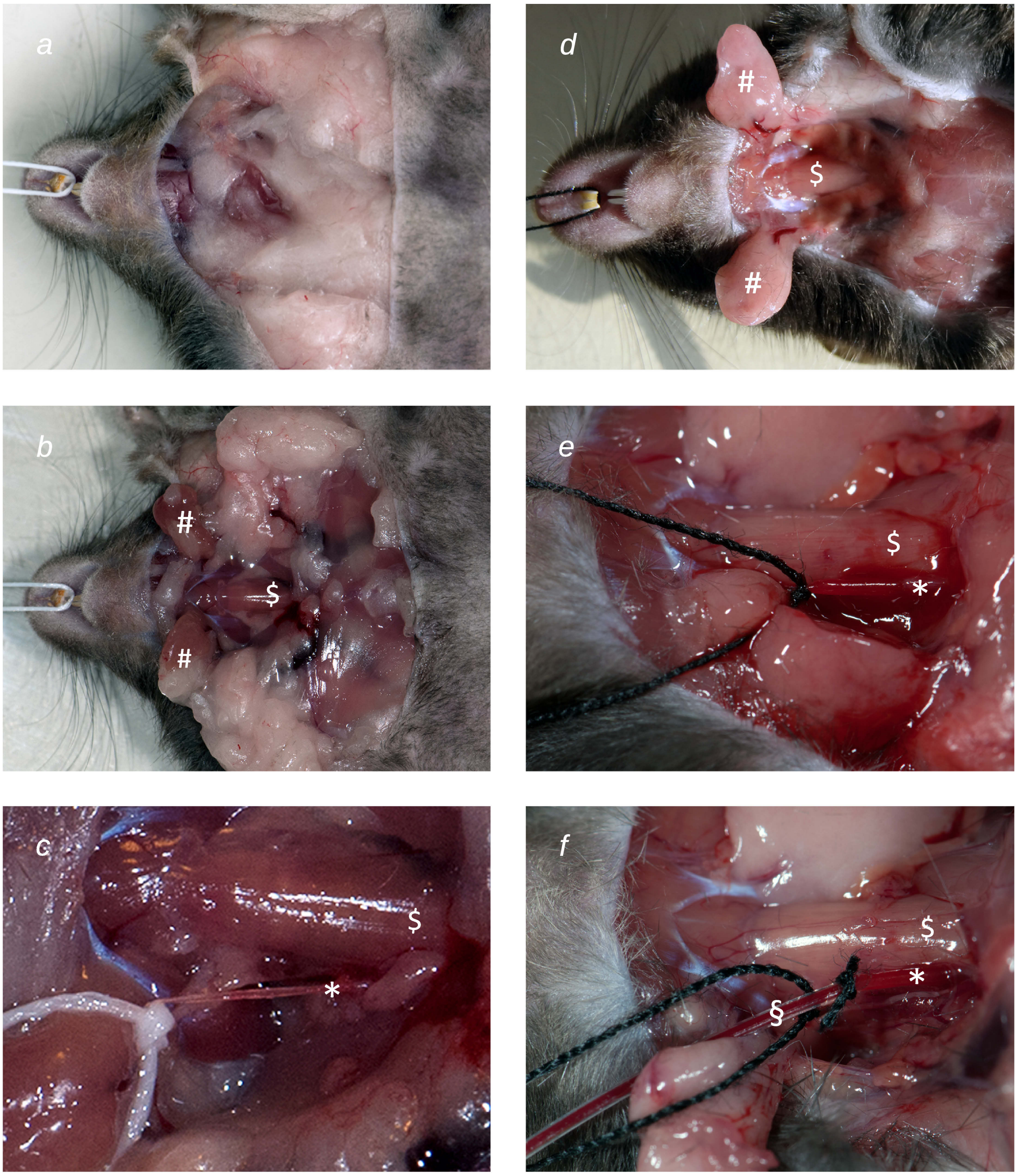
* Necessity to work under a stereomicroscope (ZEISS Stemi DV4 SPOT)

Figure 4 | Cecal ligation and puncture photo gallery



panel a | Skin incision for the midline laparotomy (left=cranial) **panel b** | Exteriorized intestines; *Ileocecal valve **panel c** | Ligation and *puncture of the cecum with a 21-g needle in the top third **panel d** | Squeezing out *feces at the bottom third **panels e-f** | Continuous sutures of the rectus abdominis muscle and the skin

Figure 5 | Carotid artery cannulation



panels a-c | ob-mouse

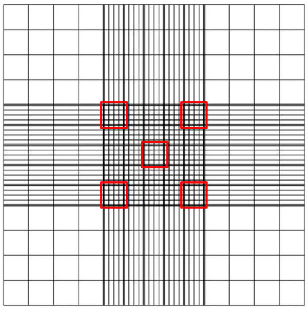
panels d-f | WT-mouse

∗ Carotid artery | # Salivary glands | $ Trachea | § Catheter

Table 8 | Platelet dilution fluid

Chemical	**Amount**
Sodium Citrate	3.8 g
Brilliant Cresyl blue	0.1 g
Formaline 35%	0.2 ml
Distilled water	100 ml

Figure 6 | Hemocytometer



About 1 ml of whole blood was drawn into a 1 ml syringe prefilled with 0.1 ml of 'Acid-Citrate-Dextrose' *(ACD)* buffer. The blood was transferred to a 1.5 ml Eppendorf tube, mixed two times with the syringe and immediately centrifuged at 1,200 rounds per minute *(rpm)* for 8 minutes. Serum and buffy coat were pipetted in a new 1.5 ml Eppendorf tube. The serum was then spun at 1,200 rpm for 3 minutes. The platelet rich plasma was taken without the pellet (consisting of leukocytes and erythrocytes) and centrifuged at 3,000 rpm for 10 minutes. The resulting pellet was slowly resuspended with 500 µl 'Phosphate-Buffered Saline'[1] *(PBS)* according to Sørensen (pH 7.38). In order to count the number of platelets 5 µl of the suspension was taken und mixed with 495 µl freshly filtered platelet dilution fluid (**T**able **8**). The suspension was incubated for 5 minutes at room temperature. A small amount was manually transferred to a hemocytometer and incubated for 10 to 40 minutes. The platelets were counted within the marked 5 squares (**F**igure **6**) and the number was multiplied with 5,000 resulting in number of platelets per µl. Shortly before usage, the suspension was filled up with PBS to 1.5 ml and 9 µl of CFSE[2] were added to give a concentration of 90 µM. The solution was gently mixed and incubated for 10 minutes in the dark at room temperature. The tube was then centrifuged at 3,000 rpm for 10 minutes. The resulting pellet was gently diluted with PBS to give a concentration of 100×10^6 platelets per 120 µl. The platelets isolated of one donor mouse usually were sufficient for two experiments. This isolation process does not cause activation of platelets as assessed by flow cytometry.[88]

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[1] Obtained from MORPHISTO® Evolutionsforschung und Anwendung GmbH, Frankfurt am Main, Germany

[2] Obtained from Sigma Aldrich, Vienna, Austria

3.7 Hepatic Intravital Microscopy

3.7.1 Fluorescent Substances Administration

Six hours following either induction of sepsis or the sham procedure animals were re-anesthetized with ketamine hydrochloride (150 mg/kg) and xylazine (7.5 mg/kg) and placed on a sensor controlled heating pad (37°C). One of the tail veins was cannulated with a 1 ml insulin syringe and the CFSE marked platelets (100x10^6 in 120 µl) were infused over 5 minutes using a syringe pump[1].

Five minutes thereafter rhodamine 6G[2] (120 µl over 5 minutes) was infused to get fluorescently labeled leukocytes. The stem solution of rhodamine 6G was prepared as shown in **T**able **9**. Both syringes were protected from light using aluminum foil.

Table 9 | Rhodamine 6G stem solution

Chemical	Amount
Rhodamin 6G	0.2 g
Distilled water	100 ml

Before infusion the stem solution was diluted tenfold with PBS

3.7.2 Surgical Procedure and Experimental Setup

The upper abdomen was shaved, an upper median midline laparotomy was performed and the skin was removed in a circular manner. The abdominal layers were opened and the mouse placed in a supine position on a Plexiglas microscope stage. Parts of liver lobe were carefully exteriorized applying pressure on the back of the mouse and placed on the cover glass of the Plexiglas (**F**igure **7**) stage moistened with 0.9% NaCl. The liver was covered with moist gauze to protect from dehydration. The mouse was then transferred to the intravital microscope[3] equipped with a X-Cite® 120 PC fluorescent illumination system with a 120W high pressure metal halide arc lamp and a dual fluorescent filter set[4]. Microscopic images were obtained using a 20x objective lens and digitized on a computer

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[1] Model 540060; Obtained from TSE Systems, Bad Homburg, Germany [2] Obtained from Sigma Aldrich, Vienna, Austria [3] Olympus® IX 71 inverted microscope, Olympus, Vienna, Austria [4] U-N51004v2 - FITC/TRITC

through a monochrome video camera[1] and were recorded with an image processor[2] with following basic settings (**T**able **10**).

Figure 7 | Liver lobe exteriorization procedure

panel a | Upper median midline skin incision (12 - 15 mm) **panel b** | Removed skin in a circular manner **panel c** | Carefully removing of the abdominal layers using a bipolar forceps[3] **panel d** | Opened peritoneal cavity directly over the liver **panels e-f** | Exteriorization of the liver without direct manipulation

Animals were sacrificed by cervical dislocation after recordings from up to eight videos.

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[1] Olympus® XM10, 1.4 Mpx

[2] Olympus® cellSens® image acquisition software

[3] Model: VIO 100 C; Obtained from ERBE® Elektromedizin GmbH, Tübingen, Germany

Table 10 | Software settings

Settings	Description
Dark application skin	To avoid scattered radiation from light
Exposure mode	Manual 50 ms | results in around 12 – 15 frames per second
Resolution	1376 x 1032 px
Duration	At least 1107 single frames (about 1 minute and 30 seconds)
Device settings	XM10 contrast and brightness settings changed if necessary

3.7.3 Microcirculation Analyses

The liver surface was scanned for well representable postcapillary terminal hepatic venules or collecting venules. Depending on the generally health status[1] of each individual mouse 3 - 8 venules could be recorded. According to the resulting video-quality (sharpness, motion, brightness) 3 - 5 venules were selected for a digital post-production process which was performed with Adobe Premiere Pro CS6®[2]. At this process the videos selected for statistical analysis were cut to a length of 1 minute and 6 seconds[3], enlarged up to 25% and centered. Other settings like brightness, contrast, shadow/highlight and lightning effects were set if they had positive effects to the interpretability of the videos. The analyses was made with the free, open-source video-player VLC[4] considering the following aspects. Terminal hepatic venules or collecting venules with diameters ranging from 23.2 to 69.5 µm (mean 40.4 µm) were observed on segments of one-hundred micron (100 µm) and cells were classified according to their interaction with the vessel wall as either rolling or adherent as depicted in **F**igures **8** and **9**. Leukocytes and platelets were considered as rolling if they were moving along the endothelial cells of the vessel wall in at least 3 seconds for the whole distance but not remaining more than 10 seconds stationary on a segment of the vascular wall or connected to other adherent cells. Leukocytes and platelets which were tightly adherent to the endothelium or other stationary cells for more than 10 seconds were counted as adherent. Sticking cells in inlets of the sinusoid network were excluded if they remained stationary over the period considered (**F**igure **9**).

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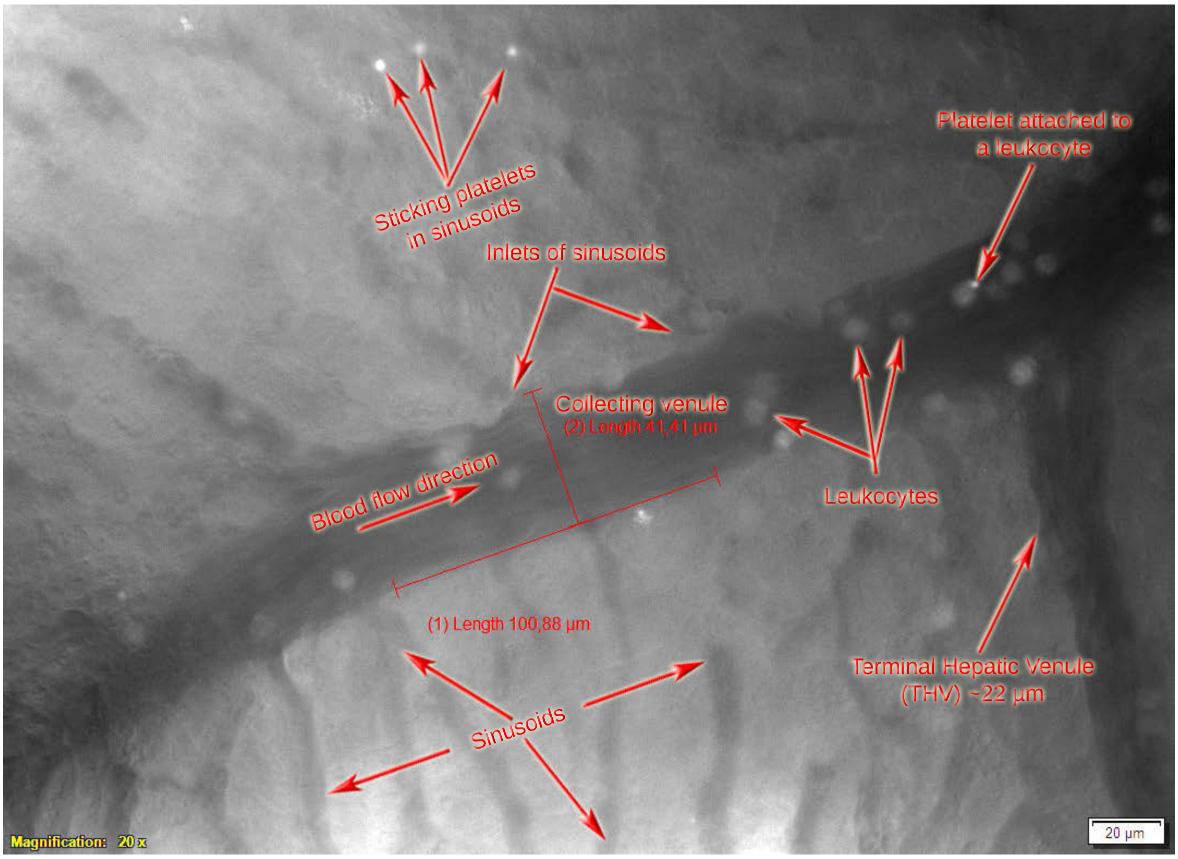
[1] Depends on the surgical procedure (sham or CLP), the experimental strains (WT, db/db, ob/ob) and also on the general circulatory situation as a function of time [2] Obtained from Adobe Systems GmbH, München, Deutschland [3] 3 seconds each for in- and outro [4] VideoLAN organization, Paris, France

Rolling cells (leukocytes and platelets) were expressed as number per minute per 100 µm vessel length, whereas adherent cells were quantified as the number per mm^2 venular wall (calculated assuming cylindrical vessel geometry). The mean of the average values of either leukocyte– and platelet adhesion or rolling determined in each mouse was used to generate the group mean.

3.7.4 Statistical Analysis

For statistical analysis SPSS 22.0[1] was used. Data are described as means plus/minus 'Standard Deviation' *(SD)*. The data for intravital microscopy were analyzed using a one-way ANOVA with Fisher's 'Least Significant Difference' *(LSD)* post-hoc test. A p-value of $p < 0.05$ was considered statistical significant.

Figure 8 | Liver segment overview



Snapshot captured with Olympus® IX71 microscope | Length (1) and (2) labeled with cellSens® image acquisition software

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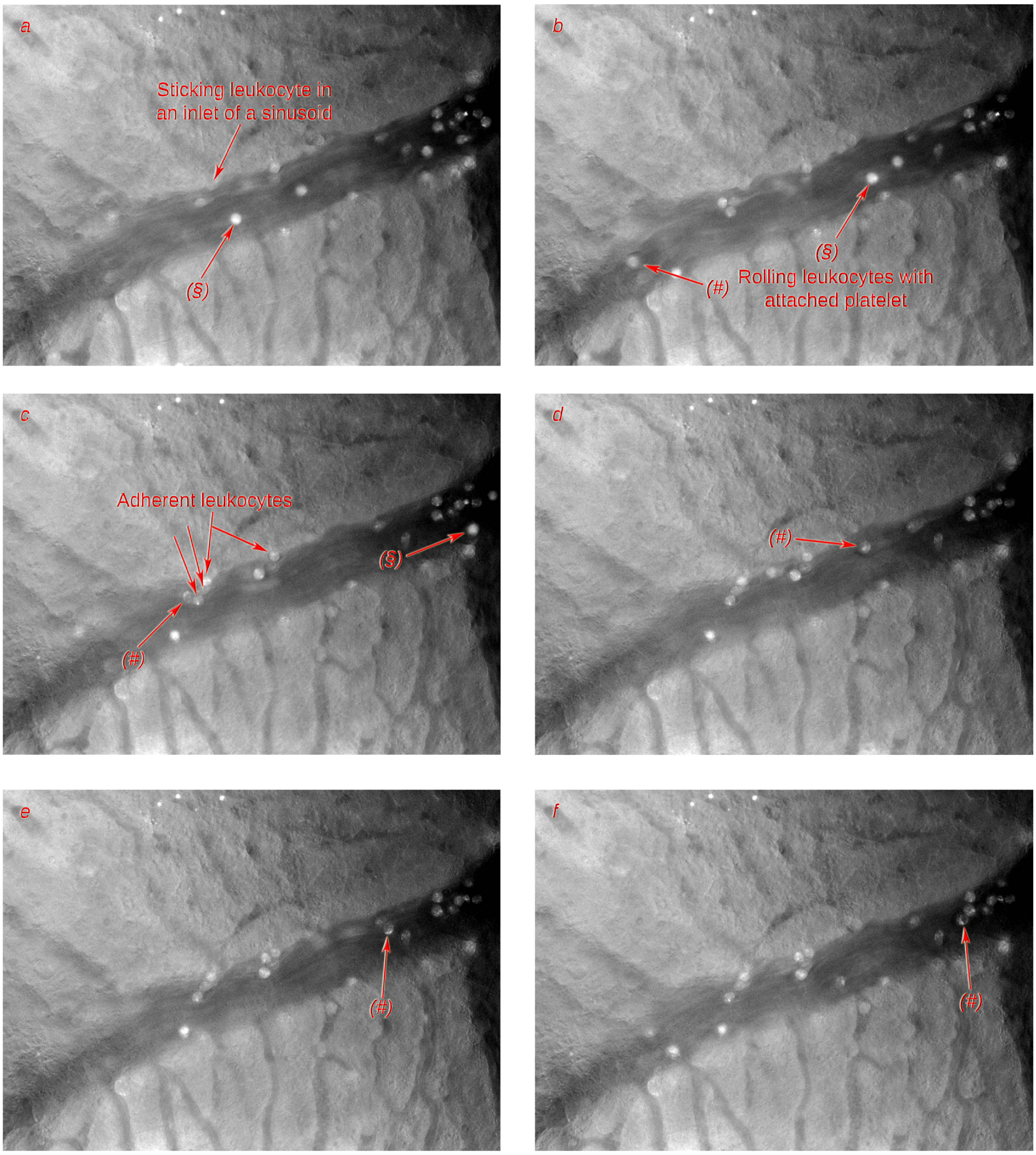
[1] IBM Corp, Armonk, NY, USA

Figure 9 | Microcirculation example from a DIO CLP mouse



panels a–e | Illustration of 6 snapshots made with VLC–video-player captured in 15 seconds (1 frame every 2.5 seconds)
#... in statistic (≥ 3 sec rolling) | §... not in statistic (≤ 3 sec rolling)

4 | Results

4.1 Standard Parameters

The weight of all examined groups is presented in **T**able **11**. All tested sham and septic mice (DIO, db/db, ob/ob and ob/ob + leptin) were significantly heavier compared with WT mice. However, treatment of ob/ob with leptin led to a significant weight reduction when compared with the ob/ob mice.

Table 11 | Mean weight by group

Group	Number of animals	Weight [g]
WT sham	5	21.9 ± 0.9
WT CLP	4	21.8 ± 1.1
DIO sham	5	28.4 ± 1.4*
DIO CLP	4	26.9 ± 1.2*
db/db sham	5	47.0 ± 4.7*
db/db CLP	4	51.2 ± 2.4*
ob/ob sham	4	41.4 ± 1.8*
ob/ob CLP	4	37.0 ± 3.5*
ob/ob + leptin sham	4	28.7 ± 2.4*#
ob/ob + leptin CLP	4	29.8 ± 3.6*#

Comparison of the weight of the animals studied in sham and septic mice of WT, DIO, db/db, ob/ob and ob/ob + leptin mice | WT. . . **W**ild **T**ype; DIO. . . **D**iet **I**nduced **O**besity; CLP. . . **C**ecal **L**igation and **P**uncture | Values are expressed as mean ± SD | * $p < 0.05$ vs. corresponding WT | # $p < 0.05$ vs. corresponding ob/ob.

The venular diameter of the vessels studied is depicted in **T**able **12**. The venular diameter did not differ among the studied groups.

Table 12 | Mean venular diameter by group

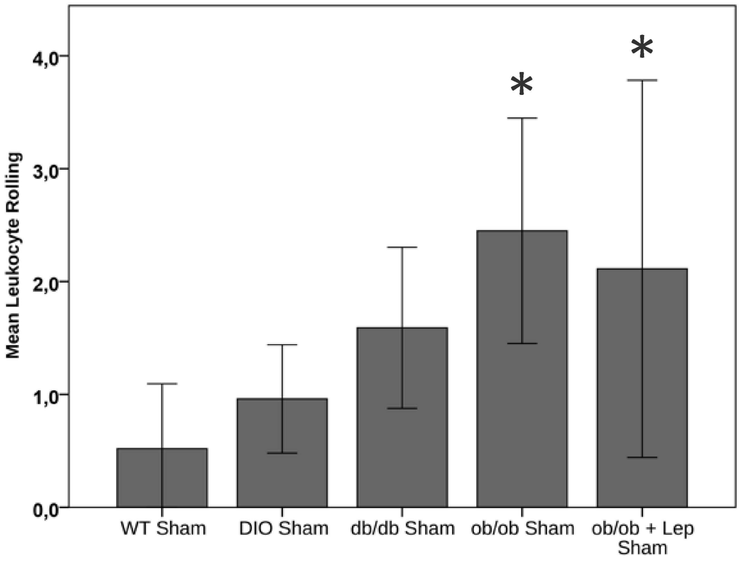
Group	Number of animals	Venular diameter [µm]
WT sham	5	39.8 ± 5.9
WT CLP	4	37.7 ± 2.0
DIO sham	5	42.6 ± 5.7
DIO CLP	4	41.7 ± 2.2
db/db sham	5	39.8 ± 7.0
db/db CLP	4	38.0 ± 2.7
ob/ob sham	4	41.5 ± 5.6
ob/ob CLP	4	43.6 ± 1.6
ob/ob + leptin sham	4	40.9 ± 2.7
ob/ob + leptin CLP	4	38.5 ± 2.1

Comparison of the venular diameter of the animals studied in sham and septic mice of WT, DIO, db/db, ob/ob and ob/ob + leptin mice | WT. . . **W**ild **T**ype; DIO. . . **D**iet **I**nduced **O**besity; CLP. . . **C**ecal **L**igation and **P**uncture | Values are expressed as mean ± SD

4.2 Leukocyte Rolling

First a comparison of leukocyte rolling was performed, which was assessed in all groups. Even though a tendency towards an increase in leukocyte rolling in obese sham groups versus WT mice was seen, the increase reached statistical significance only in the ob/ob group and the ob/ob mice treated with leptin (**F**igure **10**).

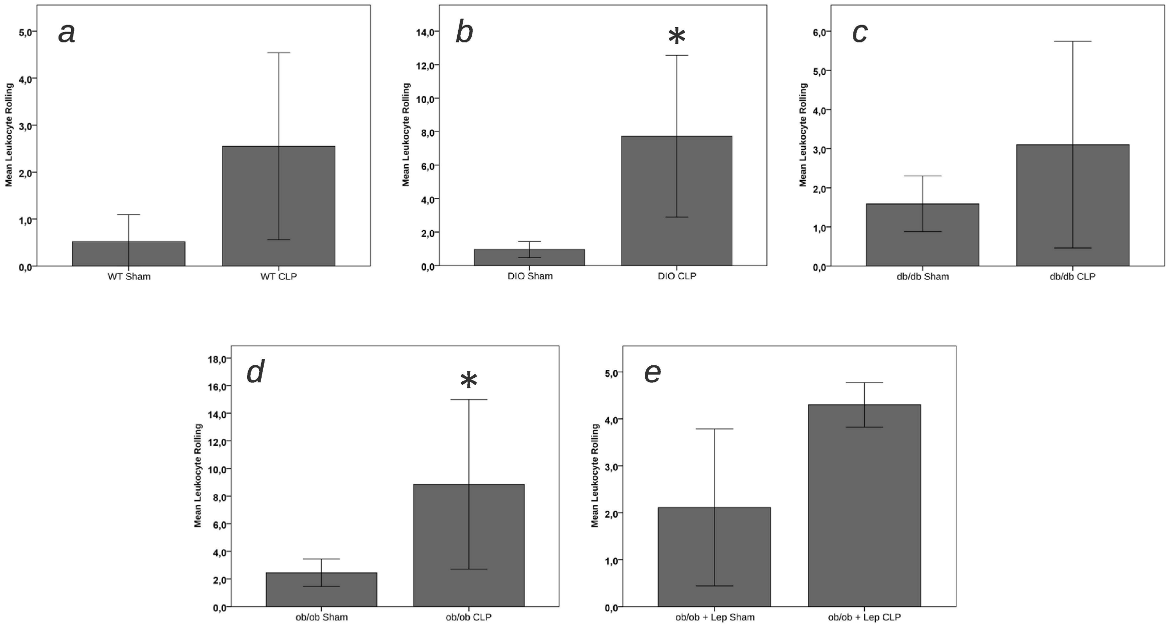
Figure 10 | Mean leukocyte rolling | Sham vs. WT sham



Leukocyte rolling in WT, DIO, db/db, ob/ob and ob/ob + leptin mice following sham operation | * $p < 0.05$ vs. WT sham

In a next step, a groupwise comparison of sham versus CLP procedure was performed in the different groups. Polymicrobial sepsis caused increases in leukocyte rolling in all groups with statistical significance in the diet induced obesity and ob/ob animals. The results are depicted in **F**igure **11**.

Figure 11 | Mean leukocyte rolling | Sham vs. CLP



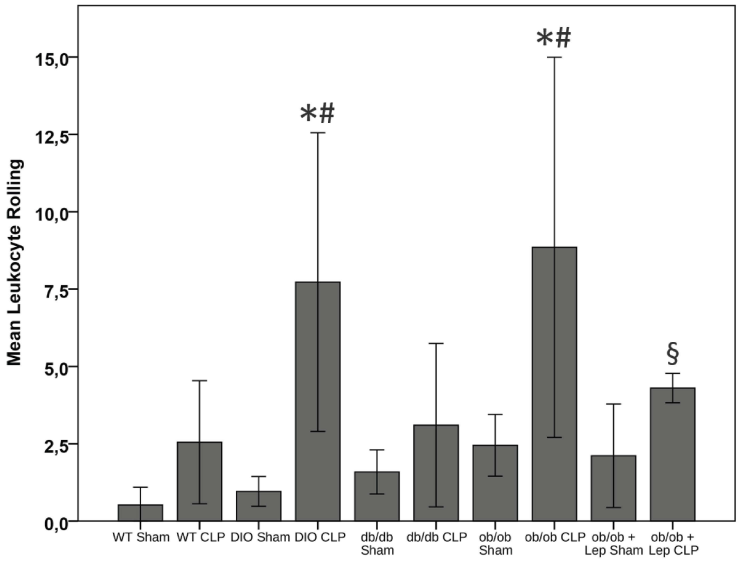
panels a–e | Leukocyte rolling in sham versus CLP mice (WT, DIO, db/db, ob/ob, ob/ob + leptin) | * $p < 0.05$ vs. corresponding sham animals

Statistical analysis of all groups revealed that both diet induced obesity and obesity on an ob/ob background responding with a significant increase in leukocyte rolling 6 hours following induction of sepsis with CLP. Leptin treatment in the ob/ob strain resulted in a significant decrease in leukocyte rolling compared to the ob/ob CLP group as seen in **F**igure **12**.

4.3 Leukocyte Adhesion

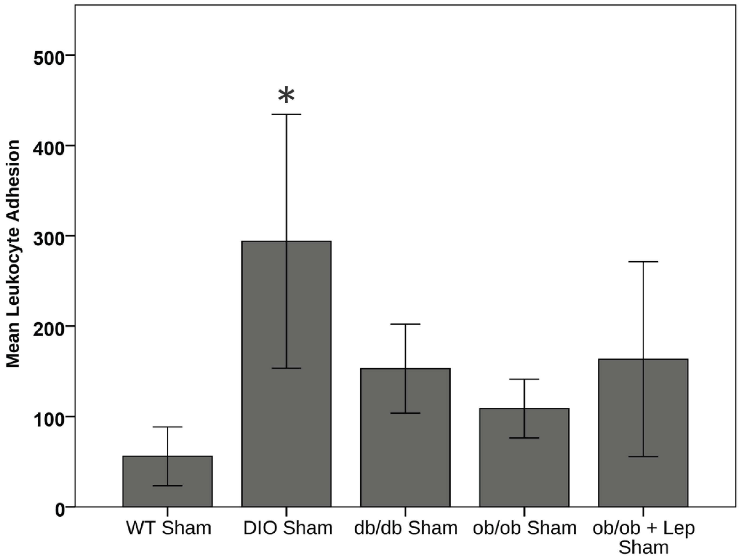
All sham mice had a trend towards augmented leukocyte adhesion. However, values reached statistical significance in diet induced obesity (**F**igure **13**).

Figure 12 | Mean leukocyte rolling | Sham vs. CLP – all groups



Leukocyte rolling in WT, DIO, db/db, ob/ob, ob/ob + leptin 6 hours following either a sham or a CLP procedure | * p < 0.05 vs. corresponding sham animals | # p < 0.05 vs. WT CLP | § p < 0.05 vs. ob/ob CLP

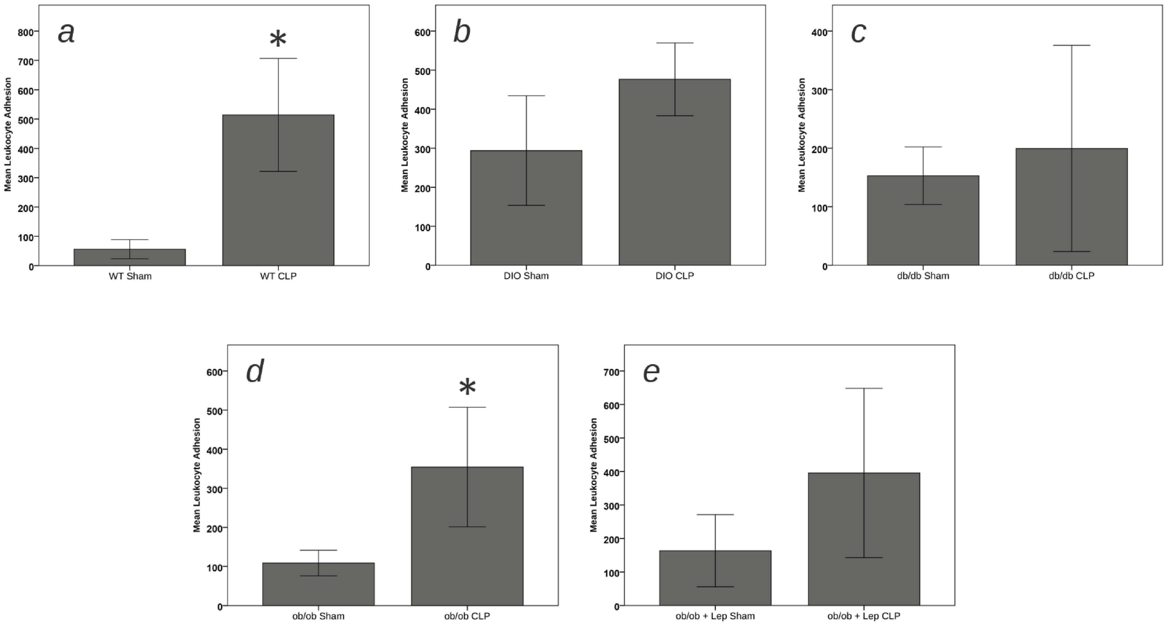
Figure 13 | Mean leukocyte adhesion | Sham vs. WT sham



Leukocyte adhesion in WT, DIO, db/db, ob/ob and ob/ob + leptin mice 6 hours following sham operation | * p < 0.05 vs. WT sham

Figure **14** shows that comparison of leukocyte adhesion in a groupwise manner revealed that polymicrobial sepsis caused by CLP led to increases in leukocyte-endothelium interaction in WT and ob/ob mice (Panels a and d).

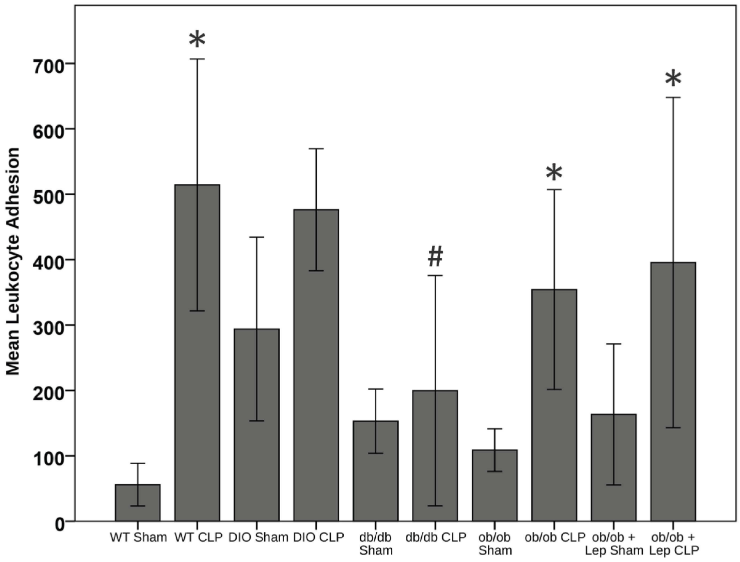
Figure 14 | Mean leukocyte adhesion | Sham vs. CLP



panels a–e | Leukocyte adhesion in sham versus CLP mice (WT, DIO, db/db, ob/ob, ob/ob + leptin) | * $p < 0.05$ vs. corresponding sham animals

Treatment of ob/ob mice with leptin could not reverse these microcirculatory disturbances elicited by sepsis. Interestingly, septic db/db mice had significantly less leukocyte adhesion when compared to the WT CLP group (**F**igure **15**).

Figure 15 | Mean leukocyte adhesion | Sham vs. CLP – all groups

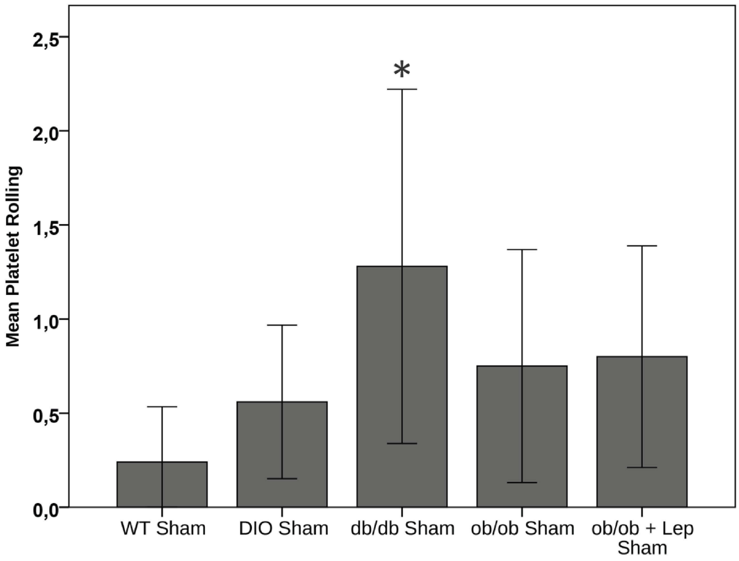


Leukocyte adhesion in WT, DIO, db/db, ob/ob, ob/ob + leptin 6 hours following either a sham or a CLP procedure | * $p < 0.05$ vs. corresponding sham animals | # $p < 0.05$ vs. WT CLP

4.4 Platelet Rolling

Platelet rolling was unchanged in nearly all studied sham groups. Only the db/db strain had increases in hepatic platelet rolling (**F**igure **16**).

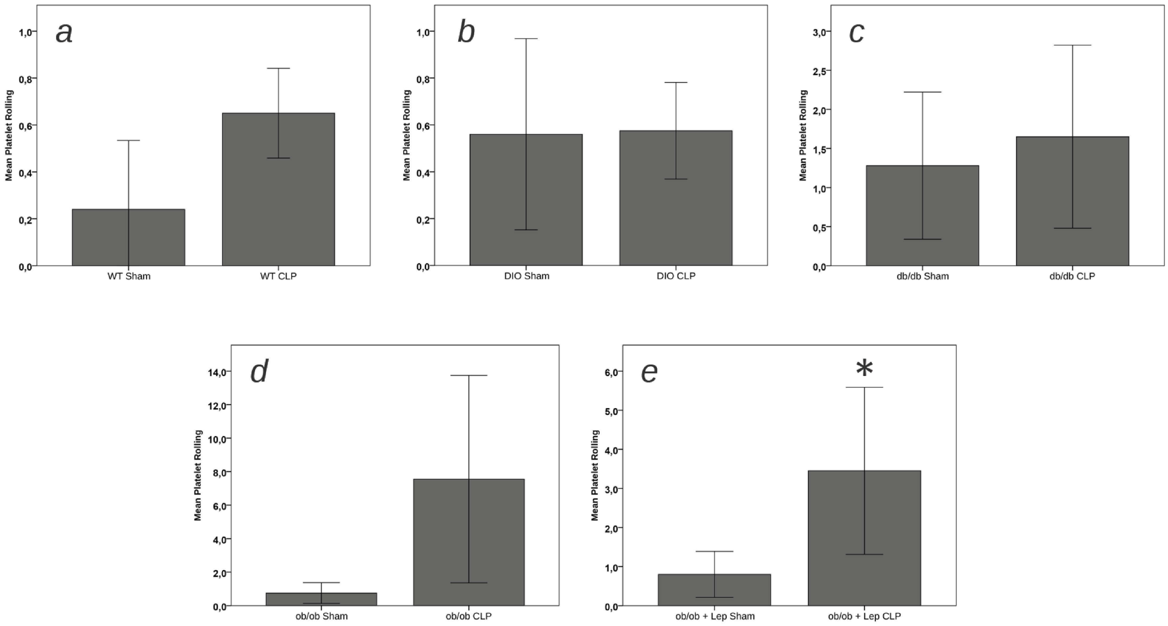
Figure 16 | Mean platelet rolling | Sham vs. WT sham



Platelet rolling in WT, DIO, db/db, ob/ob and ob/ob + leptin mice 6 hours following sham operation | * $p < 0.05$ vs. WT sham

Sepsis caused significant increases of platelet rolling in the ob/ob + leptin group whereas there was only a tendency towards an increase in WT and ob/ob animals 6 hours following CLP (**F**igure **17**).

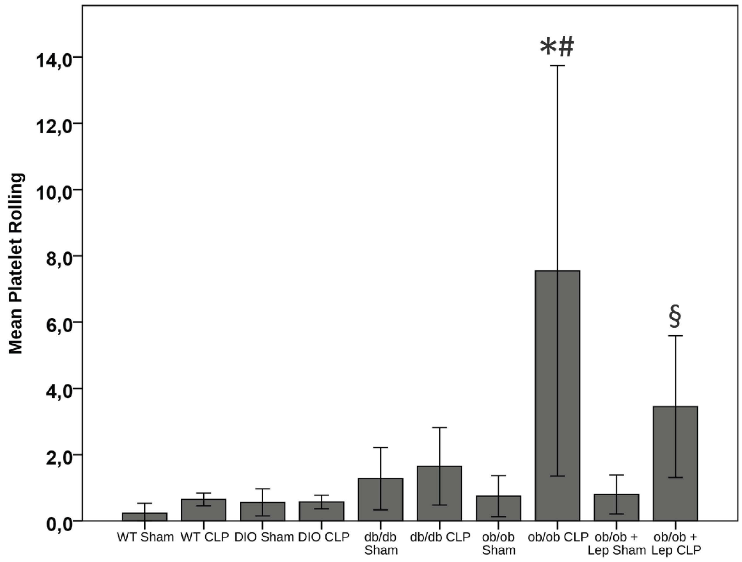
Figure 17 | Mean platelet rolling | Sham vs. CLP



panels a–e | Platelet rolling in sham versus CLP mice (WT, DIO, db/db, ob/ob, ob/ob + leptin) | * $p < 0.05$ vs. corresponding sham animals

Figure **18** demonstrates that there were significantly more platelets rolling in ob/ob mice following sepsis when compared with the corresponding sham and WT CLP animals. Treatment of ob/ob mice with leptin caused a significant reversal of these microcirculatory disturbances.

Figure 18 | Mean platelet rolling | Sham vs. CLP – all groups

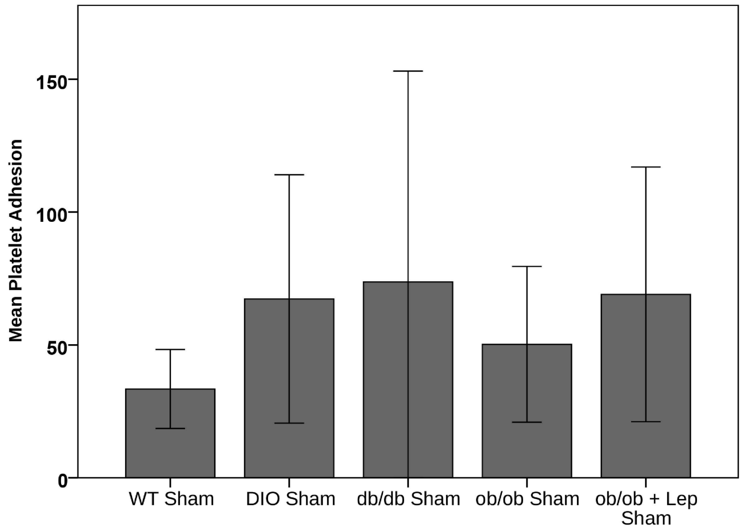


Platelet rolling in WT, DIO, db/db, ob/ob, ob/ob + leptin 6 hours following either a sham or a CLP procedure | * p < 0.05 vs. corresponding sham animals | # p < 0.05 vs. WT CLP | § p < 0.05 vs. ob/ob CLP

4.5 Platelet Adhesion

Platelet adhesion was unchanged in all sham groups tested suggesting that obesity per se does not lead to microcirculatory disturbances as seen by changes in platelet adhesion (**F**igure **19**).

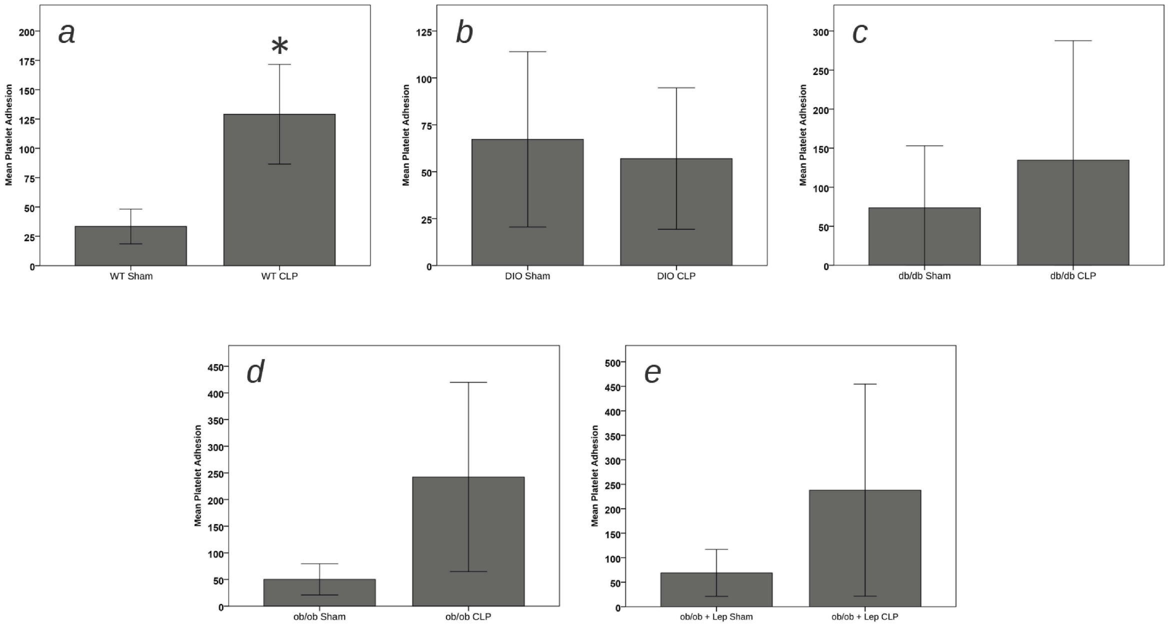
Figure 19 | Mean platelet adhesion | Sham vs. WT sham



Platelet adhesion in WT, DIO, db/db, ob/ob and ob/ob + leptin mice 6 hours following sham operation

Figure 20 depicts the changes in platelet adhesion in WT, DIO, db/db, ob/ob and ob/ob + leptin 6 hours following cecal ligation and puncture. Sepsis was associated with increased platelet adhesion in WT animals and a trend towards an increase in ob/ob and ob/ob + leptin animals.

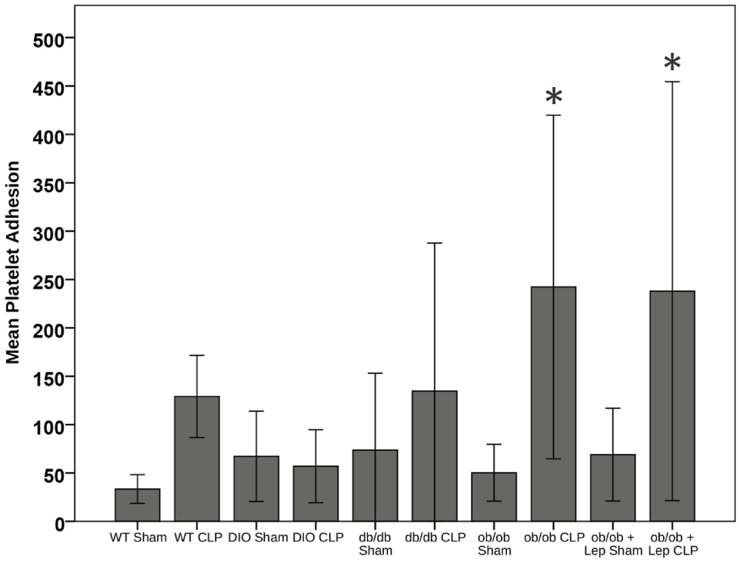
Figure 20 | Mean platelet adhesion | Sham vs. CLP



panels a–e | Platelet adhesion in sham versus CLP mice (WT, DIO, db/db, ob/ob, ob/ob + leptin) | * $p < 0.05$ vs. corresponding sham animals

Statistical analysis including all 10 groups of animals tested revealed significantly elevated levels of platelet adhesion in ob/ob and ob/ob + leptin animals as shown in **Figure 21**.

Figure 21 | Mean platelet adhesion | Sham vs. CLP – all groups



Platelet adhesion in WT, DIO, db/db, ob/ob, ob/ob + leptin 6 hours following either a sham or a CLP procedure | * $p < 0.05$ vs. corresponding sham animals

5 | Discussion

5.1 Obesity | Inflammation

Obesity represents a major health problem in the industrialized world in both adults and children. In the United States almost 35% of the adults (≥20 yrs, age-adjusted) and about 17% of children and adolescents (2 – 19 yrs, age-adjusted) are obese.[45] The obesity epidemic across the OECD area (average 18% obesity) spreads further, albeit more slowly than before, whereas more than one in three adults in Mexico, New Zealand and the United States are obese compared to Asian countries were the rates are just 2 to almost 5%. In Greece, Italy, U.S. and Slovenia more than 30% of boys as well as girls (except Slovenia) are overweight or obese.[43] A WHO estimation stating that globally over 42 million children under 5 years of age were overweight or obese in 2013.[40] These children are at greater risk of poor health in adolescence, as well as in adulthood, at which point cardiovascular disease, certain forms of cancer, diabetes, osteoarthritis, a reduced quality of life and premature death become health concerns.[89] Although contradictory opinions, there is evidence that obesity likely exacerbate sepsis morbidity as well as mortality. Obese patients acquire more often nosocomial– and wound infections, postoperative complications as well as more serious complications of common infections[67] which often results in increased admissions into hospitals and intensive care units with a higher mean length of stay and need of mechanical ventilation.[64,68] An increased ICU mortality rate in obese adults as well as in morbidly obese patients was determined at least in a risk-adjusted matched cohort [66] and a retrospective study.[64] It has been proposed that obesity exacerbates the body's responses to sepsis because increased obesity results in a chronic state of low-grade inflammation in white adipose tissue and at systemic level that may prime blood and endothelial cells to respond more vigorously to the additional inflammatory stimulus that results from sepsis.[84,90]

For creating the precondition of obesity we used established genetically altered mouse models of obesity, namely ob/ob and db/db mice lacking leptin and the leptin receptor, respectively. But since exceedingly few humans have genetic mutations we also used a diet induced obesity model, with mice that were exclusively fed with a high fat and carbohydrates diet for at least 4 weeks. Additionally, to account for the role of leptin we used ob/ob mice treated with leptin (0.3 mg/kg/day), whereby we could show a significant weight reduction as described in literature before.[54,56,91] The route of administration for leptin with implanted osmotic pumps subcutaneously in the upper back[92] or the abdomen[53] are established, and the first mentioned method worked for us as well.

Despite significant weight gain of all obese groups compared with WT mice and a significant weight loss in the ob/ob + leptin group compared to the genetically altered mouse models (ob/ob and db/db; compare **T**able **11**) there were no differences in the recorded venular diameters in all groups (**T**able **12**).

5.2 Leukocyte Recruitment

Rodent models offer important knowledge about metabolic abnormalities such as altered concentrations of hormones, metabolic factors, and nutrients. Altered levels of circulating immunomodulatory adipokines including leptin and adiponectin, as well as the pro-inflammatory cytokines TNFα, IL-6, and IL-1β or even CRP are responsible for the low grade chronic inflammation in obese individuals. In addition, there are decreased levels of adiponectin in obesity, which acts potently immunosuppressive.[51,57,58,71] This state may prime endothelial cells to over-express adhesion molecules and therefore promote subsequent adhesion of leukocytes and platelets. This has already been shown in genetically modified obesity in the microvasculature of the brain[93] and the small bowel[84] as well as in terminal hepatic venules in a DIO model.[94] Although the liver plays a key role and is one of the most commonly affected organs during sepsis, the microcirculation in genetically and dietary obese mouse models together were not determined until now.

In the obese *sham groups* of the present study mean *leukocyte rolling* was significantly elevated in the models with ob/ob background (**F**igure **10**). In the genetically modified groups more rolling compared to the DIO model was seen. Even though treatment with leptin in ob/ob mice led to weight reduction it did not modify leukocyte rolling. This may be as a consequence of the low grade inflammation in excess obesity and due to the fact that leptin per se acts as a pro-inflammatory agent, as Shapiro et al. have demonstrated in their study. The authors concluded that exogenously administered leptin in normal lean mice increased sepsis mortality, whereas leptin receptor-deficient mice (db/db) were protected during sepsis. Furthermore, leptin was associated with elevated expression of coagulation and adhesion molecules, macrophage infiltration into the liver and kidney, and endothelial barrier dysfunction.[53] Leptin administration also impaired T cell-mediated hepatic inflammation induced by Concanavalin A *(Con A)* by mediating T cell activation as well as cytokine production and maintaining high numbers of hepatic NK T cells.[95] Together, these findings support a pathogenic role for exogenously supplied leptin.

However, in DIO *shams* more *adhesion of leukocytes* was seen compared to genetically altered murine models of obesity and with a significantly difference to WT mice (**F**igure **13**). Because of a relatively short period of the western type diet the DIO group had just little weight differences compared to WT and ob/ob + leptin (**F**igure **10**). In fact the fur as well as the fat storage of the DIO mice looked different and more affected by unhealthy mixture of nutrients. These findings, combined with the later discussed fact that lean mice had more leukocyte adherence to the endothelium in sepsis and a presumed higher circulating leptin level compared to genetically altered obesity models (except leptin treated ob/ob group), might be the reason for the significant increase of leukocyte adhesion in sham operated DIO mice.

Mean *leukocyte rolling in polymicrobial sepsis* induced by CLP was significantly elevated in DIO mice and the ob/ob group referring to corresponding shams and to WT CLP mice (**F**igure **12**). Leptin treatment in septic mice reduced leukocyte rolling significantly compared with the ob/ob strain which had the highest mean value of all groups.

Surprisingly, most *leukocyte sticking* in the hepatic microcirculation was seen in septic WT mice, whereas all other groups tended to lower values, however, a statistically significant reduction of leukocyte adhesion was found in db/db mice, maybe due to the fact that the circulating leptin cannot act because of the lack of leptin receptors (**F**igure **15**). This might be one of the effects for better outcome in obese individuals during critical illness (described previously in ‘Obesity – A Protective Impact?’), besides clinically described hypothesis[60] for this apparent survival effect, which is called obesity paradox. Interestingly, the three strains with quite similar little weight, namely WT, DIO, and ob/ob + leptin, had the most adhesion of leukocytes in hepatic THVs and collecting venules. These findings suggest that white blood cells in the microvascular bed of the liver in various mouse models respond differently to the behavior of corresponding groups in the small bowel[84] or the brain[93].

5.3 Platelet Recruitment

Following an inflammatory stimulus WBCs recognize pathogens via pattern recognition receptors inciting a variety of changes such as release of cytokines and adipokines, leading to an activation of complement– and coagulation cascades. Generation of thrombin and fibrin are the consequences of these pathways resulting in stabilized platelet plugs which immobilize the pathogen on the surface of leukocytes and the neighboring endothelial cells for further intracellular absorption and disposal. In addition there is downregulation of anti-coagulant responses in sepsis and a shift towards a pro-coagulant environment as well as consumption of coagulation factors which can result in an acute life-threatening state of disseminated intravascular coagulation *(DIC)*.[27] Singer et al. demonstrated an impaired hepatic perfusion with increased residence time *(sequestration)* of leukocytes in both sinusoids and terminal hepatic venules of the liver microcirculation accompanied by the adhesion of platelets as well as a neutrophil-dependent platelet response to sepsis.[32]

In our *sham* experiments we have seen almost no changes in *platelet rolling and adhesion* suggesting that the pro-inflammatory condition of obesity per se does not cause enough endothelial or platelet activation (**F**igures **16** + **19**). These results are comparable with findings in other organs such as intestine[84], liver[32] and the mouse brain[93].

Sepsis induced by CLP caused a significant increase in *platelet rolling* in the ob/ob group compared to corresponding shams and WT CLP mice, whereas leptin replacement had the same reduction effect on cell rolling as described before with leukocyte rolling compared to the septic ob/ob strain (**F**igures **18** + **21**). Significantly more platelet adherence was seen in the septic ob/ob mice checked against its shams. No protective effect was seen in terms of platelet adhesion by exogenously supplied leptin which is likely the same result as in the leukocyte adhesion ob/ob + leptin CLP group.

6 | Conclusion

Exogenously administered leptin lead to a significant weight reduction in genetically altered leptin deficient ob/ob mouse model in our experiments as described before in current literature. Diet induced obesity, although a relatively short period of high caloric feeding, as well as the leptin receptor deficient mice (db/db) and the ob/ob group reached significant weight gain compared to the lean WT mice, whereas no difference in diameter of the recorded post-capillary venules could be observed.

Excess obesity had almost no influence on leukocyte rolling in sham groups, although significantly higher cell counts were measured in the ob/ob strain, however, these findings suggest minor influence of obesity for leukocyte rolling. DIO is associated with significantly more adherent leukocytes in sham operated mice with mean sticking cells of around 300 cells per mm^2 which is about twice as much as in the genetically altered models of obesity. Higher circulating leptin levels with its associated pro-inflammatory effects described by Shapiro et al. might be the reason for this in high caloric fed obese mice.

Polymicrobial sepsis induced by CLP increased the number of rolling leukocytes in DIO and ob/ob groups, whereas leptin treatment in ob/ob mice reduced the amount of rolling cells significantly from approximately 9 to 4 cells per 100 µm venule per minute. Although septic db/db mice had almost as less rolling as septic WT mice in this lean group the most leukocyte sticking with over 500 cells per mm^2 was observed, whereby the heaviest group of db/db mice had the lowest values. This protective fact might be because of the impossibility of leptin to interact with white blood cells and the endothelium. Septic mice of DIO group were just behind the WT mice with second most leukocyte adherence followed by ob/ob + leptin group and untreated ob/ob mice. These findings suggest an exacerbating state in the liver microcirculation in lean subjects maybe due to increased circulating pro-inflammatory leptin levels.

In our sham processed obese individuals there were nearly no changes in platelet recruitment. Septic leptin deficient ob/ob mice with almost 8 rolling platelets per 100 µm venule per minute reached by far the highest values, whereas exogenously supplied leptin reduced the rolling about 50%.

Both, ob/ob and ob/ob + leptin, had, with around 250 cells per mm^2 each, the highest number of adherent platelets which is around twice as high as in the next ranked db/db group and closely behind WT groups and reached significance compared to their corresponding shams. These finding suggests that in genetically altered mouse models of obesity a shift into a pro-coagulant environment occurs which might be an independent risk factor for achieving a DIC more easily.

In conclusion, the results of this study indicate that obesity induced by high caloric feeding acts differently than genetically altered models of obesity at least in the hepatic microcirculation. It looks like leptin, mainly produced by adipocytes, and its long– or short-form receptors, that are expressed in most WBCs as well as endothelial cells, may play a key role in the mediation of inflammatory changes. Sepsis is associated with the recruitment of both leukocytes and platelets in the liver microcirculation, whereas leukocyte adhesion was more common in the 'lean' groups (WT, DIO and ob/ob + leptin) and platelet recruitment more common in genetically altered models of obesity (db/db and ob/ob). These findings suggest a different intravascular environment in hepatic capillary bed in various groups of obesity may be caused by a changed production of bioactive substances in white adipose tissue and activation of endothelial– and white blood cells, resulting in an imbalance of pro– and anti-inflammatory cytokines and increased circulating leptin levels as well as less immunosuppressive adiponectin levels. To confirm these assumptions, additional research of the underlying patholophysiological mechanisms are mandatory to determine and precisely describe the pro-inflammatory environment associated with obesity and to assess whether this pro-inflammatory environment is caused by leptin deficiency or the milieu of obesity. This knowledge may lead to the development of innovative treatment options in septic individuals.

7 | References

1. Bone RC, Balk RA, Cerra FB, 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;101:1644–55.
2. Vincent JL. Dear SIRS, I'm sorry to say that I don't like you... Critical care medicine 1997;25:372–4.
3. Abraham E, Matthay MA, Dinarello CA, et al. Consensus conference definitions for sepsis, septic shock, acute lung injury, and acute respiratory distress syndrome: time for a reevaluation. Critical care medicine 2000;28:232–5.
4. Levy MM, Fink MP, Marshall JC, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Critical care medicine 2003;31:1250–56.
5. Goldstein B, Giroir B, Randolph A, and International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatric critical care medicine: a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies 2005;6:2–8.
6. Moerer O and Quintel M. Sepsis in adult patients - definitions, epidemiology and economic aspects. Der Internist 2009;50:788, 790–4, 796–8.
7. Murphy SL, Xu J, and Kochanek KD. Deaths: Final Data for 2012. 2015. URL: `http://han.medunigraz.at/han/pubmed/www.cdc.gov/nchs/products/nvsr.htm` (visited on 2015).
8. Melamed A and Sorvillo FJ. The burden of sepsis-associated mortality in the United States from 1999 to 2005: an analysis of multiple-cause-of-death data. Critical care 2009;13:R28.
9. Proulx F, Fayon M, Farrell CA, Lacroix J, and Gauthier M. Epidemiology of sepsis and multiple organ dysfunction syndrome in children. Chest 1996;109:1033–37.

10. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive care medicine 2013;39:165–228.

11. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Critical care medicine 2008;36. Ed. by Hora P:296–327.

12. Barton P, Kalil AC, Nadel S, et al. Safety, Pharmacokinetics, and Pharmacodynamics of Drotrecogin Alfa (Activated) in Children With Severe Sepsis. Pediatrics 2004;113:7–17.

13. Vincent JL, Bernard GR, Beale R, et al. Drotrecogin alfa (activated) treatment in severe sepsis from the global open-label trial ENHANCE: Further evidence for survival and safety and implications for early treatment*. Critical care medicine 2005;33.

14. Dombrovskiy VY, Martin AA, Sunderram J, and Paz HL. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. Critical care medicine 2007;35:1244–50.

15. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, and Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Critical care medicine 2001;29:1303–10.

16. Watson RS, Carcillo JA, Linde-Zwirble WT, Clermont G, Lidicker J, and Angus DC. The epidemiology of severe sepsis in children in the United States. American journal of respiratory and critical care medicine 2003;167:695–701.

17. Annane D, Aegerter P, Jars-Guincestre MC, and Guidet B. Current epidemiology of septic shock: the CUB-Réa Network. American journal of respiratory and critical care medicine 2003;168:165–72.

18. Pavon A, Binquet C, Kara F, et al. Profile of the risk of death after septic shock in the present era: an epidemiologic study. Critical care medicine 2013;41:2600–9.

19. Ranieri VM, Thompson BT, Barie PS, et al. Drotrecogin Alfa (Activated) in Adults with Septic Shock. New England Journal of Medicine 2012;366:2055–64.

20. DuPont HL and Spink WW. Infections due to gram-negative organisms: an analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958-1966. Medicine 1969;48:307–32.

21. Pollack MM, Fields AI, and Ruttimann UE. Distributions of cardiopulmonary variables in pediatric survivors and nonsurvivors of septic shock. Critical care medicine 1985;13:454–9.
22. Carcillo JA, Davis AL, and Zaritsky A. Role of early fluid resuscitation in pediatric septic shock. JAMA : the journal of the American Medical Association 1991;266:1242–5.
23. Carcillo JA. Pediatric septic shock and multiple organ failure. Critical care clinics 2003;19:413–440.
24. National Institute of General Medical Sciences. Sepsis Fact Sheet. 2014. URL: `http://www.nigms.nih.gov/education/pages/factsheets.aspx` (visited on 2015).
25. UpToDate. Pathophysiology of sepsis. 2014. URL: `http://www.uptodate.com/contents/pathophysiology-of-sepsis?source=search%5C_result%5C&search=sepsis+pathophysiologie%5C&selectedTitle=1~150` (visited on 2015).
26. Schulte W, Bernhagen J, and Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets—an updated view. Mediators of inflammation 2013;2013.
27. Vachharajani V. Influence of obesity on sepsis. Pathophysiology: the official journal of the International Society for Pathophysiology / ISP 2008;15:123–134.
28. Cavaillon JM. Pro- versus anti-inflammatory cytokines: myth or reality. Cellular and molecular biology (Noisy-le-Grand, France) 2001;47:695–702.
29. Mayo Clinic. Sepsis Causes - Diseases and Conditions. 2014. URL: `http://www.mayoclinic.org/diseases-conditions/sepsis/basics/causes/con-20031900` (visited on 2015).
30. Bateman RM, Sharpe MD, and Ellis CG. Bench-to-bedside review: microvascular dysfunction in sepsis–hemodynamics, oxygen transport, and nitric oxide. Critical care (London, England) 2003;7:359–73.
31. Fries M, Weil MH, Sun S, et al. Increases in tissue Pco2 during circulatory shock reflect selective decreases in capillary blood flow. Critical care medicine 2006;34:446–52.

32. Singer G, Urakami H, Specian RD, Stokes KY, and Granger DN. Platelet recruitment in the murine hepatic microvasculature during experimental sepsis: role of neutrophils. Microcirculation 2006;13:89–97.

33. Trzeciak S, Dellinger RP, Parrillo JE, et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Annals of emergency medicine 2007;49:88–98, 98.e1–2.

34. Bacon SL, Lavoie KL, Arsenault A, et al. The research on endothelial function in women and men at risk for cardiovascular disease (REWARD) study: methodology. BMC cardiovascular disorders 2011;11:50.

35. Tiruppathi C, Naqvi T, Sandoval R, Mehta D, and Malik AB. Synergistic effects of tumor necrosis factor-alpha and thrombin in increasing endothelial permeability. American journal of physiology. Lung cellular and molecular physiology 2001;281:L958–68.

36. WHO. WHO | Obesity and overweight. 2014. URL: `http://www.who.int/mediacentre/factsheets/fs311/en/` (visited on 2015).

37. CDC. Obesity and Overweight for Professionals: Adult: Defining - DNPAO - CDC. 2014. URL: `http://www.cdc.gov/obesity/adult/defining.html` (visited on 2015).

38. Harvard. Obesity Definition | Obesity Prevention Source | Harvard School of Public Health. 2014. URL: `http://www.hsph.harvard.edu/obesity-prevention-source/obesity-definition/` (visited on 2015).

39. Flegal KM, Kit BK, Orpana H, and Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. JAMA: the journal of the American Medical Association 2013;309:71–82.

40. WHO. WHO | Childhood overweight and obesity. 2014. URL: `http://www.who.int/dietphysicalactivity/childhood/en/` (visited on 2015).

41. Ogden CL, Yanovski SZ, Carroll MD, and Flegal KM. The epidemiology of obesity. Gastroenterology 2007;132:2087–2102.

42. Onis M de and Blössner M. The World Health Organization Global Database on Child Growth and Malnutrition: methodology and applications. International journal of epidemiology 2003;32:518–26.
43. OECD. Health, Obesity Update. 2014. URL: `http://www.oecd.org/els/health-systems/Obesity-Update-2014.pdf` (visited on 2015).
44. OECD. Prevalence of Overweight, Obesity, and Extreme Obesity Among Adults: United States, 1960–1962 Through 2011–2012. 2014. URL: `http://www.cdc.gov/nchs/data/hestat/obesity%5C_adult%5C_11%5C_12/obesity%5C_adult%5C_11%5C_12.pdf` (visited on 2015).
45. Ogden CL, Carroll MD, Kit BK, and Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA : the journal of the American Medical Association 2014;311:806–14.
46. Encyclopedia Britannica. Adipose Tissue | Anatomy | Encyclopedia Britannica. 2014. URL: `http://www.britannica.com/EBchecked/topic/5948/adipose-tissue` (visited on 2015).
47. Kershaw EE and Flier JS. Adipose tissue as an endocrine organ. The Journal of clinical endocrinology and metabolism 2004;89:2548–56.
48. Kajimura S and Saito M. A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis. Annual review of physiology 2014;76:225–49.
49. Moschen AR, Wieser V, and Tilg H. Adiponectin: key player in the adipose tissue-liver crosstalk. Current medicinal chemistry 2012;19:5467–73.
50. Li FYL, Cheng KKY, Lam KSL, Vanhoutte PM, and Xu A. Cross-talk between adipose tissue and vasculature: role of adiponectin. Acta physiologica (Oxford, England) 2011;203:167–80.
51. Ronti T, Lupattelli G, and Mannarino E. The endocrine function of adipose tissue: an update. Clinical endocrinology 2006;64:355–65.
52. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, and Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–32.

53. Shapiro NI, Khankin EV, Van Meurs M, et al. Leptin exacerbates sepsis-mediated morbidity and mortality. Journal of immunology (Baltimore, Md. : 1950) 2010;185:517–24.
54. Soukas A, Cohen P, Socci ND, and Friedman JM. Leptin-specific patterns of gene expression in white adipose tissue. Genes & development 2000;14:963–80.
55. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. Cell 2004;116:337–50.
56. Zhang W, Della-Fera MA, Hartzell DL, Hausman D, and Baile CA. Adipose tissue gene expression profiles in ob/ob mice treated with leptin. Life sciences 2008;83:35–42.
57. Genoni G, Prodam F, Marolda A, et al. Obesity and infection: two sides of one coin. European journal of pediatrics 2014;173:25–32.
58. Wallace AM, McMahon AD, Packard CJ, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 2001;104:3052–6.
59. Katzmarzyk PT, Reeder BA, Elliott S, et al. Body mass index and risk of cardiovascular disease, cancer and all-cause mortality. Canadian journal of public health = Revue canadienne de santé publique 2012;103:147–51.
60. Amundson DE, Djurkovic S, and Matwiyoff GN. The obesity paradox. Critical care clinics 2010;26:583–96.
61. Sakr Y, Madl C, Filipescu D, et al. Obesity is associated with increased morbidity but not mortality in critically ill patients. Intensive care medicine 2008;34:1999–2009.
62. Hogue CW, Stearns JD, Colantuoni E, et al. The impact of obesity on outcomes after critical illness: a meta-analysis. Intensive care medicine 2009;35:1152–70.
63. Abhyankar S, Leishear K, Callaghan FM, Demner-Fushman D, and McDonald CJ. Lower short- and long-term mortality associated with overweight and obesity in a large cohort study of adult intensive care unit patients. Critical care (London, England) 2012;16:R235.
64. El-Solh A, Sikka P, Bozkanat E, Jaafar W, and Davies J. Morbid obesity in the medical ICU. Chest 2001;120:1989–97.
65. Abir F and Bell R. Assessment and management of the obese patient. Critical care medicine 2004;32:S87–91.

66. Bercault N, Boulain T, Kuteifan K, Wolf M, Runge I, and Fleury JC. Obesity-related excess mortality rate in an adult intensive care unit: A risk-adjusted matched cohort study. Critical care medicine 2004;32:998–1003.

67. Falagas ME and Kompoti M. Obesity and infection. The Lancet. Infectious diseases 2006;6:438–46.

68. Desruisseaux MS, Nagajyothi, Trujillo ME, Tanowitz HB, and Scherer PE. Adipocyte, adipose tissue, and infectious disease. Infection and immunity 2007;75:1066–78.

69. Martino JL, Stapleton RD, Wang M, et al. Extreme obesity and outcomes in critically ill patients. Chest 2011;140:1198–206.

70. Kolyva AS, Zolota V, Mpatsoulis D, et al. The role of obesity in the immune response during sepsis. Nutrition & diabetes 2014;4:e137.

71. Singer G and Granger DN. Inflammatory responses underlying the microvascular dysfunction associated with obesity and insulin resistance. Microcirculation 2007;14:375–387.

72. Hwang S. Microcirculation of the Liver. In: *Venous Embolization of the Liver*. Springer, 2011:9–13.

73. Dardenne A. The microcirculation of the liver: literature study and micro CT-imaging of its architecture and hemodynamic properties. Master Thesis. Universiteit Gent, 2014:1–51. URL: `http://lib.ugent.be/fulltxt/RUG01/002/061/659/RUG01-002061659%5C_2013%5C_0001%5C_AC.pdf`.

74. Kan Z and Madoff DC. Liver anatomy: microcirculation of the liver. Seminars in interventional radiology 2008;25:77–85.

75. Nesseler N, Launey Y, Aninat C, Morel F, Mallédant Y, and Seguin P. Clinical review: The liver in sepsis. Critical care (London, England) 2012;16:235.

76. Nilsson K. Cells in Peripheral Blood. 2000. URL: `http://www.cellavision.com/?id=3651` (visited on 2015).

77. Pries AR, Wenger RH, and Zakrzewicz A. Physiologie. In: *Elsevier GmbH*. Ed. by Deetjen P, Speckmann EJ, and Hescheler J. 4. vollstä. München: Urban & Fischer, 2005. Chap. 06 | 07:339–415.

78. Petri B and Bixel MG. Molecular events during leukocyte diapedesis. The FEBS journal 2006;273:4399–407.

79. Carman CV and Springer TA. A transmigratory cup in leukocyte diapedesis both through individual vascular endothelial cells and between them. The Journal of cell biology 2004;167:377–88.

80. Katayama T, Ikeda Y, Handa M, et al. Immunoneutralization of glycoprotein Ibalpha attenuates endotoxin-induced interactions of platelets and leukocytes with rat venular endothelium in vivo. Circulation research 2000;86:1031–7.

81. Gawaz M, Langer H, and May AE. Platelets in inflammation and atherogenesis. The Journal of clinical investigation 2005;115:3378–84.

82. Varga-Szabo D, Pleines I, and Nieswandt B. Cell adhesion mechanisms in platelets. Arteriosclerosis, thrombosis, and vascular biology 2008;28:403–12.

83. Baatz H, Steinbauer M, Harris AG, and Krombach F. Kinetics of white blood cell staining by intravascular administration of rhodamine 6G. International journal of microcirculation, clinical and experimental / sponsored by the European Society for Microcirculation 1995;15:85–91.

84. Singer G, Stokes KY, Terao S, and Granger DN. Sepsis-induced intestinal microvascular and inflammatory responses in obese mice. Shock 2009;31:275–279.

85. Vachharajani V, Russell JM, Scott KL, et al. Obesity exacerbates sepsis-induced inflammation and microvascular dysfunction in mouse brain. Microcirculation (New York, N.Y. : 1994) 2005;12:183–94.

86. Vachharajani V, Wang SW, Mishra N, El Gazzar M, Yoza B, and McCall C. Curcumin modulates leukocyte and platelet adhesion in murine sepsis. Microcirculation (New York, N.Y.: 1994) 2010;17:407–416.

87. Spezialdiäten G ssniff. Experimental diets for laboratory animals. 2014. URL: `http://www.ssniff.de/index.php?pcid=9%5C&pdid=15` (visited on 2014).

88. Tailor A, Lefer DJ, and Granger DN. HMG-CoA reductase inhibitor attenuates platelet adhesion in intestinal venules of hypercholesterolemic mice. American journal of physiology. Heart and circulatory physiology 2004;286:H1402–7.

89. OECD. Overweight and obesity among children. URL: `http://www.oecd-ilibrary.org/docserver/download/8113161ec016.pdf?expires=1424086006%5C&id=id%5C&accname=guest%5C&checksum=94E0D5C8FDC962D58FF9A4F8707E632E`.

90. Fantuzzi G. Adipose tissue, adipokines, and inflammation. The Journal of allergy and clinical immunology 2005;115:911–9, quiz 920.

91. Margetic S, Gazzola C, Pegg GG, and Hill RA. Leptin: a review of its peripheral actions and interactions. International journal of obesity and related metabolic disorders: journal of the International Association for the Study of Obesity 2002;26:1407–1433.

92. Tschöp J, Nogueiras R, Haas-Lockie S, et al. CNS leptin action modulates immune response and survival in sepsis. The Journal of neuroscience: the official journal of the Society for Neuroscience 2010;30:6036–6047.

93. Vachharajani V, Cunningham C, Yoza B, Carson J, Vachharajani TJ, and McCall C. Adiponectin-deficiency exaggerates sepsis-induced microvascular dysfunction in the mouse brain. Obesity (Silver Spring, Md.) 2012;20:498–504.

94. Rivera CA, Gaskin L, Singer G, Houghton J, and Allman M. Western diet enhances hepatic inflammation in mice exposed to cecal ligation and puncture. BMC Physiol 2010;10:20.

95. Sennello Ja, Fayad R, Morris AM, et al. Regulation of T cell-mediated hepatic inflammation by adiponectin and leptin. Endocrinology 2005;146:2157–64.