UNIVERSITY OF COPENHAGEN
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CEREBRAL HAEMODYNAMIC FUNCTION IN EXPERIMENTAL AND CLINICAL SEPSIS

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DOCTORAL THESIS (DRMEDSCI)

FACULTY OF HEALTH AND MEDICAL SCIENCES
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This thesis was submitted to The Faculty of Health and Medical Sciences, University of Copenhagen on August 31, 2018. The thesis is based on the following papers:

I. Taudorf S, Krabbe KS, **Berg RMG**, Pedersen BK, Møller K. Human models of low-grade inflammation: bolus versus continuous infusion of endotoxin. *Clinical and Vaccine Immunology* 2007; 14: 250-255.

II. **Berg RMG**, Taudorf S, Bailey DM, Lundby C, Larsen FS, Pedersen BK, Møller K. Cerebral net exchange of large neutral amino acids after lipopolysaccharide infusion in healthy humans. *Critical Care*. 2010; 14: R16.

III. **Berg RMG**, Plovsing RR, Bailey DM, Holstein-Rathlou NH, Møller K. The dynamic cerebral autoregulatory adaptive response to noradrenaline is attenuated during systemic inflammation in humans. *Clinical and Experimental Pharmacology and Physiology*. 2015; 42: 740-746.

IV. **Berg RMG**, Plovsing RR, Greve AM, Christiansen CB, Toksvang LN, Holstein-Rathlou NH, Møller K. Spontaneous blood pressure oscillations in mechanically ventilated patients with sepsis. *Blood Pressure Monitoring*. 2016; 21: 75-79.

V. **Berg RMG**, Plovsing RR. Effects of short-term mechanical hyperventilation on cerebral blood flow and dynamic cerebral autoregulation in critically ill patients with sepsis. *Scandinavian Journal of Clinical and Laboratory Investigation*. 2016; 76: 226-233.

VI. **Berg RMG**, Plovsing RR, Bailey DM, Holstein-Rathlou NH, Møller K. Dynamic cerebral autoregulation to induced blood pressure changes in human experimental and clinical sepsis. *Clinical Physiology and Functional Imaging*. 2016; 36: 490-496.

VII. **Berg RMG**, Taudorf S, Bailey DM, Dahl RH, Lundby C, Møller K. Transcerebral net exchange of vasoactive peptides and catecholamines during lipopolysaccharide-induced systemic inflammation in healthy humans. *Canadian Journal of Physiology and Pharmacology*. 2018; 96: 313-316.

VIII. Dahl RH, **Berg RMG**, Taudorf S, Bailey DM, Lundby C, Larsen FS, Møller K. A reassessment of the blood-brain barrier transport of large neutral amino acids during acute systemic inflammation in humans. *Clinical Physiology and Functional Imaging*. 2018; 38: 656-662.

The papers are referred to by their Latin numbers in the thesis. They are part of a series of related publications that also include two papers that formed the basis of my PhD thesis "Cerebral Autoregulation in Sepsis"[52,53] which I defended on June 17, 2014.

Content

Foreword

I have been wanting to write this thesis for more than a decade, but it was not until my paternity leave in the autumn of 2017 that I really got around to it. While my parental duties rendered this period anything but a relaxing vacation, I soon realised that my son Falke's mid-day nap was a recurring window of opportunity. During my daily stroller walks, I would therefore watchfully wait for the moment when Falke closed his eyes, and then immediately park the stroller, sit on the nearest bench (typically a lovely bench just outside the Arnfred Law Firm in Hellerup), and get down to business. I wrote approximately 1½ pages per day in the wear and tear of the Scandinavian autumn weather, thus often wearing my wet water clothes with an umbrella conveniently covering my computer.

The experimental work that forms the basis of studies included in the present thesis was mainly conducted over a 7-year period between 2005 and 2012 during my employments as a pregraduate research associate (kindly funded by the Danish Medical Research Council and the P. Carl Petersen Foundation), then as a research assistant (kindly funded by the Research Board at Rigshospitalet and the P. Carl Petersen Foundation), and lastly as a PhD-student (kindly funded by the Faculty of Health and Medical Sciences at the University of Copenhagen) at the Copenhagen Muscle Research Centre and the Centre of Inflammation and Metabolism (CIM), now the Centre for Physical Activity Research, at Rigshospitalet. Additional work was done during my subsequent fellowship in clinical physiology and nuclear medicine at Rigshospitalet and at Bispebjerg and Frederiksberg Hospitals. Christian Larsen and Judge Ella Larsen's Grant, the A. P. Møller Foundation, the Classen Trust Jubilee Foundation, the Copenhagen Hospital Corporation, the Foundation of 1870, the Foundation of Merchant Jakob Ehrenreich and Grete Ehrenreich, the Højmosegaard Foundation, the Jensa la Cour Foundation, the Larsen Founda-

tion, the Laerdal Foundation, the P. Carl Petersen Foundation, and the Toyota Foundation all generously provided financial support for the studies.

I have many people to thank; without them, none of these studies would have been possible, and I would have no thesis to write.

First, I want to thank Professor Kirsten Møller, who more than anyone is the person that gave me a taste for science. I first met Kirsten when I was looking for a supervisor for my bachelor thesis as a 2nd-year medical student. It was Professor Gitte Moos Knudsen who referred me to Kirsten, but she was not immediately available, as she turned out to have travelled to Nepal to save distressed trekkers and mountaineers with the Himalayan Rescue Association. Even though I was admittedly a somewhat bold 22-year old at the time, that really impressed me! By the kind help of Kirsten's family, my request for supervision made it all the way to the Himalayas. We have worked together since then, and of all people, it is first and foremost Kirsten who has raised me as a physiologist and scientist and trained me rigorously in the art of hypothetical deduction. She involved me in the experiments that led to the development of the continuous lipopolysaccharide infusion method, which has formed the basis of several of my subsequent studies, and indeed the present thesis. Kirsten was furthermore my master thesis and PhD supervisor, and over the more than 15 years known each other by now, she has proven herself not only to be a great mentor and role model, but also a friend and a confidante that I can always trust and count on.

I first met the esteemed scientist, athlete, and adventurer Professor Damian M. Bailey during the infamous *2006 Copenhagen Brain Study*, which apart from focusing on the effects of intravenous lipopolysaccharide and inspiratory hypoxia on cerebral haemodynamics, also elucidated the effect of months of sleep deprivation combined with a restricted diet (green and red curry in random order) on a small group of scientist (it mainly causes malaise and nausea, not very dif-

ferent from that observed after intravenous lipopolysaccharide). Since then, he has served as my unofficial mentor, and he has selflessly helped promote my career. It thus largely his achievement that I now serve as Visiting Professor at the University of South Wales. Needless to say, I am deeply grateful to Damian, and I am honoured to be his scientific collaborator.

After I completed my bachelor thesis (on static cerebral autoregulation), Professor Bente Klarlund Pedersen, the visionary and incontestable regent queen of integrative human physiology, offered me a spot at the burgeoning CIM to continue my work with Kirsten. It has been an honour to work in Bente's world-leading laboratory for so many years. From the beginning, it really struck me how much the scientific environment at CIM resembled that of the Krogh Laboratory as portrayed in Bodil Schmidt-Nielsen's *August and Marie Krogh – Lives in Science*. August Krogh was a pioneer in physiology – in my view, Bente is the August Krogh of our time.

The renowned physiologist Professor Niels-Henrik Holstein-Rathlou served as my PhD supervisor and his deep insight into physiological and mathematical modelling has been indispensable during the studies, and we have had many valuable discussions over the years. Indeed, the transfer function analysis-based method used for assessing dynamic cerebral autoregulation in the studies of the present thesis and in hundreds of other studies was conceived by Niels-Henrik and initially used in his now classical studies of tubulo-glomerular feedback.

I am deeply grateful to my 'fellow stormtrooper' Dr. Ronni R. Plovsing with whom I conducted most of my studies. Some would probably kindly characterise me as a rather theoretical person, while others would be franker, and call me a downright *uatamáiler*. In contrast, Ronni is both a brilliant theoretician, well-organised, and practically minded. He played a critical role for keeping things on track, and the studies could not have been done at all were it not for his skillset

as an anaesthesiologist, which were required due to the nature of the procedures and interventions in the different studies. For the most part, we developed these (and other) studies together, conducted them together, analysed the data together, and wrote up the papers together (at all times of the day and night). Ronni is the best team mate I could have asked for, and I hope we'll do many more studies together in the future. Apart from Ronni, the other co-authors both of the papers of my PhD thesis and of the eight papers of the present thesis deserve mention: Dr. Claus B. Christiansen, Dr. Kevin A. Evans, Dr. Karen Suarez Krabbe, Professor Fin Stolze Larsen, Professor Carsten Lundby, Dr. Andreas Ronit, Dr. Sarah Taudorf, and in particular my close collaborator Mr. Rasmus H. Dahl, an extremely talented medical student, who was kind enough to help me prepare many of the figures in the thesis, and who also provided constructive feedback to some of the early drafts of the thesis.

I am grateful to Professor Anders Perner and Dr. Jan Bonde from Department of Intensive Care at Rigshospitalet, where of the experiments were conducted. Even though the studies were very demanding, both for the staff, patients and relatives at the department, Anders and Jan have always made me feel extremely welcome and never hesitated to help when needed. Anders created a very enriching and inspiring research environment, and while I was in the department his research group made several major scientific breakthroughs and became world-leading in conducting controlled, randomised trials in the intensive care setting. I must also thank the patients at the Department of Intensive Care and their relatives who selflessly chose to contribute to our studies at a most difficult time, as well as the healthy volunteers who willingly and patiently participated in our experiments.

I am also grateful to Professor Olaf B. Paulson; I have read his work since I was a young student, and all my studies on cerebral haemodynamics are to a great extent based on concepts and principles

that he has developed. Olaf kindly accepted to serve as Chair of my PhD thesis evaluation committee, and he has been extremely helpful with feedback and discussions in relation to the subsequent studies.

As described in the thesis, studies on patients admitted to the intensive care unit are not only technically and logistically difficult, but also ethically challenging. While my studies were still at a very early stage, I therefore contacted the esteemed historian and ethicist Associate Professor Peter Rossel. This evolved into a very fruitful collaboration and friendship with Peter and his wife Bente who hospitably opened up their home to me. Peter's contributions to medical ethics, which he founded as a mandatory subject for medical graduates at the University of Copenhagen, are paramount, and it is not only me, but all who have had the privilege of having Peter as their teacher over the past four decades, which owe him gratitude.

For many years, CIM was a second home to me. It was a competitive environment, but with a homely and warm atmosphere. Apart from Bente, this was largely due to Ms. Ruth Rovsing, Ms. Hanne Villumsen, Ms. Inge Holm, and Ms. Marie Kvistgaard; they have all helped me and other CIM employees with great many things, including various technical, practical, and administrative matters, but most of all I want to thank them for making CIM such a fantastic place, from where I have so many great memories. This being said, it was the help and support from Dr. Naja Zenius Jespersen that got me through the demanding experiments. I owe my best memories from CIM during those years to her. Another person that I got to know at CIM and who has become a close friend is Dr. Sofie Andreasen. Although she did not participate in any of the studies of the present thesis, she has looked after me like a caring older sister from our early days at CIM, and helped mature me as a scientist, and has always been someone that I can trust and count on, both in relation to scientific and personal matters.

While a huge part of my research hitherto has focused on the mechanisms that function to ensure a sufficient oxygen and substrate supply to the brain, my focus has slightly changed over the past ~5 years, as I have become increasingly interested in respiratory physiology, and thus the mechanisms that ensure a sufficient supply of oxygen to the organism as a whole. Serendipitously, the acclaimed and visionary respiratory physiologist and nuclear pulmonologist Professor Jann Mortensen is based at the Department of Clinical Physiology, Nuclear Medicine, and PET at Rigshospitalet, and since I first started working there in 2014 he has served as a mentor to me, both in clinical nuclear medicine and scientifically. While we are currently doing a number of studies together, Jann has not been directly involved in any of the studies in the present thesis, but he has kindly provided insightful feedback and comments to the review. Professor Liselotte Højgaard, who apart from being Chairman of the Board of the National Research Foundation and President of the Copenhagen Research Forum and numerous other things, is Head of the Department of Clinical Physiology, Nuclear Medicine and PET at Rigshospitalet, has also been extremely kind and helpful in relation to my work with this thesis, and provided valuable feedback. It is an honour that such a capacity within science such as Liselotte has taken the time to critically review my work.

I must also thank my great colleagues Drs. Louise Brinth, Carsten Hædersdal, and Niels Wiinberg, with whom I worked at the Department of Clinical Physiology and Nuclear Medicine at Frederiksberg Hospital. We had great companionship, and most times it was them that selflessly did all the hard work at the department, so that I could do my research. It was also at Frederiksberg Hospital that I met Mr. Chi-Han Henry Ma, a medical laboratory technician that is also a skilled medical illustrator, and who provided most of the illustrations for this thesis (including the cover). Furthermore, the artist Matt Kish

kindly permitted me to use a drawing from his illustrated edition of *Heart of Darkness*.

I must also thank all my friends who have put up with me for all these years – both before and after I became a scientist. I cannot mention them all here, but Drs. Anders M. Greve and Sopha Hammer-Hansen cannot go unmentioned. It was them that heroically saved my near-complete PhD thesis back in the day when I temporarily lost it to ransomware hackers, who had sent me a fake doublet e-mail from Amazon after I had purchased the 2nd edition of Haldane's *Respiration* online. My whole hard drive was encrypted, and the hackers demanded that I paid the ransom via MoneyPac, a cash card that can only be purchased in the US. Anders and Sophia who were US residents at the time rushed to my assistance and disbursed the ransom of 200$ n a local store in Bethesda. Bitter money, some may say; to me, it was the sweetest deal I ever made. And thanks to the hackers, I have deliberately not procrastinated by purchasing ancient physiology textbooks on Amazon since then.

In the foreword to my PhD thesis, I expressed my gratitude to Mr. Georg Carstensen, my primary school teacher of mathematics, physics, and chemistry. The same gratitude goes for this thesis. It is largely our teachers that spark our interest in the surrounding world, and provide us with the fundamental tools to explore it. I sent my thesis to Georg, and to my delight he responded. We have been communicating since then, and I must say that although I am not the most reliable pen friend, my new-found friendship with Georg is one of the best things that came out of my PhD thesis.

Lastly, I want to thank Kim and Marianne for all the love and parental support they have provided me for all these years. But – above all – I must thank Linea and Falke: you carried me through all this, and none of it would be worthwhile if it weren't for you. This thesis is as much yours as it is mine.

Ronan M. G. Berg

Summary of thesis

The present thesis is based on eight original articles and a review, the scope of which were to elucidate whether changes in cerebral haemodynamic function may predispose to cerebral ischaemia in sepsis.

Based on studies using intravenous lipopolysaccharide infusion in healthy volunteers, which was considered an experimental model of the acute systemic inflammatory response during early sepsis, as well as clinical studies of critically ill septic patients admitted to the intensive care unit, various aspects of cerebral haemodynamic function were examined. These included changes in global cerebral blood flow and cerebrovascular resistance, as well as the cerebrovascular reactivity to changes in arterial blood gases, cerebral autoregulation, the transcerebral net exchange of selected vasoactive peptides, and aspects of blood-brain barrier function related to catecholaminergic homeostasis within the brain. Given that the cerebrovasculature is an integral component of the cardiovascular system, and the blood supply to the brain ultimately depends on the ability of the cardiovascular system to generate a sufficient and stable blood pressure, the autonomic regulation of cardiovascular function was also examined.

Together with other studies, the findings of the present thesis indicate that cerebral vasoconstrictive influences lead to a state of cerebral hypoperfusion relative to oxidative metabolism during the systemic inflammatory response triggered by intravenous lipopolysaccharide. The cerebrovascular reactivity to changes in $PaCO_2$ is maintained, and accordingly these changes appear to be related to hyperventilation. The fever response and an enhanced cerebrovascular release of the potent cerebral vasoconstrictor endothelin-1 also appear to contribute. Cerebral autoregulation concurrently responds faster and more effectively to an acute change in blood pressure, while the autonomic regulation of cardiovascular function becomes less effective with a reduced sympathetic output to the heart and peripheral blood

vessels and slowing of the arterial baroreflex. Intravenous lipopolysaccharide does not appear to trigger changes in the passage of catecholamines or large neutral amino acids across the blood-brain barrier to such an extent that changes in cerebral haemodynamic function may ensue. In accordance with findings from other studies, the findings of the present thesis furthermore show that the cerebrovascular resistance is likewise high, while the ability of the cerebrovasculature to respond to changes in $PaCO_2$ is preserved in patients admitted to the intensive care unit with sepsis. Although the cerebrovasculature remains capable of exerting autoregulatory responses, the responses are slower than in healthy conditions, and are neither enhanced by hyperventilation or by noradrenaline infusion. The autonomic regulation of cardiovascular function is concurrently impaired, with changes in arterial baroreflex function like those encountered after lipopolysaccharide infusion.

The findings of the present thesis indicate that cerebral vasoconstriction during the early stages of sepsis is part of a neuroprotective mechanism that renders the cerebrovasculature more agile, in the sense that it responds faster and more effectively to sudden changes in blood pressure. This conceivably functions to prevent the concurrently unstable blood pressure due to the impairment of the autonomic regulation of cardiovascular function from causing cerebral ischaemia. In contrast, the slower autoregulatory responses observed during more advanced stages of sepsis, may permit the more unstable blood pressure to trigger cerebral ischaemia. This may provide a mechanistic basis for the widespread cerebral ischaemic damage that has previously been documented in septic patients, and which supposedly contributes both to encephalopathy, long-term cognitive deficits, and the progression of septic shock.

Resumé af afhandling på dansk

Nærværende afhandling er baseret på otte originalartikler og en oversigt, der har til hensigt at belyse om der forekommer ændringer i cerebral hæmodynamisk funktion ved sepsis som kan disponere til cerebral iskæmi.

På grundlag af studier baseret på intravenøs lipopolysakkarid-infusion i raske forsøgspersoner, som blev betragtet som en eksperimentel model for det akutte systemisk inflammatoriske respons ved tidlig sepsis, samt kliniske studier af patienter indlagt på intensivafdeling med sepsis, undersøgte vi forskellige aspekter af cerebral hæmodynamisk funktion. Disse omfattede ændringer i hjernens globale gennemblødning og cerebrovaskulær modstand, de cerebrale kars følsomhed for ændringer i arterielle blodgasser, cerebral autoregulation, hjernekarrenes frigivelse af udvalgte vasoaktive peptider, og aspekter af blod-hjernebarrierens funktion relateret til katekolaminerg homøostase i centralnervesystemet. Eftersom hjernens blodkar er en integreret del af hjerte-kredsløbet, og hjernens blodtilførsel i sidste ende afhænger af hjerte-kredsløbets evne til at generere et tilstrækkeligt og stabilt blodtryk, blev den autonome kontrol af hjerte-kredsløbets funktion også undersøgt.

Sammen med andre studier peger fundene i nærværende afhandling på, at vasokonstriktive faktorer fører til en tilstand af cerebral hypoperfusion relativt til hjernens iltbehov under det systemisk inflammatoriske respons udløst af intravenøs lipopolysakkarid-indgift. Hjernens kar er fortsat følsomme for ændringer i $PaCO_2$, og disse ændringer synes således at være relateret til hyperventilation. Endvidere synes feberresponset og en øget frigivelse af den potente cerebrale vasokonstriktor endotelin-1 fra hjernens kar at bidrage. Samtidig responderer cerebral autoregulation hurtigere og mere effektivt på akutte ændringer i blodtryk, mens den autonome kontrol af hjerte-kredsløbets funktion er svækket med reduceret sympaticus-aktivitet til hjerte og

perifere kar og en langsommere arteriel barorefleks. Lipopolysakkarid-infusion synes ikke at udløse ændringer i passage af katekolaminer eller store neutrale aminosyrer over blod-hjernebarrieren i en sådan grad at det kan forventes at føre til ændringer i cerebral hæmodynamisk funktion. Sammen med andre studier viser fundene i nærværende afhandling endvidere at den cerebrovaskulære modstand ligeledes er høj, mens hjernens kars evne til at respondere på ændringer i $PaCO_2$ er bevaret hos kritisk syge patienter indlagt på intensivafdeling med sepsis. På trods af at hjernens kar fortsat kan autoregulere er den cerebrale autoregulation langsommere end hos raske, og forbedres ikke af hverken hyperventilation eller noradrenalin-indgift. Den autonome kontrol af hjerte-kredsløbets funktion er samtidig svækket med ændringer i den arterielle barorefleks svarende til dem der ses efter lipopolysakkarid-infusion.

Fundene i nærværende afhandling peger på, at cerebral vasokonstriktion ved tidlig sepsis er del af en neuroprotektiv mekanisme, der gør hjernens kar mere agile i den forstand, at de responderer hurtigere og mere effektivt på pludselige ændringer i blodtryk. Dette har formentlig til hensigt at forhindre det mere ustabile blodtryk, der opstår som følge af den samtidige svækkelse af den autonome kontrol af hjerte-kredsløbets funktion, i at forårsage cerebral iskæmi. Grundet den mere langsomt reagerende cerebrale autoregulation ved mere avancerede stadier af sepsis kan det ustabile blodtryk udløse cerebral iskæmi. Dette kan udgøre det mekanistiske grundlag for de udbredte cerebral iskæmiske forandringer der tidligere er dokumenteret hos septiske patienter, og som antages at bidrage til encefalopati, permanent kognitiv dysfunktion og progression af septisk shock.

Abbreviations

Terms that are strictly used as mathematical operators in footnotes and appendices are not included in this list.

AAA:	aromatic amino acid
AAAD:	aromatic L-amino acid decarboxylase
AC:	adenylate cyclase
a-jvDO_2:	arterial-to-jugular venous oxygen content difference
a-jvD_{glc}:	arterial-to-jugular venous glucose difference
a-jvD_{lac}:	arterial-to-jugular venous lactate difference
Amy:	central nucleus of the amygdala
APACHE II:	acute physiology and chronic health evaluation II
BBB:	blood-brain barrier
BCAA:	branched-chain amino acid
BH_2:	dihydrobiopterin
BH_4:	tetrahydrobiopterin
CAI:	cerebral autoregulation index
cAMP:	cyclic adenosine monophosphate
CaO_2:	arterial oxygen content
C_b:	concentration in brain extracellular fluid (of a given LNAA)
CBF:	cerebral blood flow
CGRP:	calcitonin-gene related peptide
CLR:	calcitonin receptor-like receptor
$CMRO_2$:	cerebral metabolic rate of oxygen
CPP:	cerebral perfusion pressure

CVC:	cerebrovascular conductance
$CVCO_2R$:	cerebrovascular carbon dioxide reactivity
CVLM:	caudal ventrolateral medulla
CVO_2R:	cerebrovascular oxygen reactivity
CVRi:	cerebrovascular resistance index
DAG:	diacylglycerol
DBH:	dopamine β-hydroxylase
DHA:	dehydroascorbic acid.
DMV:	dorsal nucleus of the vagal nerve
ECE:	endothelin-converting enzyme
EO_2:	cerebral extraction fraction
eNOS:	endothelial nitric oxide synthase
ET-1:	endothelin-1
ET_A:	endothelin receptor A
ET_B:	endothelin receptor B
F_IO_2:	inspired oxygen fraction
GC:	guanylate cyclase
cGMP:	cyclic guanine monophospate
HR:	heart rate
ICA:	internal carotid artery
ICP:	intracranial pressure
ICU:	intensive care unit
IL-1β:	interleukin-1β
IL-6:	interleukin-6
IMLT:	intermediolateral tract
IP_3:	inositol trisphosphate
J_A:	transcerebral net exchange of adrenaline
J_{ET-1}:	transcerebral net exchange of endothelin-1
J_{CGRP}:	transcerebral net exchange of calcitonin gene-related peptide
J_{Dop}:	transcerebral net exchange of dopamine

J_{NA}:	transcerebral net exchange of adrenaline
k_e:	elimination constant
K_m:	Michaelis-Menten constant
LAT1:	large neutral amino acid transporter 1
LBP:	lipopolysaccharide-binding protein
LC:	locus coeruleus
LGI:	cerebral lactate-glucose index
LL:	lower limit of autoregulation
LNAA:	large neutral amino acid
LOI:	cerebral lactate-oxygen index
LPS:	lipopolysaccharide
MAP:	mean arterial blood pressure
MCA:	middle cerebral artery
MCAv:	middle cerebral artery blood flow velocity
MD-2:	myeloid differentiation factor 2
mRoR:	modified rate of regulation
Mx:	time correlation index (based on cerebral perfusion pressure)
Mxa:	time correlation index (based on mean arterial blood pressure)
NA:	noradrenaline
Nam:	nucleus ambiguous
NFκB:	nuclear factor κB
NIRS:	near-infrared spectroscopy
NO:	nitric oxide
NTS:	nucleus of the solitary tract
OGI:	cerebral oxygen-glucose index
$PaCO_2$:	arterial carbon dioxide tension
PAH:	phenylalanine hydroxylase
PaO_2:	arterial oxygen tension

$P_{ET}CO_2$:	end-tidal carbon dioxide tension
PI:	Gosling's pulsatility index
PLC:	phospholipase C
PKA:	protein kinase A
PKC:	protein kinase C
PKG:	protein kinase G
PS:	permeability surface area product
PVN:	paraventricular nucleus
RAMP:	receptor modifying protein
RCP:	receptor component protein
RI:	Pourcelot's resistive index
RoR:	rate of regulation
RVLM:	rostral ventrolateral medulla
SaO_2:	arterial oxygen saturation
SIRS:	systemic inflammatory response syndrome
SOFA:	sequential organ failure assessment
TH:	tyrosine hydroxylase
TLR4:	toll-like receptor 4
TNF-α:	tumour necrosis factor α
UL:	upper limit of autoregulation
V_c:	volume of distribution
V_{max}:	maximal transport velocity

Figures, tables, and textbox

Chapter 1. Introduction

"The Brain – is wider than the sky –" writes Emily Dickinson,[135] a statement that is true not only for poets, but also for scholars and scientist. Hence, the complexity of the human brain has been a source of fascination and bewilderment since it was first proposed as the seat of the mind in antiquity.[98,398,497] This complexity also applies to cerebral haemodynamics, as cerebral blood flow (CBF) comprises ~15 % of total cardiac output while the brain is responsible for ~20 % of the human body's oxygen consumption, despite comprising only 2–3 % of body weight.[590] The high metabolic requirements of the brain combined with its limited oxygen reserves,[a] necessitates a tight control of CBF to maintain a stable supply of substrates, despite changes in cerebral metabolism, cerebral perfusion pressure (CPP), and arterial blood gases.[88,195,228,299,300,412,413,419,516,590]

If the homeostatic mechanisms that control CBF fail, cerebral ischaemia and consequent neuronal damage may ensue.[8,257,411,419,590] As will be outlined in the present thesis, this may be particularly relevant in the context of sepsis,[b] the systemic inflammatory response to infection,[267,308,487,597] a condition that carries a substantial burden in terms of mortality and morbidity.[17,268,345,567,568] According to magnetic resonance imaging-based[420,430,490] and neuropathological studies,[252,346,490,491,494,598] ischaemic strokes, including occasional watershed infarcts as well as vasogenic and cytotoxic oedema, are common in septic patients. Neuropathological studies have furthermore shown that diffuse ischaemic changes that are undetectable by conventional neuroimaging are to some extent present in all patients dying from septic shock.[151,491,494] Accordingly, cerebral ischaemia is thought to contribute

[a] If the substrate supply is entirely abolished, unconsciousness evolves in seconds and the brain's total energy pool is consumed in minutes.[138,220]

[b] The word sepsis is Greek (σηπσις), and literally means putrefaction.

Systemic inflammatory response syndrome (SIRS):

- ≥ 2 of the following:
  - Heart rate > 90 beats min^{-1}
  - Body temperature < 36 °C or > 38 °C
  - Respiratory rate > 20 breaths min^{-1} or $PaCO_2$ < 4.3 kPa
  - White blood cell count < 4 10^9 cells L^{-1} or > 12 10^9 cells L^{-1}, or the presence of > 10 % immature neutrocytes

Sepsis:

- The presence of SIRS with documented or suspected infection.

Severe sepsis:

- Sepsis complicated by organ dysfunction or hypoperfusion.

Textbox 1.1. Sepsis definitions.
These criteria are based the 2001 International Sepsis Definitions Conference criteria,[70,308] which was effectual when the studies of the present thesis were conducted. It must, however, be noted that the sepsis definitions have recently been revised.[500]

both to sepsis-associated encephalopathy,[2,66,68,199,493,530] long-term cognitive deficits in sepsis survivors,[19] and to the progression of septic shock.[17,492]

The conspicuous link between febrile illness and brain dysfunction, now termed sepsis-associated encephalopathy,[c] has been noted by physicians since the dawn of clinical medicine more than two millennia ago.[3,89,168,306,390] With the rise of intensive care medicine from the mid-20th century and the resultant introduction of sepsis as a formal clinical diagnosis[39,308] (*Textbox 1.1*), the clinical relevance of encephalopathy as a complication in this context was reinvigorated.[66,68,146,199,245,403,598,600] The phenomenology of encephalopathy is so inherently difficult to convey that even the *Oxford Handbook of Clinical Medicine*[318] resorts to Joseph Conrad's *Heart of Darkness* (*Figure 1.1*) in the section on delirium. Charles F. Bolton and coworkers nevertheless made a fair attempt at describing the natural history of sepsis- associated encephalopathy based on systematic obser-

[c] The word encephalopathy originates from the Greek εν (inside), κεφαλη (head), and παθος (suffering).

Figure 1.1. Fever and encephalopathy in classical literature. In the finale of Joseph Conrad's Heart of Darkness (1899), the ivory trader Kurtz suffers a severe bout of jungle fever complicated by encephalopathy:

"The wastes of his weary brain were haunted by shadowy images now – images of wealth and fame revolving obsequiously round his unextinguishable gift of noble and lofty expression.… Sometimes he was contemptibly childish. He desired to have kings meet him at railway-stations on his return from some ghastly Nowhere, where he intended to accomplish great things.…'Close the shutter,' said Kurtz suddenly one day; 'I can't bear to look at this.' I did so. There was a silence. 'Oh, but I will wring your heart yet!' he cried at the invisible wilderness."

Classical literature provides numerous other illustrative examples of the link between fever and encephalopathy, such as Falstaff from Shakespeare's Henry V (1599), the poet Antonio in Hans Christian Andersen's *The Improvisatore* (1835), the mysterious Madman from Charles Dicken's *Pickwick Papers* (1836), Anna Karenina from Leo Tolstoy's eponymous novel (1877), and Bishop Gustav Trolle from Johannes V. Jensen's *The Fall of the King* (1901). As with Kurtz, the development of encephalopathy in the context of febrile illness often heralds an untimely death or a similar unfavourable outcome. Incidentally, this is consistent with contemporary scientific literature.

Illustration by Matt Kish reproduced from the illustrated edition of *Heart of Darkness*[105] with permission from the artist.

vations on patients admitted to the intensive care unit (ICU) in the 1970s and 1980s.[66,67] They found that sepsis-associated encephalopathy typically involves a change in mental status that occurs within hours of the onset of sepsis, which is in accordance with other reports.[485,495,598] This involved an initial impairment of attention, which then progressed to involve orientation and cognition, often with a gradual decrease in consciousness with concomitant agitation and con-

fusion.[d,66,67,252,598] Sepsis has previously been reported as the most common non-neurological cause of encephalopathy in ICU patients.[63]

The occurrence of encephalopathy in septic patients has been assessed in eight studies published between 1983 and 2017.[63,152,426,506,508,567,587,598] In these studies, the prevalence varies greatly (from less than 10 to approximately 70 %), conceivably due to differences in the diagnostic definitions, patient demography, and changes in the cause and treatment of sepsis over the more than three decades where the studies were conducted. Consequently, the prognostic impact of encephalopathy is also difficult to address in these studies, even though six of the studies reported data on mortality.[152,426,508,567,587,598] Together, the studies nevertheless indicate that the development of encephalopathy in septic patients increases the risk of death.

Studies focusing on survivors of sepsis complicated by the acute respiratory distress syndrome have found that these patients often exhibit permanent cognitive deficits after discharge, notably memory loss and impaired learning.[235–237,251,253] In one cohort, some aspects of cognitive function had recovered one year after discharge, but memory loss and impaired learning were nevertheless largely irreversible.[235–237] In another study, more than 60 % of survivors still exhibited cognitive dysfunction eight years after discharge.[251]

Lastly, several central nervous system nuclei that are involved in the autonomic regulation of cardiovascular function consistently show signs of ischaemic damage in patients that have died from septic shock.[491,494] This has led to the theory that damage to these nuclei contributes to the progression of the distributive shock that may evolve in

[d] Some authors have asserted that the term sepsis-associated encephalopathy should be replaced by *sepsis-associated-delirium*.[146] However, according to the observations by Bolton and co-workers, the gradual decrease in consciousness does not necessarily involve delirium.[66,67,252] Accordingly, it has been argued that encephalopathy and delirium are not necessarily synonymous terms, as delirium encompasses specific diagnostic criteria, while encephalopathy may be used in a broader sense to describe the presence of diffuse brain dysfunction.[60065,379,596,602]

sepsis.[17,492] Approximately one third of patients with severe sepsis develop shock, which is associated with an increase of the ICU mortality rate by up to 20 %.[567]

The studies of the present thesis aimed at investigating various aspects of cerebral haemodynamic function in experimental and clinical sepsis and are briefly outlined in *Appendix 1*. We developed a human-experimental model of the acute systemic inflammatory response of sepsis by means of a continuous lipopolysaccharide (LPS) infusion (*Study A*), and this method was used in three subsequent studies (*Studies B, D, and E*), while the two remaining studies (*Studies C* and *F*) included septic patients admitted to the ICU.

In the next chapter, the use of LPS-infusion as a human-experimental model of early sepsis is reviewed, including a discussion of how this model relates to other experimental models of sepsis. In the following six chapters, various aspects of cerebral haemodynamic function after LPS infusion and in patients with clinical sepsis are reviewed. Furthermore, given that the cerebrovasculature is an integral component of the cardiovascular system, and the blood supply to the brain ultimately depends on the ability of the cardiovascular system to generate an adequate and stable blood pressure,[590] the thesis also includes a chapter on the autonomic regulation of cardiovascular function in sepsis. On this basis, I will seek to identify changes in cerebral haemodynamic function that may predispose to cerebral ischaemia in sepsis, with focus on the changes that occur between the very early stages, as modelled by LPS infusion in healthy volunteers, and more advanced stages, as encountered in critically ill, mechanically ventilated patients admitted to the ICU.

Chapter 2. Experimental and clinical sepsis

In the clinical setting, sepsis may be caused by a plethora of pathogens, and the clinical presentation is markedly influenced by the site of infection, temporal factors, various therapeutic interventions, as well as patient characteristics including genetic predisposition and comorbidity.[16,433] Clinical studies on sepsis are thus burdened with very heterogeneous study populations, in which the onset time of disease is far from well-defined. For logistic and ethical reasons, it is therefore difficult to study septic patients for scientific purposes until the septic condition has progressed beyond its very early stages.[48,321] This complicates the identification of the fundamental mechanism of disease, and thus necessitates the use of experimental models of sepsis.[431,483]

The most widely used human-experimental model of sepsis is the human endotoxaemia model, in which a human volunteer receives an intravenous bolus injection of the endotoxin LPS (*Figure 2.1*).[172,290,352,432] LPS comprises the lipocarbohydrate component of the outer membrane of most Gram-negative bacteria,[359] and is a potent activator of the innate immune system (*Figure 2.2*). The resultant immune response is considered responsible for most of the toxic effects of Gram-negative bacteria.[58,125,361]

After intravenous injection, LPS is contained in the blood compartment, where it is mainly carried by specific carrier proteins and platelets.[359,389,484] Of note, LPS does not pass the BBB,[52,53] but is predominantly bound in the liver,[86,96] and degraded within minutes.[e,134,342]

[e] In Deventer et *al.*,[134] six healthy volunteers receive an intravenous bolus injection of LPS (2 ng kg^{-1}); Figure 2 in the paper provides a clearance curve for the injected LPS, from which the elimination constant (k_e) for LPS may be estimated to be 0.14 minute^{-1}. The plasma half-life, $T_{½}$ is consequently:

$$T_{½} = \frac{ln2}{k_e} = \frac{ln2}{0.14} \sim 5\ minutes$$

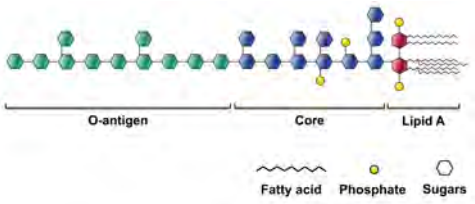


Figure 2.1. Structure of lipopolysaccharide (LPS).
The innermost region of the LPS molecule contains lipid A, a phosphorylated glucosamine disaccharide to which multiple β-hydroxy fatty acids are bound. Lipid A anchors LPS to the bacterial membrane, so that the rest of the LPS projects from the cell surface.[408,443,456] It has been estimated that there are around 106 lipid A residues per *Eschericia coli* bacterium.[186] A nonrepeating oligosaccharide component is attached to lipid A, and comprises the 'core' of LPS.[408,443,456] The outermost part of the LPS molecule, the so-called O-antigen, is bound to the other end of the core oligosaccharide, and is thus exposed on the outer surface of the bacterial cell. It varies between strains and is consequently target for recognition by host antibodies.[443] While lipid A accounts for most of the biological effects of LPS, including the systemic inflammatory response,[442,456,482] the O-antigen influences the magnitude of the response.[169,269]

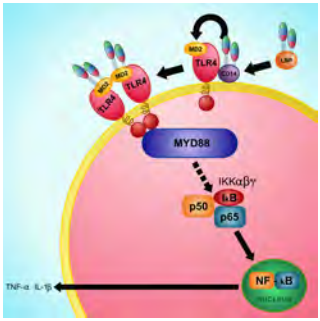


Figure 2.2. Molecular basis of the lipopolysaccharide (LPS)-triggered immune response.
LPS monomers are carried by the LPS-binding protein (LBP) in plasma,[408,484] and LBP subsequently transfers LPS to CD14, a pattern recognition receptor (PRR) that is found on innate immune cells.[364] CD14 acts as a co-receptor with myeloid differentiation factor 2 (MD-2) and the toll-like receptor 4 (TLR4),[227,278,364,408,498] of which the latter is another PRR that specifically binds lipid A.[4,319] The association of CD14 with the TLR4-MD-2 complex leads to dimerisation of TLR4, which then activates the intracellular TLR4 pathway.[319,437] This involves the upstream activation of MyD88, and which eventually leads to the translocation of nuclear factor κB (NFκB) into the nucleus.[319,348,408] This induces the synthesis and release of a wide variety of inflammatory mediators including tumour necrosis factor α (TNF-α) and interleukin-1β (IL-1β).[4,396,518]

Despite this, an intravenous bolus injection of LPS triggers an hour-lasting systemic inflammatory response by initiating toll-like receptor 4 (TLR4)-dependent pathways in resident tissue macrophages and endothelial cells (*Figure 2.2*).

The minimal pyrogenic dose of well-characterised reference LPS prepared from *Eschericia coli* has been identified as 0.1 ng kg^{-1},[155] whereas the maximum tolerated dose before hepatotoxicity ensues in humans is 4.0 ng kg^{-1}.[164] The highest known dose of LPS administered in man is 1.0 mg kg^{-1} (self-administration), which led to a state of shock and multiorgan failure.[538] As of now, no adverse reactions to intravenous LPS administration have been reported in the human-experimental setting.[184] At doses of 2.0 ng kg^{-1}, which are often used in this setting, the plasma concentration of LPS briefly increases to a level similar to that encountered in septic patients early after the onset of a Gram negative infection (approximately 10 ng L^{-1}).[44,134] However, in patients admitted to the ICU with advanced sepsis, much higher plasma LPS concentrations (in the mg L^{-1} range) are often observed.[44,340,389]

The TLR4-dependent systemic inflammatory response to an intravenous injection of an LPS bolus involves the release of numerous pro- and anti-inflammatory cytokines to the bloodstream, which will only briefly be reviewed here. Notably, plasma tumour necrosis factor α (TNF-α) increases almost immediately and peaks 1½ to two hours post-injection, and typically returns to normal levels within three hours.[165,172,352,520] Relative to this, a slightly delayed increase in interleukin-6 (IL-6) takes place between 1½ and 3 hours post-injection. [246,428] This delay occurs because the IL-6 response is mainly triggered by TNF-α in an autocrine and paracrine fashion.[246,428] IL-6 usually peaks at about two hours and remains elevated up to 24 hours post-injection.[165,172,290,434,520] It acts in concert with the closely related IL-1β to induce fever[139,198,221,322,572] and stimulate the release of acute phase reactants from the liver.[97,133,190,322] This is accompanied by

changes in circulating immune cells; even though white blood cells are recruited from the bone marrow, a reduced total white blood cell count is usually observed within the first 1½ hours of injection due to neutropenia.[544] This involves enhanced endothelial adhesion[13,205,290] and extravascular migration of neutrocytes triggered by TNF-α.[326,571] Neutrocytes subsequently accumulate in various organs, particularly the liver and the lungs.[429,544] Within the next hours, the initial leukopenia is converted to a marked leucocytosis, which is facilitated by an increase in plasma cortisol and adrenaline,[155,289,365,520] as these hormones inhibit neutrocyte adhesion and migration.[512,557] The neutrocyte count consequently typically peaks approximately eight hours post-injection.[172,429,520] Lymphocytopenia develops within the first 1½ hours after an LPS injection,[164,287,288] and this involves migration of lymphocytes from the blood stream into splanchnic lymph nodes, where activated mature T-cells undergo apoptosis.[288,545] A gradual recovery in lymphocyte counts is evident four to five hours post-LPS, and normal values are reached between eight and 24 hours.[288,461]

In humans, the systemic inflammatory response to an intravenous bolus injection of LPS is highly dose-dependent. Hence, at subpyrogenic doses, subjects do not experience symptoms, and no significant change in temperature, heart rate or blood pressure can be detected.[14] LPS doses of 2.0-4.0 ng kg^{-1} increase core temperature by about 2 °C, and are accompanied by characteristic flu-like symptoms, which peak at 1½ to two hours post-injection.[287,366,429] Furthermore, the heart rate increases, while mean arterial pressure may decrease, and the subject becomes tachypnoeic with evidence of respiratory alkalosis.[155,289,365,429,519–521] The systemic inflammatory response may thus briefly resemble that encountered clinically during the early stages of sepsis (*Textbox 1.1*). However, the sudden and brief primary insult elicited by an intravenous LPS bolus injection differs fundamentally from clinical sepsis, where the insult is persistent and evolves over time.[119–121,341] Compared to various clinical conditions,

the systemic inflammatory response to an LPS bolus is consequently very short-lived, and although representative cytokine concentrations are briefly reached, they may not be of a sufficient duration to induce representative pathophysiological changes in various target organs. This also poses a problem when using sub-pyrogenic LPS doses to study low-grade inflammation, defined as a two- to three-fold increase in circulating pro-inflammatory cytokines and acute-phase reactants, which is considered a key player in the pathogenesis of various chronic non-communicable diseases.[49,417,614] Human endotoxaemia has therefore been widely criticised as being insufficient as a clinical model, notably in relation to the systemic inflammatory response associated with sepsis.[12,14,184]

Due to these fundamental problems, we developed a continuous LPS infusion-model in humans.[I] As summarised in *Figure 2.3*, we found that LPS at a dose of 0.3 ng kg^{-1} administered over four hours induced a less pronounced TNF-α and IL-6 response than a 0.3 ng kg^{-1} bolus injection. The neutrocyte response followed the same pattern, while lymphocytes decreased similarly in the two groups. As expected at this LPS dose, the slight increase in core temperature was too small to be significant in point-by-point comparisons. However, notable differences in the kinetics of the cytokine responses were evident, as TNF-α peaked one hour after cessation of the continuous infusion, while IL-6 peaked within the following hour, and neutrocytes peaked after three hours. The systemic inflammatory response thus evolved more gradually and was more sustained than following bolus injection.[I]

A recurring challenge when using LPS bolus injection is defining the appropriate time point for a given physiological assessment. In many studies, measurements are made at 1½ to two hours after the bolus injection, at which time TNF-α, IL-6, and flu-like symptoms approximately peak. However, this time point precedes the peak core temperature by several hours,[287,365,429] while neutrocytes are typically

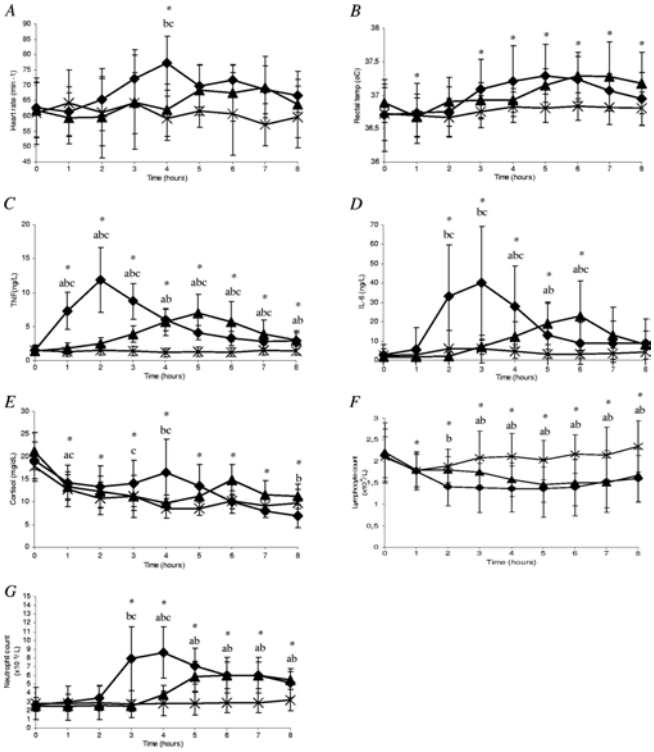


Figure 2.3. Time course of clinical and biochemical variables after a bolus injection vs. continuous infusion of lipopolysaccharide (LPS).
Ten healthy male volunteers received a bolus injection (♦) or a 4-hour continuous infusion (▲) of LPS (total dose 0.3 ng kg^{-1}), or placebo (×) in a double-blinded crossover study. A: Heart rate; B: Rectal temperature; C: Plasma tumour necrosis factor α (TNF-α); D: Plasma interleukin-6 (IL-6); E: Plasma cortisol; F: Lymphocyte count; G: Neutrocyte count. Data are presented as mean ± SD. *Overall difference between baseline (0 hours) and the specific time point, as evaluated by ANOVA; [a]significant difference between continuous infusion and placebo by *post hoc* comparison at the given time-point; [b]significant difference between bolus injection and placebo by *post hoc* comparison at the given time-point; [c]significant difference between bolus injection and continuous infusion by *post hoc* comparison at the given time-point. Reproduced from *Paper I* with permission.

	0.3 ng kg^{-1}		2.0 ng kg^{-1}	
	Bolus injection (n=10)	**4h infusion (n = 22)**	**Bolus injection (n = 8)**	**4h infusion (n = 19)**
Temperature (ºC)	0.1 ([-0.2] – 0.3)	0.9*† (0.5 – 1.4)	0.7*$^{\#}$ (0.4 – 0.9)	2.1*†$^{\#}$ (1.8 – 2.3)
White blood cell count (10^9 L^{-1})	-0.7 ([-2.2] – 0.9)	3.4*† (2.3 – 4.1)	-0.8 ([-2.2] – 0.8)	1.0*†$^{\#}$ ([-0.1] – 1.9)
Neutrocyte count (10^9 L^{-1})	0.70 ([-2.3] – 1.7)	4.2*† (3.2 – 5.0)	0.6 ([-0.8] – 2.1)	2.7*†$^{\#}$ (1.4 – 3.6)
Lymphocyte count (10^9 L^{-1})	-0.7* ([-1.0] – [-0.5])	-0.7* ([-0.9] – [-0.6])	-0.7* ([-0.8] – [-0.5])	-1.0*†$^{\#}$ ([-1.3] – [-0.9])
TNF-α (fold change)	8* (5 – 13)	8* (6 – 12)	371*$^{\#}$ (143 – 963)	40*†$^{\#}$ (32 – 49)
IL-6 (fold-change)	23* (14 – 40)	57* (27 – 122)	2922*$^{\#}$ (1705 – 5009)	956*†$^{\#}$ (735 – 1247)
Heart rate (beats min^{-1})	3 ([-2] – 8)	12* (1 – 20)	14*$^{\#}$ (7 – 22)	37*†$^{\#}$ (31 – 40)
MAP (mmHg)	0 ([-5] – 5)	- 3 ([-12] – 4)	3 ([-6] – 12)	-9*† ([-17] – [-3])
$PaCO_2$ (kPa)	n/a	-0.7* ([-0.9] – [-0.5])	-0.8* ([-1.0] – [-0.6])	-0.8* ([-1.0] – [-0.7])
pH (units)	n/a	0.03* (0.00 – 0.05)	0.03 ([-0.01] – 0.08)	0.04* (0.03 – 0.05)

Table 2.1. Changes in systemic inflammatory response variables to bolus injection vs. four-hour continuous infusion of lipopolysaccharide (LPS) at two different doses.

Data are presented as changes between baseline and 1½ hours post-LPS for bolus injections, and between baseline and 1-1½ hour after cessation of the continuous infusions, respectively. For all variables, absolute mean changes with 95 % CI are presented, except for tumour necrosis factor α (TNF-α) and interleukin-6 (IL-6) where mean fold-changes with 95 % CI are presented, because the data had to be logarithmically transformed. Data are pooled from *Studies A-D* and Møller *et al.* 2002.[365] n/a: not available. *Significant change from baseline; †significant change from bolus injection at the same LPS dose; $^{\#}$significant change from lower LPS dose (0.3 ng kg^{-1}) when using the same mode of administration (bolus injection or continuous infusion).

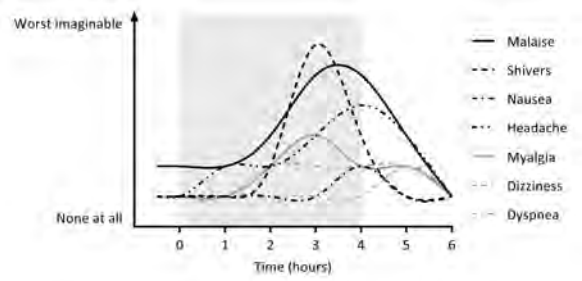


Figure 2.4. Symptoms during a four-hour lipopolysaccharide (LPS) infusion.

LPS was infused from 0–4 hours (shaded area) at a dose of 2.0 ng kg^{-1}. Symptoms were evaluated by a visual analogue scale, represented by a horizonal line, ranging from 'none at all' to 'worst imaginable'. The presented data are median values from *Studies D and E* (n = 19).

within the normal range as they have just recovered from the initial neutropenia.[289,429] The associated increase in heart rate and ventilation likewise continue to increase beyond the cytokine peak and reach their maximal values more than four hours after the bolus injection,[289,365,429,519,521] at which time the mean arterial blood pressure (MAP) nadir is also reached.[289,429] Given that the systemic inflammatory response syndrome is defined by temperature, white blood cell counts, and cardio-respiratory changes (*Textbox 1.1*), the 1½ to two-hour time point is clearly not ideal for replicating several aspects of the acute systemic inflammatory response of sepsis.

In *Table 2.*1, LPS-induced changes in the various parameters of the systemic inflammatory response syndrome are reported as differences from baseline at the post-LPS cytokine peak following either a bolus injection or a continuous LPS infusion. At a dose of 0.3 ng kg^{-1}, the TNF-α and IL-6 increase and the lymphocyte decrease are similar after bolus injection and continuous infusion. In contrast, changes in core temperature, neutrocyte count, and heart rate were only observed after the continuous infusion. Hence, even though 0.3 ng kg^{-1} is typically considered a sub-pyrogenic dose, volunteers receiving this dose as a four-hour continuous infusion showed evidence of acute systemic inflammation, both in terms of temperature, cytokine and immune cell responses, as well as the associated cardio-respiratory changes. At 2.0 ng kg^{-1}, the cytokine response was less pronounced after continuous infusion than following bolus injection, but the core temperature increase was larger,[f] and the changes in white blood cell counts and cardio-respiratory parameters were more pronounced. Furthermore, the associated flu-like symptoms peaked around the cessation of the continuous infusion (*Figure 2.4*).

[f] In *Study E*, we continually monitored skin temperature. We found that a sudden temperature increase set in 2 hours and 22 minutes (mean, 95 % CI 2 hours and 5 minutes to 2 hours and 38 minutes) into the infusion, and peaked 37 minutes (mean, 95 % CI 7 to 66 minutes) after the cessation of the infusion. This indicates that the one-hour post infusion time point is close to the peak increase in core temperature, at least at a dose of 2.0 ng kg^{-1}.

In the present thesis, the continuous infusion model was used in *Studies B, D*, and *E*, and in all studies post-LPS cerebral haemodynamic assessments were made within two hours after cessation of the infusion (*Appendix 1*). At this time, all volunteers showed evidence of acute systemic inflammation with fever, increases in TNF-α, IL-6, and white blood cell counts with associated cardio-respiratory changes and flu-like symptoms.

While the emphasis is on human studies in the present thesis, these are discussed in relation to animal studies as appropriate, based on the presumption that the validity of a finding in human-experimental or clinical studies is strengthened if it is supported in animal models.[184] These animal models in various species include intravenous infusion of LPS or live bacteria, as well as peritonitis induced by either caecal ligation and puncture or by the injection of bacteria or LPS into the peritoneal cavity.[92,178,453,454,483,585] As with the human endotoxaemia model, animal models make it possible to standardise the experimental conditions, so that the study group and the septic condition become less heterogeneous than in the clinical setting, while also allowing the study of the very early stages of disease. However, they additionally permit the induction of a much more severe and protracted systemic inflammatory response than human endotoxaemia, so that the progression of sepsis can be studied beyond the early stages. The direct translation from human to animal studies is, however, complicated by vast interspecies differences in immune physiology, including LPS sensitivity.[106,453,454]

There is currently no consensus as to how the different animal models of sepsis should be compared to human endotoxaemia and clinical sepsis. In the present thesis, I handle this by defining five basic stages of the acute systemic inflammatory response, which is based on the composite changes in temperature, white blood cell counts, circulating cytokines, as well as cardio-respiratory changes as

available in the different studies, and which takes the species and type of infectious insult into account:

Stage 0: Refers to the healthy control state or baseline conditions.

Stage I: Observed immediately after a bolus injection of LPS or bacteria and is probably more of an experimental curiosity or artefact than representative of any clinical stage. Within 20 minutes of the LPS bolus, and before any significant changes in core temperature and circulating white blood cells are observed, a sudden drop in arterial blood pressure with an increase in heart rate is observed, lasting for about 5 minutes.[13,205,290] Blood pressure and heart rate then quickly reach near-baseline levels.

Stage II: A gradual increase in core temperature, heart rate, and ventilation are observed, and prodromal symptoms of malaise may set in (in humans). This stage is observed from 20–30 minutes to one hour after an intravenous bolus injection of LPS in humans and animals, during one to three after the initiation of four-hour continuous LPS infusion in humans, and three to four hours after the induction of peritonitis in animal models.[12,453,483]

Stage III: Encountered one to three hours after a bolus injection of LPS in both human and animal studies, from one hour before to two hours after the cessation of a continuous LPS infusion in humans, and four to six hours after the induction of peritonitis in most animal mod-

els. This stage is characterised by fever with associated changes in white blood cells (which differ depending on species), hyperventilation, and tachycardia, and in some instances a reduction in arterial blood pressure. TNF-α and IL-6 peak during this stage.[178,453,483] In human-experimental studies, volunteers quickly recover after the peak of this stage, and neither bolus injection or continuous infusion of LPS thus progress beyond this stage.[12,432]

Stage IV: A transitional stage between stage III and V, as encountered in some animal models of sepsis using high-dose endotoxemia, peritonitis-, or bacteraemia-studies, as well as in some clinical patient studies.[90,178,240,453,483] During this stage, the systemic inflammatory response reaches a plateau, with continued fever, persistently high white blood cell counts, hyperventilation, and IL-6 levels, while TNF-α levels slowly decrease. Clinical deterioration with progressive organ failure is observed at this stage.

Stage V: In the present thesis, this is the stage of manifest advanced sepsis, when patients have been admitted to the ICU, or when sepsis has been present for more than 20 hours in animal models.[132,483,540] During this stage a systemic inflammatory response is maintained with fever, increased white blood cell counts, tachycardia, and high IL-6 levels, while TNF-α levels are only moderately increased.[341,540] At this stage, shock and multiorgan failure may have evolved.

This staging of the systemic inflammatory response is not to be considered a model of the temporal evolution of the septic conditions as such, but rather a conceptual framework that renders it possible to compare findings from various experimental and clinical models of sepsis in a systematic fashion. Notably, it permits the identification of stage III in experimental studies, which is considered representative of early sepsis in the present thesis, because the systemic inflammatory response criteria are formally fulfilled during this stage. Meanwhile, stage V is considered representative of advanced sepsis, and the present thesis principally focuses on differences between stage 0, III, and V. In any event, it must be noted that the time course of the systemic inflammatory response cannot explain all putative differences observed between the stages, particularly not when considering the dose-dependency of the systemic inflammatory response combined with the fact that septic patients are typically exposed to a much higher LPS or bacterial load than can be achieved in most experimental studies. Regardless of the stages outlined above, notable interspecies differences exist both in terms of immune function and cerebral haemodynamic function.[g] The review in the present thesis is thus based on findings from human studies, while animal studies are mainly introduced when they add notable perspectives and/or further strength to conclusions based on human data.

[g] Notably, humans lack a carotid *rete mirabile*, which is involved in the regulation of brain temperature in many animals.[32,148,226,468] As will be outlined in the present thesis, changes in core temperature may be pertinent to the cerebral haemodynamic changes observed during febrile illness in humans. Due to presence of a carotid rete mirabile, this is not necessarily the case in dogs, cats, and pigs, that is, animals that are commonly used in experimental models of sepsis.

Chapter 3. Cerebral blood flow

We investigated global CBF by the Kety-Schmidt technique[270–272,274,537] during stage III in healthy volunteers. Global CBF was similar between conditions even though an increase in the cerebral metabolic rate of oxygen ($CMRO_2$) was concurrently observed[II] (*Figure 3.1A-B*). Given that an increase in $CMRO_2$ is normally a potent stimulus for CBF,[196,243,302,411,412,505,590] our findings suggest that an efficacious vasoconstrictive influence must concurrently be present in the cerebrovasculature.

Accordingly, we found evidence of reduced CBF during stage III in a subsequent study,[53] as middle cerebral artery blood flow velocity (MCAv, cyclic mean) determined by transcranial Doppler ultrasound was lower during stage III than at baseline (*Figure 3.2A*). Meanwhile, the transcranial Doppler-derived cerebrovascular resistance index (CVRi) showed no change (*Figure 3.2B*). The Doppler probe was, however, detached during the LPS infusion, and since both MCAv and CVRi are highly insonation angle-dependent,[588,613] these findings should be interpreted with caution. The associated changes in the less insonation angle-dependent Pourcelot's Resistive Index (RI) and Gosling's Pulsatility Index (PI),[203,439,613] nevertheless also suggest that cerebrovascular resistance is increased during stage III (*Figure 3.2C-D*).

Other human-experimental studies, based on either the Kety-Schmidt technique or transcranial Doppler ultrasound, have found that CBF is also reduced during stage III triggered by a bolus injection of LPS.[84,365] In the Kety-Schmidt-based study, $CMRO_2$ was concurrently found to be unaffected,[365] thus yielding a similar mismatch between CBF and $CMRO_2$ as in our Kety-Schmidt based study.[II]

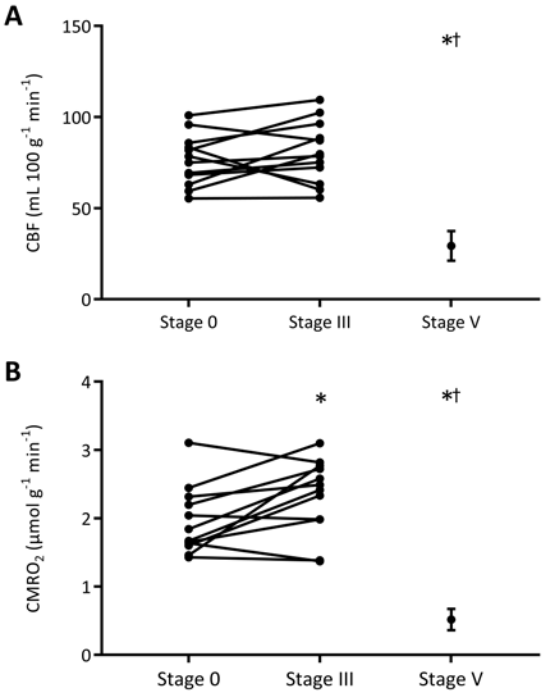


Figure 3.1. Global cerebral blood flow (CBF) and cerebral metabolic rate of oxygen ($CMRO_2$) during stage III and V.
Data on stage 0 and III are from *Paper II*, and are based on the Kety-Schmidt technique using N_2O as the tracer. Data on stage V are pooled from patients with available individual data from Bowton *et al.*[78] and Maekawa *et al.*[328] (n = 9), and are presented as mean ± SD. In these two studies, CBF and $CMRO_2$ were measured by the ^{133}Xe washout technique and the Kety-Schmidt technique (using stable argon as the tracer), of which the former is an extension of the Kety-Schmidt technique.[378] *Significantly different from stage 0; †significantly different from stage III.

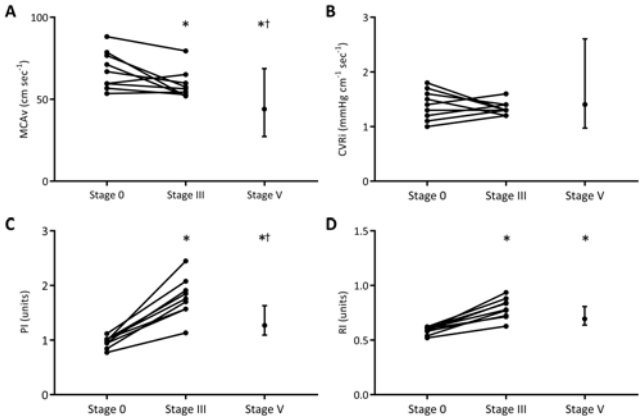


Figure 3.2. Transcranial Doppler ultrasound-based assessments of cerebral blood flow and cerebrovascular resistance during stage III and V.
Data on stages 0 and III are from *Study D*; data on stage V are from *Study C* (n = 16), and presented as median (IQR). Data on middle cerebral artery blood flow velocity (MCAv) and cerebrovascular resistance index (CVRi) have previously been reported.[53] A: MCAv (cyclic mean); B: CVRi; C: Gosling's Pulsatility Index (PI); D: Pourcelot's Resistive Index (RI). All presented values are mean values over a four-minute period. PI is normally 0.5 to 1.2, and increases as a function of cerebrovascular resistance downstream of the insonated vessel. It is, however, also affected by a number of other mutually interdependent hemodynamic parameters, such as CPP, pulse pressure, cerebral arterial compliance, and heart rate.[45,114,115,239,558,559] RI is largely dependent on the same parameters as PI; normally, RI > 0.8 is interpreted as evidence of elevated cerebrovascular resistance.[115,558,582] *Significantly different from stage 0; †significantly different from stage III.

Furthermore, based on assessments of the cerebrovascular carbon dioxide reactivity at baseline, the reduction in CBF during stage III was found to be of a magnitude that could be explained by the LPS-induced hyperventilatory response.[365] The transcranial Doppler ultrasound-based study correspondingly found that the observed reduction in MCAv correlated with the reduction in $PaCO_2$.[84]

In contrast to the abovementioned studies, one human-experimental study also based on the Kety-Schmidt technique, found no changes in either CBF or $CMRO_2$ measured hourly for five hours after an intravenous LPS bolus injection.[436] In this study, the subjects were presumably poikilocapnic, but data on $PaCO_2$ were not reported. The

statistical approach in this study was rather conservative, and the lack of any statistical difference in CBF between baseline and 1 or 2 hours post-LPS may potentially reflect a type II error.[h]

Several animal studies focusing on stage III have likewise found that CBF is unaffected while $CMRO_2$ is increased, or that CBF is reduced without any change in $CMRO_2$.[94,153,154,353,410,445,578–580] Only one study in sheep found no changes in either CBF or $CMRO_2$ during stage III.[435] Accordingly, cerebrovascular resistance has been shown to increase, even in cases where CPP is critically reduced due to the presence of shock.[94,153,154,170,353,575,578–581,592] This has been ascribed to the hyperventilatory response associated with acute systemic inflammation in most of these studies. [153,170,410,445] However, when comparing data from the same experimental model in dogs, cerebrovascular resistance also increases in isocapnic animals during stage III albeit to a lesser extent that during poikilocapnia.[153,161,410]

In terms of global CBF in clinical sepsis, we have not yet examined this in patients during stage V. However, although data from *Study C* show that CVRi is similar during stage 0 and V, MCAv is lower while RI and PI are higher in the latter, suggesting increased cerebrovascular resistance (*Figure 3.2A–D*). Findings from a more recent study that reported PI-values in septic patients also suggest that cerebrovascular resistance is frequently increased during stage V.[424] Two classic studies of unsedated patients during stage V support this, as they both reported a mean global CBF in patients of approximately 30 mL 100 g brain tissue^{-1} min^{-1}, which is lower than in healthy conditions.[78,328] $CMRO_2$ was also found to be reduced, and when considering the data from the two studies together, it is evident that $CMRO_2$ is reduced to a greater extent than CBF (*Figure 3.1A–B*). Data on global

[h] Ten volunteers participated in this study, and comparisons were made between baseline and five subsequent time points with adjustments for multiple comparisons. In order to detect a 10 ml 100 g^{-1} min^{-1} reduction between baseline and 1-2 hours as in Møller *et al.*[365] with a power of 0.80 and a similar variability in the data, this would require at least 14 subjects.

CBF and $CMRO_2$ from a study on sedated and mechanically ventilated patients during stage V show a similar pattern.[539]

The cerebral flow-metabolism relationship thus appears to dissociate in a biphasic manner between stage III and stage V. During stage III, inverse CBF and $CMRO_2$ changes take place, with a reduction in CBF, and thus an increase in cerebrovascular resistance, relative to $CMRO_2$. Given that the cerebrovascular CO_2 reactivity is preserved, hyperventilation may contribute to these changes. During stage V this appears to be reversed, since $CMRO_2$ is reduced more than CBF, notwithstanding that cerebrovascular resistance remains high.

Chapter 4. Cerebrovascular reactivity to acute changes in blood gases

A critical aspect of the regulation of CBF and the maintenance of a sufficient oxygen supply to the brain tissue is the ability of the cerebrovasculature to respond to changes in oxygen (*Figure 4.1*) and carbon dioxide (*Figure 4.2*).[8,228,257,553,590]

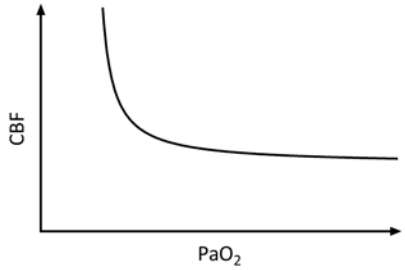


Figure 4.1. Relationship between cerebral blood flow (CBF) and PaO_2.
Hyperoxia increases while hypoxia reduces cerebrovascular resistance,[73,244,258,488,550] in such a way that a hyperbolic relationship between PaO_2 and CBF is present, with miniscule changes in CBF for PaO_2 levels ≥ 13.3 kPa and marked changes in CBF at PaO_2 levels ≤ 7.9 kPa.[257] The cerebrovascular oxygen reactivity is increased by an increase in $PaCO_2$ and *vice versa* for a reduction in $PaCO_2$.[336]

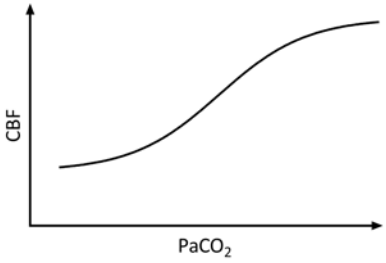


Figure 4.2. Relationship between cerebral blood flow (CBF) and $PaCO_2$.
The relationship between $PaCO_2$ and CBF is sigmoidal, with a reduction in cerebrovascular resistance upon an increase in $PaCO_2$, and an increase in cerebrovascular resistance upon a decrease in $PaCO_2$. The relationship is near-linear in the 2.5–8.0 kPa range.[223,249,273,275,283,452] If PaO_2 is reduced, the cerebrovascular carbon dioxide reactivity is increased, and *vice versa* for an increase in PaO_2.[336]

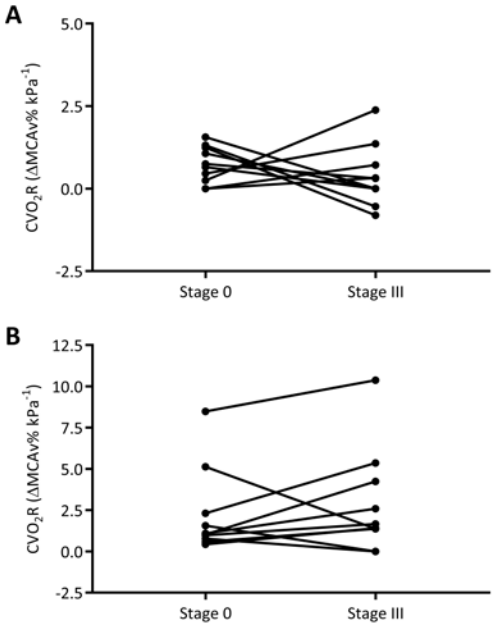


Figure 4.3. Cerebrovascular oxygen reactivity (CVO_2R) during stage III.
Data from.[52] The change in middle cerebral artery blood flow velocity (MCAv) from normoxia (F_IO_2 = 21 %) to either inspiratory hyperoxia (F_IO_2 = 40 %) or hypoxia (F_IO_2 = 12 %) is shown at each time point. A: Cerebrovascular responses to acute inspiratory hyperoxia B: Cerebrovascular responses to acute inspiratory hypoxia. The assessments were based on the cerebrovascular effects to changes in PaO_2. We obtained similar findings when the estimates were based on changes in arterial oxygen content (CaO_2).

We examined the cerebrovascular oxygen reactivity (CVO_2R) to both hyperoxia (F_IO_2 40 %) and hypoxia (F_IO_2 12 %) in humans during stage III, while monitoring and adjusting end-tidal PCO_2 to keep $PaCO_2$ constant.[52] The hyperoxia and hypoxia affected arterial oxygenation to the same extent at baseline and during stage III,[52] and we found no changes in CVO_2R to either intervention (*Figure 4.3*). In accordance with maintained CVO_2R during stage III, we have previous-

	Poikilocapnia	Isocapnia
Acid-base status		
$P_{ET}CO_2$ (kPa)	4.7 (0.7)	5.3 (0.13)†
$PaCO_2$ (kPa)	4.8 (0.5)	5.1 (0.3)†
pH (unts)	7.44 (0.02)	7.42 (0.05)†
Base excess (mM)	0.5 (2.4)	0.4 (3.7)
Oxygenation		
PaO_2 (kPa)	11.2 (1.4)	12.1 (1.1)
SaO_2 (%)	97 (1)	98 (1)
CaO_2 (mM)	8.5 (0.5)	8.4 (0.5)
Cardiovascular variables		
Heart rate (beats min^{-1})	93 (15)	95 (16)
MAP (mmHg)	82 (11)	83 (9)
Cerebral haemodynamic variables		
a-$jvDO_2$ (mM)	3.0 (0.3)	2.7 (0.3)†
EO_2 (%)	36 (3)	32 (3)†

Table 4.1. Systemic and cerebral haemodynamic effects of adding carbon dioxide to the inspired air during stage III.
Previously unpublished data from *Study D*. Data are presented as mean (SD). CO_2 was added to the inspired air to maintain isocapnia during stage III in seven of the volunteers. According to our protocol, paired arterial-jugular venous blood samples were obtained at the cessation of the four-hour lipopolysaccharide infusion ('poikilocapnia'), that is, immediately before end-tidal PCO_2 ($P_{ET}CO_2$) was adjusted, and twenty minutes later after breathing a gas mixture of normoxic air (F_IO_2 = 21 %), in which sufficient CO_2 was added to normalise $P_{ET}CO_2$. CaO_2: arterial oxygen content; EO_2: cerebral oxygen extraction fraction; MAP: mean arterial blood pressure. †Significantly different from poikilocapnia.

ly found that the CBF change to nine hours of inspiratory hypoxia (F_IO_2 12 %) in humans that receive intravenous LPS (corresponding to stage III) is similar to the change in humans that receive intravenous saline, corresponding to stage 0 (Taudorf *et al.*, unpublished findings). In a study on dogs, it was found that the CBF increase to 15 minutes of hypoxia at a targeted SaO_2 of 75 % and 50 % during stage III was similar to that of control animals.[104]

No previous studies have formally tested the cerebrovascular carbon dioxide reactivity ($CVCO_2R$) during stage III in humans. However, carbon dioxide was added to the inspired (normoxic) air for 20 minutes to maintain isocapnia during stage III in *Study D*, and the effects of the resultant increase in $PaCO_2$ may thus be assessed (*Table*

	Normoventilation	Hyperventilation
Acid-base status		
$PaCO_2$ (kPa)	5.3 (5.0 – 6.5)	4.7 (4.2 – 5.1)*
pH (units)	7.43 (7.40 – 7.44)	7.46 (7.40-7.48)
Base excess (mM)	1.6 ([-1.6] – 7.8)	0.4 ([-2.3] – 5.7)
Oxygenation		
F_IO_2 (%)	40 (38 – 48)	40 (38 – 48)
PaO_2 (kPa)	10.8 (9.9 – 13.0)	11.0 (9.9 – 12.5)
SaO_2 (%)	97 (97 – 98)	98 (97 – 98)
CaO_2 (mM)	6.1 (5.5 – 6.4)	6.3 (5.5 – 6.6)
Cardiovascular variables		
Heart rate (beats min^{-1})	74 (62 – 77)	64 (58 – 74)
MAP (mmHg)	71 (70 – 74)	75 (70 – 79)
Cerebral haemodynamic variables		
MCAv (cm sec^{-1})	57 (33 – 68)	32 (21 – 40)*
CVRi (mmHg sec cm^{-1})	1.3 (1.1 – 2.7)	2.5 (2.1 – 3.9)*

Table 4.2. Systemic and cerebral haemodynamic effects mechanical hyperventilation during stage V.
Data from *Paper V*. Data are presented as median (IQR). Seven critically ill patients admitted to the ICU with severe sepsis or septic shock were mechanically hyperventilated, targeting a $PaCO_2$ reduction of 10–20 %. CaO_2: arterial oxygen content; CVRi: cerebrovascular resistance index; MAP: mean arterial blood pressure; MCAv: middle cerebral artery blood flow velocity. *Significantly different from baseline.

4.1). We obtained paired arterial-jugular venous blood samples immediately before and after this intervention. By assuming that $CMRO_2$ did not change over these 20 minutes, the resultant reduction in arterial to jugular oxygen difference (a-jvDO_2) may indicate that CBF increased by 12 (4–20) %. This corresponds to a median $CVCO_2R$ of 43 (IQR 5–68) % kPa^{-1}. There is no consensus regarding the normal range of $CVCO_2R$ in humans, with reported values ranging from 8 to 45 % kPa^{-1} in different studies.[1,77,263,338] From our findings it may nevertheless be inferred that $CVCO_2R$ is still present during stage III, although this needs to be confirmed in studies where paired comparisons are made for $CVCO_2R$ between stage 0 and stage III. According to studies in dogs $CVCO_2R$ is maintained albeit slightly reduced during stage III.[94,153,410]

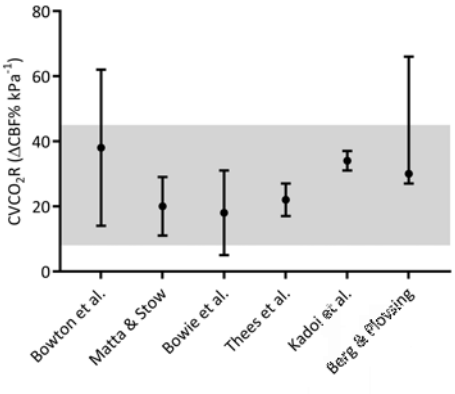


Figure 4.4. Cerebrovascular carbon dioxide reactivity ($CVCO_2R$) during stage V.
Data are obtained from Bowton *et al.* (n = 9),[78] Matta & Stow (n = 10),[343] Bowie *et al.* (n = 12),[77] Thees *et al.* (n = 10),[539] Kadoi *et al.* (n = 20)[263] and Berg & Plovsing (n = 7),[V] and are all presented as mean ± SD, except for our own findings which are presented as median (IQR). In all studies, $CVCO_2R$ was assessed during a reduction in $PaCO_2$ achieved by mechanical hyperventilation. One study used the ^{133}Xe washout technique to determine the associated CBF changes,[78] while the remaining used transcranial Doppler ultrasound. There is no well-defined normal range for $CVCO_2R$, but the range of values reported in healthy volunteers under baseline conditions in previous studies is illustrated by the shaded area.[1,77,263,338]

While CVO_2R has not yet been examined during stage V, $CVCO_2R$ has been addressed in several clinical studies.[V,77,78,263,343,539] In mechanically ventilated septic patients admitted to the ICU with sepsis, we assessed $CVCO_2R$ by increasing minute ventilation, aiming at a reduction in $PaCO_2$ of 10–20 %.[V] To ensure steady state, we maintained mechanical hyperventilation for 30 minutes; the effects of this on arterial blood gas values are summarised in *Table 4.2*. Mechanical hyperventilation caused a 36 (18–48) % increase in CVR, and a consequent 22 (11–37) % reduction in MCAv, corresponding to a median $CVCO_2R$ of 30 (IQR 27–66) % kPa^{-1}. Our findings thus indicate that $CVCO_2R$ is maintained during stage V, which is in line with previous studies (*Figure 4.4*).

Together, the available studies thus indicate that the cerebrovasculature remains capable of responding to both PaO_2 and $PaCO_2$

changes during stage III. It remains to be determined whether the cerebrovascular reactivity to changes in PaO_2 is affected during stage V, while the ability to respond to changes in carbon dioxide is maintained at this stage. However, based on our findings, the presence of a slight reduction in $CVCO_2R$ during stage III and/or stage V cannot be discarded.

Chapter 5. Cerebral autoregulation

The concept of cerebral autoregulation was first described by the Danish clinical physiologist Niels A. Lassen in a seminal paper published in *Physiological Reviews* in 1959.[299] Cerebral autoregulation refers to the ability of the cerebrovasculature to keep CBF relatively constant across a range of CPPs,[i] and may both be considered a static and a dynamic phenomenon. Hence, *static cerebral autoregulation* describes the cerebrovascular changes to changes in CPP at steady state, which encompasses the 'autoregulatory plateau,' which is enclosed by an upper and a lower limit (*Figure 5.1*).

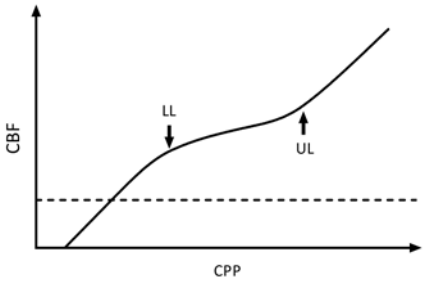


Figure 5.1. The static cerebral autoregulation curve.
Static cerebral autoregulation is mediated by adjustments in cerebrovascular resistance which reduce the impact of steady-state changes in cerebral perfusion pressure (CPP) on cerebral blood flow (CBF).[50,228,299,413] This involves cerebral vasoconstriction upon an increase in CPP, and cerebral vasodilation in response to a reduction in CPP. At the 'autoregulatory plateau,' which is enclosed by a lower (LL) and an upper limit (UL), CBF changes less than 10 % per 10 mmHg change in CPP.[53,228,320,603] The LL is located at a CPP of 60–90 mmHg and the UL at 140–150 mmHg, and outside these limits, CBF varies passively with CPP.[298,301,386,387,515] Below the LL, oxygen extraction is increased to match the cerebral metabolic requirements; when CBF reaches about 20 ml 100 g brain tissue^{-1} min^{-1} (typically at a CPP of < 25 mmHg[551]) this mechanism fails and the so-called ischaemic threshold is reached (dashed horizontal line). The curve's intersect with the abscissa is the critical closing pressure, and is normally reached at a CPP of ≤ 20 mmHg.[612]

[i] The cerebrovascular adjustments to changes in MAP and not CPP is considered in most studies of cerebral autoregulation. This may be justified because CPP varies linearly with MAP, given that that intracranial pressure (ICP) is constant:

$$\mathrm{CBF} = \frac{\mathrm{CPP}}{\mathrm{CVR}} = \frac{\mathrm{MAP} - \mathrm{ICP}}{\mathrm{CVR}}$$

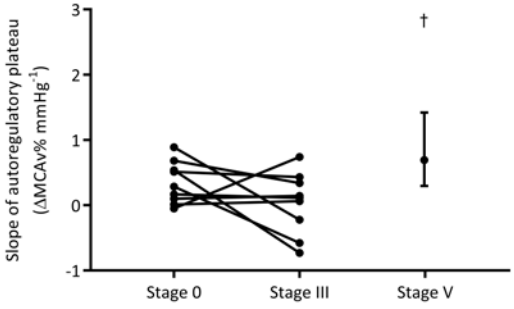


Figure 5.2. Slope of the cerebral autoregulatory plateau during stage III and V. Previously published data from *Studies C and E*.[53] Slopes of the regression lines between a noradrenaline-induced increase in mean arterial blood pressure (above the lower limit of autoregulation) and the corresponding change in middle cerebral artery blood flow velocity (MCAv) are shown. Data for stage V (n = 14) are presented as median (IQR). †Significantly different from stage III.

We examined static cerebral autoregulation during stage III by using intravenous noradrenaline to increase MAP by 25–30 mmHg while measuring MCAv by transcranial Doppler ultrasound in healthy humans at baseline and during stage III triggered by LPS.[53] At baseline, MAP was increased from 91 (86–98) to 110 (106– 116) mmHg, and during stage III, from 74 (73–83) to 104 (101–106) mmHg. On the basis of a dual linear regression method,[298,479] we inferred that all MAP values at baseline and stage III exceeded the lower limit of autoregulation, and that the slope of the autoregulatory plateau was similar between these stages (*Figure 5.2*). No other studies have examined static cerebral autoregulation during stage III in humans, but studies in both rats and dogs have likewise found that static cerebral autoregulation is maintained.[153,161,410,462]

In patients during stage V, MAP was below the lower limit of autoregulation in two of fourteen patients (at a MAP of 77 and 84 mmHg, respectively); in the remaining patients, MAP was located above the lower limit.[53] As MAP was increased from 75 (69–81) to 95 (88–110) mmHg by noradrenaline infusion, the slope of the autoregu-

latory plateau was found to be similar to the baseline slope in healthy volunteers (*Figure 5.2*).

Our findings agree with a previous transcranial Doppler ultrasound-based study by Matta & Stow, in which static cerebral autoregulation to an increase in MAP induced by phenylephrine was found to be preserved in septic patients during stage V.[343] However, in a transcranial Doppler ultrasound-based study by Taccone *et al.* where noradrenaline was used to increase MAP in a similar manner to our study, static cerebral autoregulation was reported to be impaired in two thirds of patients, particularly in the patients with the highest $PaCO_2$ levels.[529] The quantitation of static autoregulation in this study differs substantially from our approach, in that we assessed the regression line slope between MCAv and MAP during the MAP increase, while Taccone *et al.* used the cerebral autoregulation index ($CAI = \frac{\Delta MAP\%}{\Delta CVR\%}$), and dichotomously defined CAI > 2 as evidence of impaired autoregulation. This does nevertheless not readily explain the difference between the findings between studies, as a reassessment of our data yields a CAI of less than 2 in all our patients (median 0.5, IQR 0.2–0.8). However, baseline MAP values were 65 (SD 6) mmHg in the study by Taccone *et al.* and thus tended to be lower than in our study, while the lower limit of autoregulation was not accounted for. If MAP was below the lower limit of autoregulation prior to the MAP increase, this would result in a falsely high CAI. Indeed, the proportion of patients with CAI > 2 increased with $PaCO_2$, which, consistent with the known effects of hypercapnia on the cerebrovasculature, may be explained by a right-shift of the lower limit of autoregulation.[228,413] However, increased $PaCO_2$ levels are unlikely to cause the right-shift of the lower limit of autoregulation *per se*, since another study found that the lower limit of autoregulation was right-shifted during stage V in rats that were kept normocapnic.[415]

In contrast to static cerebral autoregulation, *dynamic cerebral autoregulation* specifically refers to the acute cerebrovascular changes

that occur within seconds from a change in CPP, including the cerebrovascular responses to oscillations in CPP at different frequencies.[104,400,604,613] Several conceptually different methods for assessing dynamic cerebral autoregulation exist, and the methods of relevance to this thesis are described in *Appendix 2*.

By using transfer function analysis, we found that dynamic cerebral autoregulation to spontaneous oscillations in MAP (as a proxy for CPP) was enhanced during stage III in poikilocapnic human volunteers.[53] Specifically, we found that transfer gain was reduced while phase was increased (*Figure 5.3*), which implies that the cerebrovasculature both buffers the effect of a change in MAP more effectively and responds faster to such a change.[604] When we assessed dynamic cerebral autoregulation to an acute MAP reduction triggered by thigh cuff deflation during stage III under poikilocapnic conditions,[VI] we similarly found that the cerebrovasculature responded faster than at baseline (*Figure 5.4*).

In another study based on transfer function analysis, we diminished the reduction in $PaCO_2$ during stage III by adjusting end-tidal PCO_2, so that none of the volunteers became overtly hypocapnic.[52] Gain was consequently unaffected, while an increase in phase remained, although this was less pronounced than during poikilocapnia (*Figure 5.3*). We also examined whether dynamic autoregulatory function becomes more sensitive to changes in PaO_2 in this context by exposing volunteers to an F_IO_2 of 12 % and 40 % for 20 minutes each. These interventions caused no changes in transfer function analysis-based indices of dynamic cerebral autoregulation, neither at baseline nor during stage III.[52]

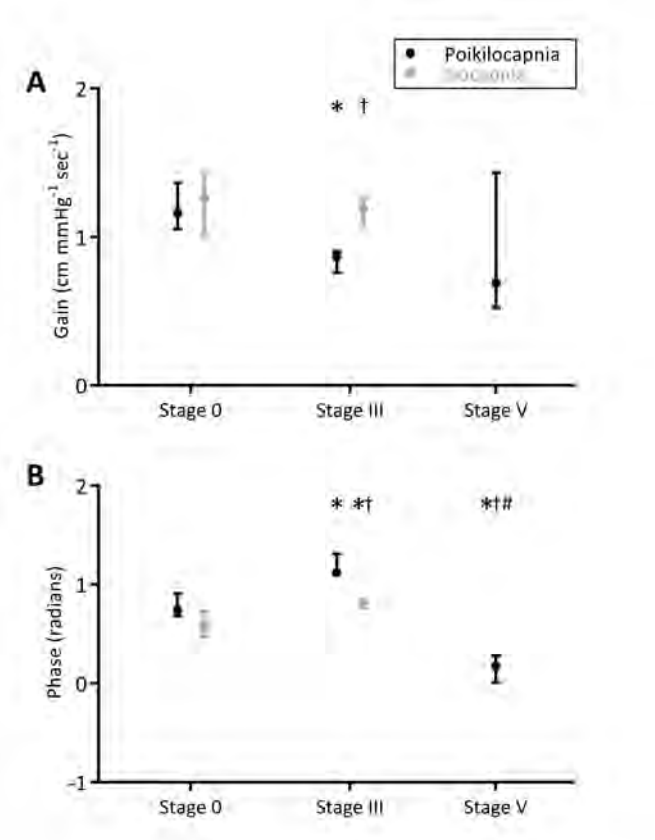


Figure 5.3. Dynamic cerebral autoregulation to spontaneous blood pressure oscillations during stage III and V.

Previously published data from *Studies C–E*.[52,53] Dynamic cerebral autoregulation was assessed by transfer function analysis, and only gain and phase values in the 0.07–0.20 Hz range where coherence ≥ 0.40 are shown. Data for stage V (n = 14) are presented as median (IQR). A: Gain; B: Phase. *Significantly different from stage 0; †significantly different from stage III during poikilocapnia; #significantly different from stage III during isocapnia.

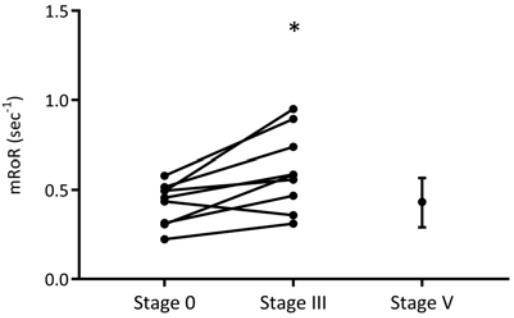


Figure 5.4. Dynamic cerebral autoregulation to a thigh-cuff deflation-induced reduction in blood pressure during stage III and V.
Data from *Paper VI*. Data for stage V (n = 6) are presented as median (IQR). mRoR: modified rate of regulation. *Significantly different from stage 0.

Since previous findings suggest that a steady state increase in MAP achieved by intravenous administration of the selective α-adrenergic agonist phenylephrine improves dynamic cerebral autoregulation,[603] we examined whether this is also the case in response to noradrenaline during stage III.[V] We found that while a noradrenaline-induced steady state MAP increase reduced gain at baseline, it did not cause any further changes in dynamic cerebral autoregulation during stage III under poikilocapnic conditions (*Figure 5.5*).

Two other studies have used transfer function analysis to examine dynamic cerebral autoregulation during stage III in humans, but in contrast to our studies they injected LPS as a bolus (2.0 ng kg^{-1}).[84,349] In accordance with our findings, one of these studies also found a reduction in gain and increase in phase during poikilocapnia, and furthermore reported that these changes correlated with the concomitant reduction in $PaCO_2$.[84] The other study assessed dynamic cerebral autoregulation at baseline and 2, 4, 6, and 8 hours after LPS, presumably under poikilocapnic conditions, and analysed trends over

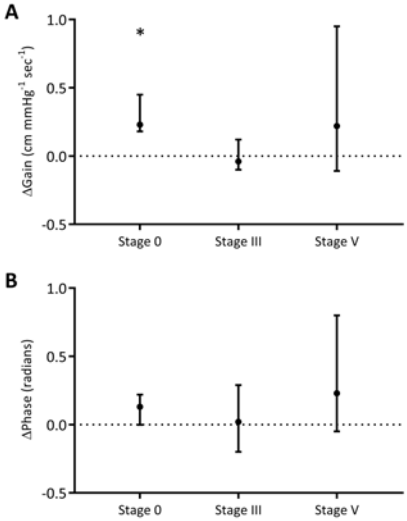


Figure 5.5. The dynamic cerebral autoregulatory adaptive response to a noradrenaline-induced blood pressure increase during stage III and V.
Data from *Studies B* and *D* which have in part been published in *Paper III*. Dynamic cerebral autoregulation was assessed by transfer function analysis during saline and noradrenaline infusion during stage 0 and III (n = 7 for both), and during an increase in the noradrenaline infusion rate during stage V (n = 6). Only gain and phase values in the 0.07–0.20 Hz range where the corresponding coherence ≥ 0.40 are shown. Data are presented as the median (95 % CI) change induced by the noradrenaline-induced increase in blood pressure. A: Change in gain; B: Change in phase. *Significant effect of a noradrenaline-induced increase in blood pressure.

time by a mixed model analysis.[349] No changes in either gain or phase were reported; however, given that only the 2 hour time point can be argued to reflect stage III, while the highly dynamic acute systemic inflammatory response to the LPS bolus had expectedly ceased at the remaining time points (see *Chapter 2*), the study design may render it difficult to detect any relevant changes between baseline and stage III.

When evaluating dynamic cerebral autoregulation by transfer function analysis in patients during stage V, we found that gain was similar while phase was lower than in healthy volunteers, and the latter furthermore correlated inversely with $PaCO_2$.[53] These findings im-

ply that the dynamic cerebral autoregulatory responses to a spontaneous change in MAP is slower than in healthy conditions, even though the magnitude of response as such is similar. In contrast to these findings, we found similar values to healthy volunteers when assessing dynamic cerebral autoregulation to an acute MAP reduction induced by thigh cuff deflation[VI] (*Figure 5.4*). The latter results were probably biased by greater intravascular volume depletion in patients, which resulted in smaller and slower MAP changes in response to thigh cuff deflation, so that the cerebrovasculature was not challenged to the same extent as in healthy volunteers.

No other studies have used transfer function analysis or thigh cuff deflation to investigate dynamic cerebral autoregulation during stage V, but five studies have used the so-called moving correlation coefficient, Mxa. These studies largely agree with our findings in that they all report a slowing of dynamic cerebral autoregulatory responses to spontaneous MAP oscillations,[418,480,481,513] and in one of this studies this was particularly noted in patients with high $PaCO_2$ levels.[513]

Since the slowing of the dynamic cerebral autoregulatory responses during stage V may be related to $PaCO_2$, we investigated whether a reduction in $PaCO_2$ by 30 minutes of mechanical hyperventilation would improve the response time of the cerebrovasculature to spontaneous oscillations in MAP.[V] However, the correlation between $PaCO_2$ and phase ceased during hyperventilation, and no improvement in either gain or phase was observed; if anything, a slight weakening in dynamic cerebral autoregulation occurred, as indicated by slightly higher gain values during hyperventilation.[V]

While the impact of acute changes in PaO_2 on dynamic cerebral autoregulation has not yet been addressed during stage V, we have examined the effect of a vasopressor-induced steady state increase in MAP.[III] Hence, the noradrenaline-induced MAP increase of approximately 20 mmHg in *Study C* was maintained for 20 minutes in seven of the patients, so that dynamic cerebral autoregulation could be

assessed by transfer function analysis. In accordance with the findings during stage III, we found no changes in either gain or phase in this subgroup (*Figure 5.5*).

In summary, the existing studies indicate that although static cerebral autoregulation is largely unaffected both during stage III and stage V, notwithstanding that the lower limit of autoregulation may be right-shifted to higher MAP-values during the latter, notable changes in the dynamic properties of the cerebrovasculature occur. Hence, the cerebrovasculature responds both faster and more effectively to acute MAP changes during stage III, which appears to be closely related to the associated hyperventilatory response. This enhancement of dynamic cerebral autoregulation is resistant to short-term changes in PaO_2 and is unaffected by a noradrenaline-induced steady state increase in MAP. During stage V, the dynamic autoregulatory responses become slower, a change that may be facilitated by higher $PaCO_2$ levels. Despite this, mechanical hyperventilation does not improve the cerebrovascular response time. Furthermore, there is no effect of a noradrenaline-induced steady state increase in MAP on dynamic cerebral autoregulation during stage V.

Chapter 6. Vasoactive peptides

Several circulating vasoactive peptides may profoundly affect cerebral haemodynamic function. The present thesis focuses on the vasoconstrictor endothelin-1 (ET-1)[542] and the vasodilator calcitonin-gene related peptide (CGRP),[81] two of the most potent vasoactive peptides in the cerebrovasculature (*Figure 6.1*).

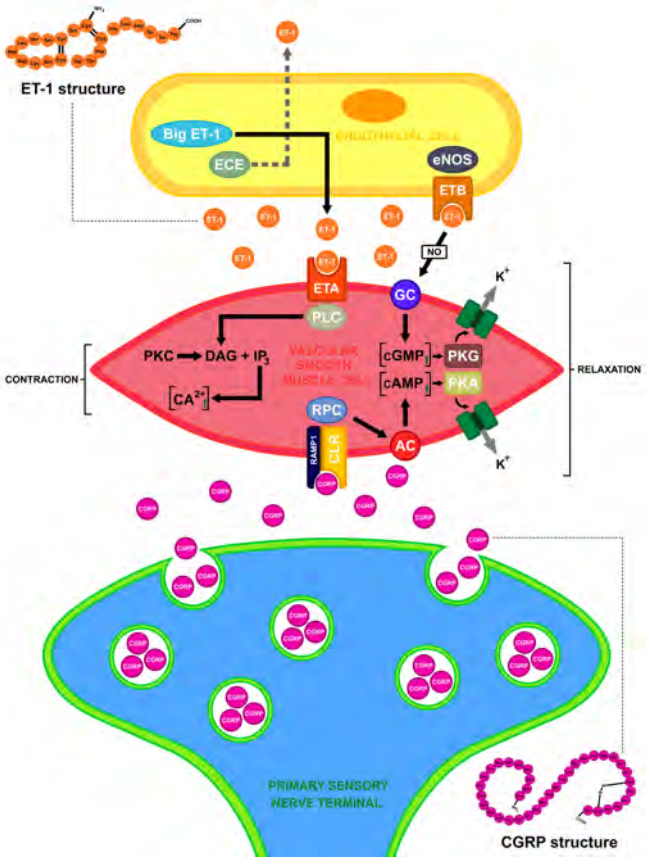
In an earlier study, we found that a cerebrovascular release of ET-1 is present in healthy volunteers at baseline, and this appeared to be abolished during stage III induced by an intravenous bolus injection of LPS (*Figure 6.2*). Due to the highly dynamic systemic inflammatory response following an LPS bolus injection, we repeated the study using a continuous LPS infusion to induce a more protracted systemic inflammatory response.[VII] Once again, we found a cerebral release of ET-1 at baseline, but in this study, we found that the cerebrovascular release was increased during stage III (*Figure 6.2*). The net cerebral release of ET-1 probably comprises a small fraction of the total production in the cerebrovasculature, because ET-1 is mainly released abluminally,[569,576] and the increased net cerebral release during stage III is thus probably associated with a substantial increase in the perivascular release within the brain. Although we found that the plasma levels of ET-1 concurrently increased,[VII] which is in accordance with findings from stage III in several previous human and animal studies,[507,576,593] the contribution of the cerebrovasculature to the increase in circulating ET-1 levels in our study is probably miniscule.[j]

[j]By assuming steady state conditions with a constant and continuous production of ET-1, the whole-body release of ET-1 ($J_{WB,\mathrm{ET-1}}$) to the plasma compartment per minute may be calculated on the basis of a previously published human plasma clearance value ($C_{\mathrm{ET-1}} = 0.066$ L kg^{-1} min^{-1})[409]:

$$J_{WB,ET1} = c_{ET1} \cdot C_{ET1} \cdot M_{body}$$

where $c_{\mathrm{ET-1}}$ is the plasma concentration of ET-1 and M_{body} is body weight. By assuming a brain weight of 1500 g (M_{brain}), the total release of ET-1 from the cerebrovasculature to plasma per minute ($J_{brain,\mathrm{ET-1}}$) may likewise be calculated:

$$J_{brain,\mathrm{ET-1}} = J_{\mathrm{ET-1}} \cdot M_{brain}$$



Where J_{ET-1} is the transcerebral net exchange of ET-1 (as provided in Figure 7.2). In *Study B*, $J_{brain,ET1}$ comprises 0.5 % (mean, SD 0.3 %) of $J_{WB,ET1}$ during stage 0 and increases to 0.8 % (mean, SD 0.4 %) ($p < 0.05$) during stage III.

Figure 6.1. Endothelin-1 (ET-1) and calcitonin-gene related peptide (CGRP).
ET-1 is a peptide composed from 21 amino acids, which is produced in the endothelium throughout the cardiovascular system, including the macro-, -meso-, and microvascular levels of the cerebrovasculature.[219,369,370] It is formed in endothelial cells from pre-proET-1, a large precursor peptide of approximately 200 amino acid residues, which is cleaved to Big ET-1, an inactive peptide of 41 amino acid residues, by a furin-like neutral endopeptidase.[219,542] Big ET-1 is then converted to the biologically active ET-1 by the endothelin-converting enzyme (ECE).[219,542]
ET-1 is continuously released from the endothelial cell, mainly to the abluminal side, from where it locally affects vascular tone,[219,383,569,576] while ET-1 released at the luminal side of the endothelium is considered a spill-over.[383,569,576] In the cerebrovasculature, ET-1 binds to endothelin receptor A (ET_A) on vascular smooth muscle cells (and pericytes),[131,425,510,599] which triggers constriction through (PLC) phospholipase C-dependent pathways.[6,250,509,535,542] ET-1-induced vasoconstriction has been documented both in large cerebral arteries, the mesovascular arterial system on the brain surface, in intracortical arterioles and in cerebral capillaries.[5,150,162,188,254,404,473] ET-1 binds with an equal affinity to ET_B receptors on the abluminal side of the endothelium, which modulates ET-1-induced vasoconstriction through pathways that involve nitric oxide (NO)-signaling.[219,425,583] ET-1 is cleared from the circulation within minutes,[409,501] mainly in the lungs,[143,144,576] but its vasoconstrictive effects may last for up to an hour.[501,566]

Calcitonin-gene related peptide (CGRP) is composed from 37 amino acids, and is generated through alternative splicing of RNA transcripts of the calcitonin gene.[81,265,467] CGRP is present in both the central and peripheral nervous system.[81,265,467] In the peripheral nervous system, it is mainly located in perivascular sensory nerves, including those from the trigeminal ganglion, which innervate the macrovascular cerebral arteries and the mesovascular arterial network at the cortical surface. CGRP causes local vasodilation by binding to a complex consisting of the seven transmembrane calcitonin receptor-like receptor (CLR), the single transmembrane 'receptor activity-modifying protein 1' (RAMP1), and receptor component protein (RCP) on vascular smooth muscle.[467] This complex then activates adenylate cyclase (AC), which increases intracellular cyclic adenosine monophosphate (cAMP), and subsequently activates protein kinase A (PKA). In cerebral vessels, this causes vascular smooth muscle cell relaxation and thus vasodilation, primarily by the opening of large conductance Ca^{2+}-activated K^+-channels.[81,265,467] The resultant vasodilation has been documented in cerebral arterial vessels, and is more prominent in smaller than in larger vessels.[265] On the vascular level, this vasodilatory effect has furthermore been found to overrule the vasoconstrictive effects of ET-1.[350,351] In the circulation, CGRP has a half-life of about 10 minutes,[81] while its vasodilatory effects may last for several hours.[82] cGMP: cyclic guanosine monophosphate; DAG: diacylglycerol; eNOS: endothelial nitric oxide synthase; ET_B: endothelin receptor B; GC: guanylate cyclase; IP_3: inositol trisphosphate; PKC: protein kinase C; PKG: protein kinase G

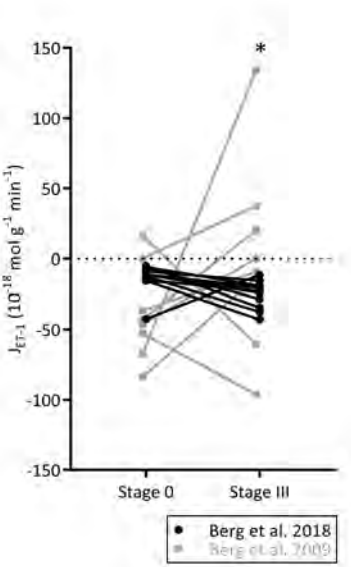


Figure 6.2. Transcerebral net exchange of endothelin 1 (J_{ET-1}) during stage III.
Data are presented from a previous study (grey data points),[54] and from *Paper VII* (black data points). By convention, a positive value indicates a net cerebral uptake (net cerebral influx), while a negative value indicates a net cerebral release (net cerebral efflux) of ET-1. *Significantly different from stage 0 in Berg *et al.* 2017.[VII]

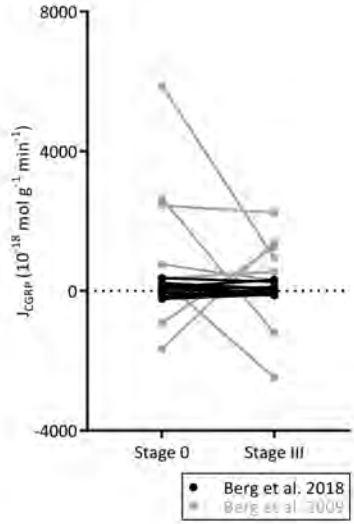


Figure 6.3. Transcerebral net exchange of calcitonin gene-related peptide (J_{CGRP}) during stage III.
Data are presented from a previous study (grey data points),[54] and from *Paper VII*. By convention, a positive value indicates a net cerebral uptake (net cerebral influx), while a negative value indicates a net cerebral release (net cerebral efflux) of CGRP.

Although a number of studies have reported that the circulating levels of ET-1 in blood are elevated in septic patients and correlate with severity of disease,[87,422,427,552,577] the transcerebral net exchange of ET-1 has not yet been examined during stage V.

In terms of CGRP, we have found no transcerebral exchange, either at baseline or during stage III, and the circulating levels in blood were also unaffected, regardless of whether stage III was triggered by a bolus injection or a continuous infusion of LPS (*Figure 6.3*). The transcerebral net exchange of CGRP has not been examined during stage V, but elevated plasma CGRP levels have been reported in several studies of critically ill septic patients, and similarly to ET-1, these correlate with severity of disease.[21,22,43,260]

In summary, the cerebrovascular release of the cerebral vasoconstrictor ET-1 is enhanced during stage III, which likely reflects an increased cerebrovascular production and abluminal release of ET-1. No concurrent changes in the transcerebral net exchange of the cerebral vasodilator CGRP are observed. Although both the circulating levels of ET-1 and CGRP in blood are elevated during stage V, it is currently unknown whether their cerebrovascular net exchange is affected at this stage.

Chapter 7. Blood-brain barrier function

The BBB plays a major role for the regulation of cerebral haemodynamics as it functions to ensure a stable microenvironment within the brain by controlling the passage of substances between the blood stream and brain extracellular compartment.[80,411,503,579] In the present thesis, the focus is the BBB-dependent catecholaminergic homeostasis within the brain, and in this context two classical hypotheses relating to cerebral haemodynamic function in sepsis are considered: the *central monoamine hypothesis* and the *false neurotransmitter hypothesis*.

The *central monoamine hypothesis* refers to the cerebral haemodynamic changes that occur during stage III and has mainly been investigated in dogs. According to this hypothesis, the inverse changes in CBF and $CMRO_2$ are caused by the passage of catecholamines, principally noradrenaline from the blood stream across a disrupted BBB into the brain extracellular space.[153,154] In contrast to normophysiological conditions, where the BBB is largely impermeable to catecholamines,[323,384,385] circulating catecholamines are thus thought to increase in $CMRO_2$ relative to CBF by activating β-adrenergic pathways within the brain.[578,580,581] In our human-experimental studies of stage III, we nevertheless did not find any support for this. Even though inverse changes in CBF and $CMRO_2$ were evident, the transcerebral net exchange of catecholamines was unaffected during stage III (*Figure 7.1*). Furthermore, an increase in the circulating catecholamines, which have been proposed to facilitate the cerebral uptake of catecholamines by generating a blood-to-brain concentration gradient,[154,580,581] is not consistently present at this stage in our studies. According to the central monoamnine hypothesis, noradrenaline infusion would expectedly increase the a-jvDO_2 during stage III; based on data from stage III in *Study D*, there is no evidence of this, and there is furthermore no other evidence of noradrenaline-induced

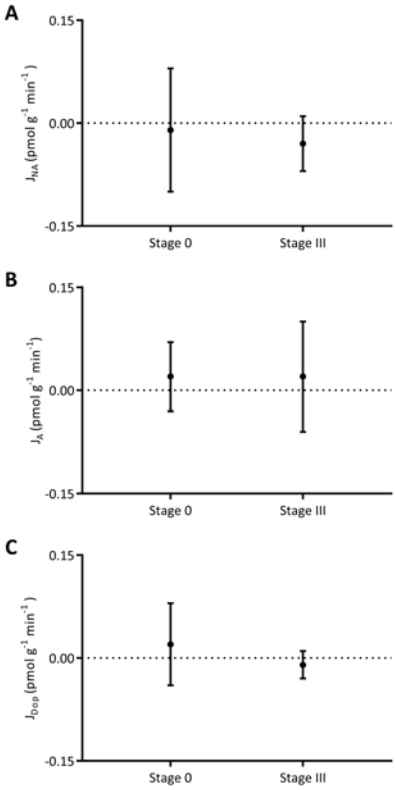


Figure 7.1. Transcerebral net exchange of catecholamines during stage III.
Data from *Paper VII*. A: Transcerebral net exchange of adrenaline (J_A); B: Transcerebral net exchange of noradrenaline (J_{NA}); C: Transcerebral net exchange of dopamine (J_{Dop}). By convention, a positive value indicates a net cerebral uptake (net cerebral influx), while a negative value indicates a net cerebral release (net cerebral efflux) of the given catecholamine.

	Saline	Noradrenaline
a-jvDO_2 (mM)	3.18 (0.46)	3.26 (0.50)
a-jvD_{glc} (mM)	0.44 (0.13)	0.48 (0.22)
a-jvD_{lac} (mM)	-0.10 (0.11)	-0.08 (0.07)
OGI (fraction)	7.73 (1.98)	7.25 (3.76)
LGI (fraction)	0.23 (0.25)	0.18 (0.19)
LOI (fraction)	0.03 (0.03)	0.02 (0.02)

Table 7.1. Effect of noradrenaline infusion on the arterial-to-jugular venous oxygen difference (a-jvDO_2) and indices of cerebral intermediary metabolism during stage III.
Previously unpublished data from *Study E*. Data are presented as mean (SD). a-jvD_{glc}: arterial-to-jugular venous glucose difference; a-jvD_{lac}: arterial-to-jugular venous lactate difference; LGI: cerebral lactate-glucose index; LOI: cerebral lactate-oxygen index; OGI: cerebral oxygen-glucose index.

changes in cerebral intermediary metabolism (*Table 7.1*). A study in pigs likewise found no effects of noradrenaline infusion on $CMRO_2$ during stage III.[353]

The *false neurotransmitter hypothesis* relates to the transport of large neutral amino acids (LNAAs; *Table 7.2*) across the BBB, which is important for neurotransmitter homeostasis within the central nervous system.[407,503] The LNAA transport system is predominantly saturable and thus competitive, so that a change in the plasma concentration of one LNAA affects the transport and intracerebral concentrations of all LNAAs.[117] According to the false neurotransmitter hypothesis, an increase in aromatic amino acids (AAAs) relative to branched-chain amino acids (BCAAs) will thus lead to the intracerebral accumulation of AAAs.[173–175] This gives rise to AAA degradation products which function as 'false neurotransmitters' which disrupt central noradrenergic neurotransmission by displacing noradrenaline from presynaptic terminals (*Figure 7.2*). This has been proposed to occur in sepsis, where increased skeletal muscle protein breakdown releases both BCAAs and AAAs to the bloodstream. A relative increase in AAAs nevertheless occurs, since BCAAs are cleared from the circulation to a greater extent than AAAs for the purpose of

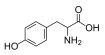
Name	Structure	Group
Phenylanine		Aromatic amino acid (neutral non-polar aromatic side chain)
Tyrosine		Aromatic amino acid (neutral polar aromatic side chain)
Tryptophan		Aromatic amino acid (neutral polar aromatic side chain)
Valine		Branched-chained amino acid (neutral non-polar branched aliphatic side chain)
Leucine		Branched-chained amino acid (neutral non-polar branched aliphatic side chain)
Isoleucine		Branched-chained amino acid (neutral non-polar branched aliphatic side chain)
Histidine		Basic amino acid (basic, aromatic side chain)
Methionine		Sulfur-containing amino acid (neutral non-polar sulfur-containing side chain)

Table 7.2. Large neutral amino acids (LNAAs).
LNAAs are characterised by large, neutral side chains and by facilitated diffusion across the blood-brain barrier by the so-called L-system.

LNAAs that are classified as *aromatic amino acids* have an aromatic ring in their side chain; apart from phenylalanine, tyrosine, and tryptophan, methionine also has an aromatic ring, but it is usually classified as a so-called sulphur-containing amino acid along with the small neutral amino acid cysteine.

LNAAs classified as *branched-chain amino acids* have an aliphatic side-chain with a branch (a central carbon atom bound to three or more carbon atoms); due to the hydrophobicity of this side chain, the individual amino acids tend to 'stick together' in large clusters *in vivo*.

Histidine is typically classified as an LNAA, because it may be neutral *in vivo*, even though it is positive at neutral pH; furthermore, its main mode of transport is through the L-system like the other LNAAs. [222,376]

Technically, glutamine is also an LNAA, but is not included in most models of the LNAA transport across the BBB, as it is mainly transported across the BBB by active transport through the so-called N-system.[225,377]

Threonine, which is a small neutral amino acid is sometimes also classified as an LNAAs because it is also transported by the L-system[222]; it is, however not included here, because its main mode of transport is active transport by the so-called ASC-system along with the other small neutral amino acids glycine, alanine, serine, and cysteine.[548]

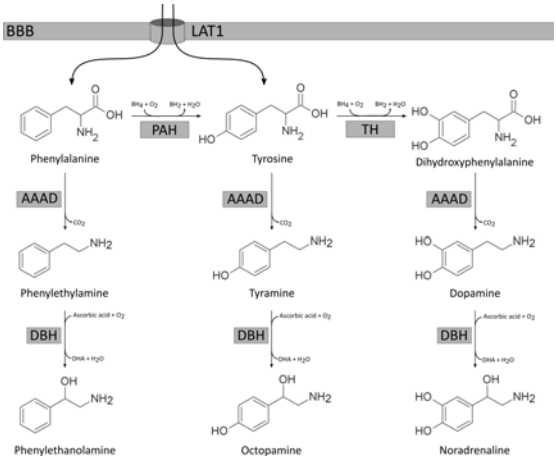


Figure 7.2. False neurotransmitters.
Within the central nervous system, catecholamine are synthesised from the aromatic amino acids phenylalanine and tyrosine through a number of decarboxylation and hydroxylation reactions.[414,528]

If the capacity of phenylalanine hydroxylase (PAH) is overwhelmed by excessive phenylalanine levels, phenylalanine is instead decarboxylated to phenylethylamine, which is converted to phenylethanolamine by the dopamine β-hydroxylase (DBH). If tyrosine levels increase beyond the capacity of the tyrosine hydroxylase (TH), tyrosine is decarboxylated to tyramine, which is a precursor of octopamine. Like catecholamines, phenylethanolamine and octopamine have a phenolic ring and a β-hydroxyl group on a short carboxyl side chain.

Noradrenergic presynaptic terminals are unable to distinguish phenylethanolamine and octopamine from catecholamines, and they are therefore designated 'false neurotransmitters'. They consequently undergo presynaptic reuptake in catecholaminergic nerve terminals, which leads to the displacement and consequent depletion of noradrenaline.[174,414] BBB: blood-brain barrier; LAT1: large neutral amino acid transporter 1; AAAD: aromatic L-amino acid decarboxylase; BH_2/BH_4: di- and tetrahydrobiopterin; DHA: dehydroascorbic acid. Reproduced from *Paper VIII* with permission.

hepatic acute phase reactant synthesis.[37,142,181,182,187,564] Although the putative cerebral haemodynamic consequences of the AAA-derived false neurotransmitters have never been explicated in detail, the involved central noradrenergic pathways principally originate from the locus coeruleus, and encompass extensive ramifications throughout the cerebrovasculature which function to optimise neurovascular coupling.[46]

	Stage 0		Stage III	
	Unidirectional influx (nmol min^{-1} g^{-1})	Unidirectional efflux (nmol min^{-1} g^{-1})	Unidirectional influx (nmol min^{-1} g^{-1})	Unidirectional efflux (nmol min^{-1} g^{-1})
Phenylalanine	10.2 (9.2 – 10.6)	10.5 (6.3 – 12.3)	12.2 (11.3 – 12.7)*	12.4 (10.9 – 14.1)
Tyrosine	4.6 (3.3 – 5.1)	3.1 ([-1.6] – 6.6)	4.5 (4.1 – 4.7)	6.4 (3.3 – 9.7)
Tryptophan	6.6 (6.1 – 7.4)	7.8 (4.9 – 9.1)	5.8 (5.3 – 6.8)	6.5 (5.2 – 7.7)
Valine	3.6 (3.5 – 3.7)	0.6 ([-6.6] – 5.0)	3.6 (3.4 – 3.8)	2.7 (1.5 – 7.1)
Leucine	15.7 (14.7 – 17.0)	13.1 (7.8 – 14.0)	15.3 (15.0 – 17.1)	12.3 (10.3 – 18.0)
Isoleucine	4.3 (3.9 – 4.6)	2.8 (0.0 – 3.7)	4.6 (4.3 – 5.2)	3.3 (2.3 – 6.2)
Histidine	3.2 (3.0 – 3.5)	4.4 (0.5 – 5.0)	2.6 (2.4 – 3.1)*	2.4 (1.8 – 7.5)
Methionine	0.6 (0.6 – 0.7)	0.1 ([-0.3] – 0.8)	0.4 (0.4 – 0.5)*	0.7 (0.5 –1.0)

Table 7.3. Blood-brain barrier transport of large neutral amino acids during stage III.
Data from *Paper VIII* (n = 12). Results are presented as median (IQR). AAA: aromatic amino acid; BCAA: branched-chain amino acid. *Significantly different from stage 0.

The generation of false neurotransmitters could thus potentially contribute to the impairment of neurovascular coupling, which has been reported in animal studies of stage III and in patient-based studies of stage V.[463,464]

We found that circulating phenylalanine levels increased, while all other LNAAs except for isoleucine, decreased during stage III.[II] This was associated with an increased cerebral delivery of phenylalanine. We estimated the BBB permeability (permeability-surface area product, PS_1) to phenylalanine from blood to brain by using average values of the maximal transport velocity (V_{max}) and the apparent Michaelis-Menten constant (K_m) of phenylamine transport across the BBB from a previous study on healthy humans.[280] While this approach did not account for the effect of competing LNAAs on the apparent K_m, our findings suggested that the combination of an increased concentration in arterial blood and an increased PS_1 led to an increased unidirectional cerebral influx of phenylalanine.[II] From our data, we could, however, not determine if this was associated with an increased intracerebral concentration of phenylalanine and/or a concurrently increased BBB permeability to phenylalanine from brain to blood (PS_2).

We later developed a mathematical model that permits the estimation of both in- and efflux of all the individual LNAAs across the BBB, as well as changes in their brain extracellular concentrations.[117] Based on this model, we critically reassessed the BBB transport of all LNAAs (*Table 7.3*). Our findings showed that while the cerebral influx of phenylalanine increased, there was only a trend towards a modest increase in its brain extracellular fluid concentration (*Figure 7.3* However, a parallel increase in the brain extracellular concentration of the other AAA tyrosine was observed (*Figure 7.3*), and since no changes in its BBB transport occurred, this was interpreted to be derived from phenylalanine.[VIII] These findings agree with a study on rats during stage IV progressing into stage V, where the cerebral influx of phenylalanine was likewise found to be increased.[256] As in our study, this was most likely converted to tyrosine, as an increase in the brain content of tyrosine concurrently took place, even though the tyrosine levels in blood were reduced.

The increase in brain extracellular fluid tyrosine observed in our study was not of a magnitude to overwhelm the capacity of the tyrosine hydroxylase, which has a K_m of 140 μM,[109] and was thus unlikely to give rise to the tyrosine-derived false neurotransmitter octopamine. We therefore interpreted the tyrosine increase to reflect a means of 'detoxification' of excess phenylalanine within the brain to prevent the formation of phenylethanolamine.[VIII] Given that the phenylalanine hydroxylase has a K_m of about 40 μM,[311,455] this pathway is expectedly insufficient during extensive increases in the brain extracellular concentrations of phenylalanine, presumably present in some septic patients with reported cerebrospinal fluid concentrations of more than 300 μM during stage V.[355,534]

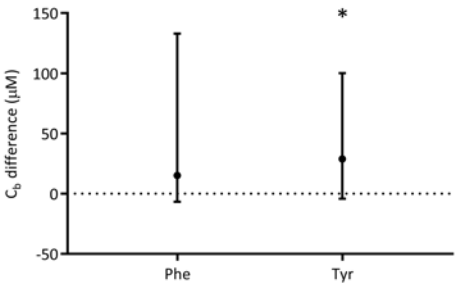


Figure 7.3. Changes in brain extracellular fluid concentrations of phenylalanine and tyrosine during stage III.
Data from *Paper VIII* (n = 12). Results are presented as median (IQR) difference in estimated brain extracellular concentrations (C_b). Phe: phenylalanine; Tyr: tyrosine. *Significant increase from stage 0 to III.

In summary, the inverse changes in CBF and $CMRO_2$ during stage III do not appear to depend on an increased cerebral influx of catecholamines across the BBB. In terms of the saturable carrier-mediated LNAA transport across the BBB, an increased cerebral influx of phenylalanine is prevented from causing intracerebral phenylethanolamine accumulation during stage III, as phenylalanine is 'detoxified' to tyrosine. When the septic condition progresses into stage V, the intracerebral levels of phenylalanine may, at least in some cases, increase to such an extent that this potentially neuroprotective pathway is overwhelmed, so that the false neurotransmitter phenylethanolamine is formed.

Chapter 8. Autonomic regulation of cardiovascular function

The focus of the present chapter is the acute regulation of MAP by the autonomic nervous system, including arterial baroreflex function. As this involves the occurrence of spontaneous blood pressure oscillations through periodic variations in heart rate and systemic vascular resistance, the mechanisms of such spontaneous oscillations and their relation to the autonomic regulation of the cardiovascular system are briefly reviewed in *Appendix 3*.

We examined the arterial baroreflex during stage III in humans by assessing the changes in heart rate to an acute reduction in MAP induced by thigh cuff deflation.[VI] The MAP reduction was similar at baseline and during stage III, but the associated cardio-acceleratory response was reduced (*Table 8.1*). In contrast, MAP was increased to the same extent by noradrenaline at baseline and during stage III, and the associated reduction in heart rate was likewise similar (*Table 8.1*), which suggests that the sensitivity of the arterial baroreflex arm is specifically impaired in relation to reductions in MAP. Another human-experimental study by Sayk *et al.* also found that arterial baroreflex function is modulated during stage III.[478] This study showed that the arterial baroreflex-mediated increase in heart rate, as well as the skeletal muscle sympathetic nervous response (recorded by microneurography in the peroneal nerve), to a MAP reduction induced by nitroprusside were both reduced during stage III. In accordance with our findings, the corresponding changes in response to a phenylephrine-induced MAP increase were concurrently unaffected.[478]

We interpreted the change in baroreflex function to specifically involve the afferent baroreflex arm,[VI] and thus to originate somewhere between the arterial baroreceptors (in the carotid sinus and aortic arch) and the nucleus of the solitary tract in the medulla oblongata. However, a recent study by Brassard *et al.* showed that the vascular response

	Stage 0	Stage III	Stage V
Thigh-cuff deflation			
ΔMAP (mmHg)	15 (12 – 18)	15 (12 – 17)	9 (8 – 11)*†
Time to MAP nadir (seconds)	4.7 (4.0 – 5.0)	5.3 (3.3 – 6.3)	10.3 (9.3 – 12.4)*†
ΔHR/ΔMAP (beats min^{-1} $mmHg^{-1}$)	1.5 (1.2 – 1.7)	0.8 (0.6 – 1.0)*	0.6 (0.4 – 0.9)*
NA infusion			
NA infusion rate increase (μg kg^{-1} min^{-1})	0.19 (0.15 – 0.26)	0.17 (0.14 – 0.24)	0.73 (0.60 – 0.78)
ΔMAP (mmHg)	19 (16 – 23)	27 (24 – 32)*	17 (15 – 20)†
ΔMAP per NA infusion rate increase (mmHg per 0.1 μg kg^{-1} min^{-1})	11 (9 – 14)	19 (9 – 24)	3 (2 – 3)*†
ΔHR/ΔMAP (beats min^{-1} $mmHg^{-1}$)	0.24 ([-0.02] – 0.77)	0.42 (0.22 – 0.88)	0.07 ([-0.18] – 0.25

Table 8.1. Cardiovascular responses to induced blood pressure changes during stage III and V.
Data from *Studies B* and *D*, that have in part previously been published.[VI,53] Based on thigh-cuff deflation in 9 healthy volunteers and 6 patients, and noradrenaline infusion in 9 healthy volunteers and 11 patients (the latter was completed in 14 patients, but data from three of these were not included in this analysis because of abundant supraventricular premature beats). HR: heart rate; MAP: mean arterial blood pressure. *Significantly different from stage 0; †significantly different from stage III.

to tyramine, which triggers the release of noradrenaline from perivascular sympathetic nerve terminals, was reduced in the femoral circulation during stage III,[85] thus indicating that changes in the efferent baroreflex arm are also present.[85] As our findings show that the cardiovascular response and thus the postsynaptic sensitivity to infused exogenous noradrenaline is concurrently maintained (*Table 8.1*), the findings by Brassard *et al.* may be interpreted to reflect noradrenaline depletion from perivascular sympathetic nerves during stage III.

We did not supplement our arterial baroreflex assessments during stage III with direct measures of sympathetic output to the cardiovascular system, but Sayk *et al.* found a reduction in both heart rate variability and skeletal muscle sympathetic nervous activity in the peroneal nerve during stage III.[478] Several animal studies have also reported that sympathetic output to the circulation is reduced from stage I and onwards through stage III with a concomitant reduction in arterial baroreflex sensitivity.[69,197,200,286,397,562]

	Stage V		Normal range§	
	Frequency (Hz)	Spectral power ($mmHg^2$)	Frequency (Hz)	Spectral power ($mmHg^2$)
High-frequency oscillations	0.31 (0.25 – 0.36)	0.69 (0.28 – 2.11)	0.20 – 0.40	0.1 – 1.0
Middle-frequency oscillations	0.02 (0.01 – 0.03)	0.49 (0.21 – 1.24)	0.07 – 0.20	2 – 6
Low-frequency oscillations	0.009 (0.006 – 0.010)	0.93 (0.48 – 1.45)	0.02 – 0.07	20 – 40

Table 8.2. Spontaneous blood pressure oscillations during stage V.
Data from *Paper IV* (n = 65). The data are presented as median (IQR). The reported frequencies and spectral powers are based on patients in which they were present. High-frequency oscillations were present in 55 (85 %) patients, middle-frequency oscillations were present in 59 (91 %) patients, and low-frequency oscillations were present in 51 (78 %) patients. §There are no established normal ranges for the spectral power in the different frequency ranges in the healthy state (stage 0). The reported spectral power values here are the normal ranges based resting continuous blood pressure recordings in the healthy volunteers from *Study C* and *Study E* (n = 19) in the established high-, middle-, and low-frequency ranges, and are consistent with those previously reported by others.[158–160,232,604]

In septic patients during stage V, we found that the cardio-acceleratory response to a thigh cuff deflation-induced reduction in MAP was reduced to a similar extent as observed during stage III[VI] (*Table 9.1*), which is supported by findings from other clinical studies.[20,489] In accordance with previous studies,[18] the systemic vessels remained sensitive to noradrenaline during stage V, but the sensitivity was reduced, as indicated by a smaller MAP increase per increment in the noradrenaline infusion rate (*Table 9.1*). This implies that the sympathetic component of the efferent baroreflex arm also is impaired on the post-synaptic level in peripheral arterial vessels at this stage. In the same group of patients, the spontaneous MAP variability in the so-called middle-frequency range was found to be remarkably low,[53] which is in agreement with other clinical studies showing that the variability of both blood pressure[20,423,594] and heart rate[20,35,99,100,189,285,423,594] is reduced in this frequency range during stage V. The reduction in heart rate and blood pressure variability has been found to be associated with the severity of sepsis,[20,35,99,189] may predict the subsequent development of shock,[20,100,189] and is furthermore reversed upon recovery from shock.[423,594] Given that the varia-

bility of the middle-frequency oscillations in heart rate and blood pressure may, at least to some extent, be interpreted to reflect the sympathetic output to the cardiovascular system (*Appendix 3*), these findings were taken to support the concept that the development of distributive shock in septic patients is facilitated by failure of the autonomic nervous system.[17,492]

Modulations of autonomic cardiovascular regulation may not only affect the spontaneous blood pressure variability at predefined frequency ranges, but also the distinct frequencies at which blood pressure oscillates. We therefore performed spectral analyses of continuous invasive blood pressure recordings during stage V in a cohort of 65 mechanically ventilated septic patients.[IV] This cohort of patients was comparable to the patient group in the abovementioned initial study (see *Table A1.1* in *Appendix 1*). The main finding in this larger cohort was that spontaneous middle- and low-frequency blood pressure oscillations were suppressed to much lower frequencies than in the healthy state, and that they were entirely absent in many patients.[IV] In the patients with maintained low- and middle-frequency oscillations, the contribution of these oscillations to the overall blood pressure variability varied markedly (*Table 8.2*).

Although our findings differ from previous studies, they do not necessarily disagree with the contention that sympathetic output to the circulation is reduced during stage V. Hence, the amplitude of the oscillations that introduce the variability in blood pressure depends not only on sympathetic output, but also on the various homeostatic control mechanisms that buffer the effects of this on blood pressure, which apart from the baroreflex, include endothelial modulation of vascular tone and vasopressin release from the posterior pituitary (*Appendix 3*), mechanisms that have also been found to be impaired during stage V.[26,242,391,489]

	Continuous LPS infusion (0.3 ng kg^{-1})		Continuous LPS infusion (2.0 ng kg^{-1})		Patients
	Stage 0	Stage III	Stage 0	Stage III	Stage V
Noradrenaline (pmol dL^{-1})	66 (51-106)	66 (51-106)	101 (90-232)	75 (50-121)	26 (11-48)
Adrenaline (pmol dL^{-1})	18 (8-23)	41 (31-64)*	25 (16-80)	50 (32-75)	546 (118-1919)

Table 8.3. Circulating catecholamine levels during stage III and V.
Data from *Studies B, C,* and *E* (n = 12, 4, and 9, respectively). The data are presented as median (IQR). Data from *Study B* are reported in *Paper VII*, while data from *Studies C* and *E* have been reported elsewhere.[391] The data from stage V is from the four patients in *Study C* that did not receive intravenous noradrenaline, adrenaline, or dopamine; due to the low number of patients, no statistical comparisons were made between this group and healthy volunteers. *Significantly different from stage 0.

Given that the pacemaker-like activity of the network of autonomic nuclei involved in cardiovascular homeostasis may be pertinent to the frequencies at which spontaneous blood pressure oscillations occur, the suppression of middle- and low-frequency oscillations to lower frequencies may reflect a reduced output from the brainstem to the cardiovascular system through the sympathetic nervous system. Accordingly, we proposed that it represent a physiological correlate of the structural neuronal damage in several areas of the central nervous system involved in the autonomic regulation of cardiovascular function,[IV] as previously reported in patients with septic shock.[491,494] Apart from brainstem nuclei (the nucleus of the solitary tract, the dorsal nucleus of the vagal nerve, and the locus coeruleus), this involves the supraoptic nucleus and paraventricular nucleus in the hypothalamus and the central nucleus of the amygdala in the temporal lobe.[491,494] In healthy conditions, these may continuously exert an excitatory influence on the pacemaker function of the network of autonomic nuclei in the brainstem, notably through their direct projections to the rostral ventrolateral medulla (*Appendix 3*). As the excitatory input to the rostral ventrolateral medulla ceases due to damage to the higher areas, the frequency of the oscillations the network of autonomic nuclei may decrease, thus resulting in the observed suppression of middle- and low-frequency blood pressure oscillations.

In our study of septic patients during stage V, we found that the 30-day mortality rate was more than threefold as high in patients with absent versus preserved low-frequency oscillations.[IV] This may reflect that the most severe cases of sepsis exhibit such extensive structural damage that the pacemaker-like function of the network of autonomic nuclei is attenuated altogether. Our findings thus lend further support to the concept that failure of the autonomic system contributes to the development and progression of shock in septic patients.

Under normophysiological conditions, circulating catecholamines are primarily released from the adrenal glands, the spleen, and lungs, while only a small proportion originates from perivascular sympathetic nerves, and catecholamine concentrations in blood thus provide a poor measure of the sympathetic output to the circulation.[166] According to our findings, this is also the case during systemic inflammation, as the reduced sympathetic output to the circulation has been found to coincide with increased noradrenaline and adrenaline levels both at stage III and V.[20,478] This likely reflects that sympathetic nerve activity to the circulation may decrease while it concurrently increases to the spleen, kidneys, and adrenal glands.[374,441,459] In our studies, a small increase in adrenaline was present during stage III induced by an LPS dose of 0.3 ng kg^{-1} but not 2.0 ng kg^{-1}, and we furthermore found that both noradrenaline and adrenaline levels were elevated during stage V (*Table 9.3*).

In summary, a reduction of the tonic sympathetic output to the circulation takes place from stage III with a concomitant reduction in the baroreflex sensitivity to acute reductions in MAP. These changes progress into stage V and are likely important to the development of shock.

Chapter 9. General discussion

In the work that forms the basis of the present thesis, we used a continuous LPS infusion model to trigger a systemic inflammatory response like that encountered during early sepsis, here designated stage III. Together with other human-experimental and animal studies of stage III, our studies indicate that an increase in cerebrovascular resistance occurs, resulting in a reduction in global CBF relative to $CMRO_2$. This is accompanied by an improvement of dynamic cerebral autoregulatory function, while static cerebral autoregulation is unaffected, and the cerebrovasculature remains capable of responding adequately to changes in $PaCO_2$. Meanwhile, the sympathetic output to the circulation is reduced, and the arterial baroreflex-dependent cardiovascular response to an acute reduction in MAP becomes less effective.

The more advanced stages of disease with manifest organ failure, here designated stage V, were assessed by clinical studies of mechanically ventilated septic patients admitted to the ICU. Along with other studies, our findings indicate that cerebrovascular resistance remains high into stage V, and that both static cerebral autoregulation and $CVCO_2R$ are maintained, although a right-shift of the lower limit of cerebral autoregulation to slightly higher CPPs may be present. In contrast to the early stages of sepsis, the dynamic autoregulatory responses become slower, while the sympathetic output to the circulation remains reduced with an impairment of the arterial baroreflex-dependent cardiovascular response to an acute reduction in MAP.

The increased cerebrovascular resistance during stage III is likely facilitated by the LPS-induced hyperventilatory response, given that the ability of the cerebrovasculature to respond to changes in $PaCO_2$ is preserved. It nonetheless remains to be established whether $CVCO_2R$ is equally effective during stage III when compared to baseline conditions, and it therefore cannot be ruled out that other vaso-

constrictive influences are also involved, particularly when considering that several animal studies have found residual cerebral vasoconstriction during stage III when animals are kept isocapnic. While our findings do not support any involvement of circulating catecholamines as proposed by the 'central monoamine hypothesis,' they do point towards the cerebral vasoconstrictor ET-1 as an additional contender in this context, as the cerebrovascular release of ET-1 is increased during stage III. The increase in core temperature due to the fever response may also cause cerebral vasoconstriction independently of changes in $PaCO_2$.[31,111] The mechanism of this is a matter of debate but may involve increased sympathetic output to the cerebrovasculature.[31,111] Indeed, increased sympathetic output to the cerebrovasculature from the superior cervical ganglion has previously been highlighted as a possible mechanism of cerebral vasoconstriction during stage III.[84,161] This remains a possibility, as sympathetic tone may theoretically be regionally increased in some vascular beds even though overall the sympathetic output to the circulation is reduced, but there is currently no experimental data that directly support this to occur in the cerebrovasculature during stage III.

The increase in cerebrovascular resistance during stage III is unlikely to predispose to cerebral ischaemia *per se*, as it is associated with an enhancement of dynamic cerebral autoregulation, involving both an improved ability of the cerebrovasculature to buffer the effect of a given MAP change on CBF as well as a faster cerebrovascular response time, thus signifying a more 'agile' cerebrovasculature. This appears to be triggered, at least in part, by the associated hyperventilatory response, which is consistent with most previous studies of dynamic cerebral autoregulation during voluntary hyperventilation.[7,140,613] Hence, findings indicate that the response time of the cerebrovasculature was improved to a lesser extent during stage III during isocapnia than during poikilocapnia. The finding that an improvement in the response time nevertheless persisted may re-

flect that the development of fever also contributed, since similar changes are observed following passive heating to comparable core temperatures.[91,141] In any event, the enhancement of dynamic cerebral autoregulation was resistant to any acute changes in PaO_2, and unlike healthy baseline conditions, no further enhancement of dynamic cerebral autoregulation was observed in response to a vasopressor-induced MAP increase, in our case by noradrenaline. This may reflect that the near-maximal capacity for enhancing dynamic cerebral autoregulation is reached during stage III, conceivably due to the combined effects of hyperventilation and fever.

The relative cerebral hypoperfusion during stage III, where CBF is reduced relatively to $CMRO_2$, appears to be converted to a state of relative hyperperfusion during the progression of sepsis, despite a consistently increased cerebrovascular resistance, as $CMRO_2$ is reduced relatively to CBF during stage V. While the lower limit of autoregulation may be shifted to higher CPPs, static cerebral autoregulation is maintained above this limit. Dynamic cerebral autoregulatory assessments also indicate that the cerebrovasculature maintains its ability to respond to a given change in CPP, but with a prolonged response time. Hence, from the 'agile' state observed during stage III, the cerebrovasculature seems to become increasingly indolent as sepsis progresses into more advanced stages. In line with the known cerebrovascular effects of $PaCO_2$ in healthy conditions,[140,149,329,401,604] results from correlation analyses suggest that this may be related to the $PaCO_2$ levels in patients, but our attempt to restore the response time of the cerebrovasculature by short-term mechanical hyperventilation was nevertheless unsuccessful. Furthermore, a noradrenaline-induced steady state increase in MAP likewise had no effect. A factor that may contribute to the slowing of dynamic cerebral autoregulation is the presence of anaemic hypoxaemia which is present in most septic patients admitted to the ICU,[53,255,477] since hour-lasting hypobaric hy-

poxia may impair dynamic cerebral autoregulation despite concomitant hyperventilation.[29]

We and others have found evidence of an increased cerebral influx of phenylalanine across the BBB due to changes in circulating LNAAs during stage III. According to our findings, this does, however, not cause changes in the brain extracellular fluid concentration of phenylalanine or its metabolite tyrosine to such an extent that significant amounts of false neurotransmitters are likely formed. Neurovascular coupling, an aspect of cerebral haemodynamic function that was not specifically addressed in our studies, has recently been found to be impaired during stage III in rats,[464] and while this remains to be confirmed in humans, it is not likely to be caused by any effect of false neurotransmitters on catecholaminergic homeostasis within the brain. As the septic condition progresses and the changes in circulating LNAAs become more pronounced, the intracerebral levels of phenylalanine may in some cases increase to such an extent that the false neurotransmitter phenylethanolamine is formed. This theoretically contribute to the impairment of neurovascular coupling which remains impaired at this stage.[464] It must, however, be noted, that although several animal studies have provided evidence of noradrenaline depletion within the brain during stage V,[179,180,264] which is consistent with the false neurotransmitter hypothesis, elevated false neurotransmitter levels have only been reported in plasma[181] but not within the brain at any stage of sepsis.

Changes in the autonomic regulation of cardiovascular function are already present during stage III and persist into stage V, and involve reduced sympathetic output to the circulation with a slower and less effective arterial baroreflex. This will predispose to more random, extensive, and protracted reductions in MAP than under normophysiological conditions, and thus impose a greater strain on cerebrovascular homeostasis.[339,382,554] The more agile dynamic autoregulation observed during stage III may prevent this from critically affect-

ing oxygen availability to the brain tissue. However, cerebral ischaemia may ensue if this mechanism fails and the cerebrovasculature instead becomes slower as observed during stage V. The impact of this may be compounded by cerebral microvascular perfusion heterogeneity, which increases as sepsis progresses according to animal studies,[101,531,532] conceivably due to changes in haemorheology, endothelial function, pericyte contraction and nitric oxide scavenging.[47,610] This renders capillary gas exchange less effective owing to so-called *functional shunting* of red blood cells that pass through the capillary bed at transit times that are too short to permit sufficient oxygen extraction.[15,444,609] This is consistent with the state of relative cerebral hyperperfusion observed during stage V, and furthermore increases the cerebral ischaemic threshold to higher CPP-values.[609,610] This is particularly critical in the septic brain, where the ability of the mitochondria to utilise oxygen may concurrently be reduced.[47,79]

The reported changes in cerebral haemodynamic function provide a mechanistic basis for the occurrence of cerebral ischaemia in septic patients. Hence, a slowly reacting cerebrovasculature in terms of dynamic cerebral autoregulatory responses, and increased cerebral microvascular perfusion heterogeneity in combination with disordered autonomic regulation of cardiovascular function may give rise to the signs of diffuse cerebral ischaemia observed in almost all septic patients.[490,491,494] Furthermore, a right-shifted autoregulation curve combined with an upward shift of the ischaemic threshold may predispose to global cerebral ischaemia, particularly in cases with large-amplitude blood pressure low-frequency oscillations that may traverse the lower limit of autoregulation.[V] This may give rise to the occasional watershed infarctions observed in some patients.[346,353,430,490] The implications of this in relation to sepsis-associated encephalopathy is supported by studies in septic patients showing that patients with encephalopathy have slower dynamic cerebral autoregulatory responses than patients without encephalopathy,[15] that slower dynamic autoregulatory

responses at admission to the ICU is associated with the subsequent development of encephalopathy,[16] and that the severity of encephalopathy is associated with the degree of hypotension.[508,587,598] Moreover, a recent study reported that patients who subsequently died from sepsis had slower dynamic cerebral autoregulatory responses than patients who survived.[59] Although this study was not appropriately designed to draw any definitive conclusions regarding the prognostic impact of changes in dynamic cerebral autoregulation, it is consistent with studies showing that the development of encephalopathy is an independent predictor of death in septic patients. Hence, a slowly reacting cerebrovasculature may both incite symptoms of encephalopathy and trigger ischaemia in autonomic nuclei, of which the latter will deteriorate cardiovascular function further, thus imposing a greater strain on the cerebrovasculature, in effect comprising a *circulus vitiosus* that may ultimately lead to septic shock and death.

As of now, no interventions that may restore cerebral haemodynamic function in ICU patients with sepsis have been identified. An obvious contender is the maintenance of a sufficient MAP to ensure adequate cerebral perfusion, notably by treatment with intravenous noradrenaline which is currently the vasopressor of choice in septic patients.[28] One randomised study examined the effects of targeting a MAP of 80–85 mmHg vs. 65–70 mmHg by intravenous noradrenaline,[24] that is, expectedly just above and below the lower limit of autoregulation in most patients. There were no effects of this on 28-day mortality, but even though neurocognitive measures were allegedly obtained in a sub-study,[25] these findings have not yet been reported. If this is found to reduce the occurrence of encephalopathy and/or improve long-term cognitive function, the findings of the present thesis suggest that this is due to other effects than changes in dynamic cerebral autoregulation, such as an increase of MAP above a slightly right-shifted lower limit of autoregulation or perhaps a restoration of neurovascular coupling.[464] Hyperventilation is theoretically another poten-

tially advantageous intervention in this context, but even though it may enhance dynamic cerebral autoregulation during stage III, we found no evidence that an approximate1 kPa reduction in $PaCO_2$ had a similar effect during stage V. It nevertheless cannot be ruled out that this would have been the case if a greater reduction in $PaCO_2$ had been targeted and/or the reduction had been maintained for a longer period. Moreover, although it needs to be examined in formal clinical trials, there may be other yet unexplored beneficial effects of hyperventilation-induced cerebral vasoconstriction, such as a left-shift of the lower limit of autoregulation or reduced cerebral microvascular perfusion heterogeneity.[413,610] Lastly, due to the possible involvement of anaemic hypoxaemia in the observed sepsis-associated changes in dynamic cerebral autoregulation, blood transfusion may potentially improve cerebrovascular function and cerebral oxygenation, but this has not yet been examined in any studies.

When considering the changes in cerebral haemodynamic function during sepsis as highlighted in the present thesis, any interventions implemented in the ICU to prevent cerebral ischaemia would expectedly be optimised by individualising treatment according to continuously monitored physiological variables. Several reviews have therefore recommended that standard ICU monitoring of systemic haemodynamics and respiration should be supplemented with monitoring of cerebral haemodynamics, notably dynamic cerebral autoregulation.[27,202,530] However, no studies have yet documented any beneficial effect of continuous cerebral haemodynamic monitoring on clinical outcome in septic patients, which may partly reflect the lack of a suitable monitoring modality. While transcranial Doppler remains the method of choice for assessing changes in CBF at the bedside,[588] it is not suitable for continuous monitoring in the clinical setting, particularly not in unsedated and potentially encephalopathic patients, as it requires a fixation rack which is uncomfortable for the patient and easily displaced, thus rendering the continuous signal unusable. At-

tempts have therefore been made to substitute transcranial Doppler by near-infrared spectroscopy (NIRS) in this context.[59,418,513] NIRS is easy to use and poses no immediate discomfort to the patient as it only involves the placement of an optode, typically on the patient's forehead.[33,362,526] It theoretically provides a real-time index of cerebral oxygenation, by estimating the relative concentrations of oxy- and deoxyhaemoglobin in the cerebral cortical microvasculature through their absorption of near-infrared light emanated from the optode. Given that the signal is not contaminated by blood in extracerebral tissues, and that arterial blood gases and core temperature as well as the cerebral metabolic rate of oxygen are stable, changes in the derived index of cerebral oxygenation will principally depend on CBF.[33,362,526] It may thus both provide a snapshot estimate of the oxygen availability to the brain tissue at a given time, as well a means to assess temporal changes in cerebral haemodynamic function indirectly when continuously recorded. However, as outlined in the present thesis, neither arterial blood gases, core temperature, or the cerebral metabolic rate of oxygen can be assumed to be constant during the clinical course of sepsis. Furthermore, a number of studies in healthy volunteers indicate that the NIRS-derived signal is readily contaminated by blood flow changes in the forehead skin, which leads to opposing changes in CBF and cerebral oxygenation assessed by NIRS during intravenous infusion of vasopressors, including noradrenaline.[381,527]

Given that vasopressor-induced changes in CBF are particularly relevant in the ICU setting, we examined the effects of noradrenaline infusion on transcranial Doppler- vs. NIRS-derived estimates of CBF changes in septic patients.[546] We found that for a given noradrenaline-induced change in CBF estimated by transcranial Doppler, the corresponding NIRS estimate was anything from 18 % lower to 45 % higher.[546] In terms of the cerebral haemodynamic effects of spontaneous oscillations in blood pressure, a previous study on septic patients reported a high correlation between dynamic cerebral autoregu-

lation assessments by transcranial Doppler and NIRS.[513] We subsequently compared the two modalities by appropriate Bland-Altman plots in a similar group of patients, and conversely found that the agreement between transfer function analysis-based dynamic cerebral autoregulatory assessments was unequivocally poor.[51] We interpreted this to reflect the impact of sepsis-associated microvascular dysfunction on the NIRS-signal, as this may render the cutaneous blood flow changes to blood pressure oscillations entirely unpredictable.[51] In any event, these findings do not support NIRS as a suitable modality for monitoring cerebral haemodynamics in septic patients. It may be a better option to monitor brain tissue oxygenation invasively and supplement this with continuous assessments of CPP for guiding various therapeutic interventions to prevent cerebral ischaemia. This has previously been suggested for patients with traumatic brain injury, in which changes in cerebral autoregulation, neurovascular coupling, and cerebral microvascular perfusion heterogeneity are also thought to predispose to cerebral ischaemia.[609] However, further studies are required to identify the target values for brain tissue oxygenation and CPP and to elucidate if monitoring of these variables improves clinical outcome in septic patients.

Chapter 10. Methodological limitations

"Perhaps no other organ of the body is less adapted to an experimental study of its circulation than the brain," the American physiologist Carl Wiggers notoriously wrote in a paper on the cerebrovascular effects of adrenaline in 1905.[586] Despite more than a century of methodological advances, this statement does in many respects still seem relevant today, at least in human studies. While the limitations of LPS-infusion as a human-experimental model of early sepsis has been discussed in detail in *Chapter 2*, and spectral analysis for assessing autonomic regulation of cardiovascular function is discussed in *Appendix 3*, the following section will mainly focus on the potential caveats of the Kety-Schmidt and transcranial Doppler ultrasound techniques used in *Studies B-E*.

Two fundamental assumption of the Kety-Schmidt technique are that 1) there is tracer diffusion equilibrium between blood and brain tissue at the initiation and termination of blood sampling, and 2) that blood from the internal jugular vein represents mixed cerebral venous blood from the whole brain.[476] In terms of the first assumption, the Kety-Schmidt technique yields an average whole-brain CBF value. However, blood flow differs between the different compartments within the brain, and areas with a relatively low perfusion may not reach diffusion equilibrium with the arterial blood during the saturation phase. For example, grey and white matter contribute differently to the measured average global CBF value, as blood flow is four times higher in grey than in white matter.[177,248,297] Consequently, incomplete diffusion equilibrium between arterial blood and white matter may lead to an under-representation of this compartment, and thus lead to a systematic overestimation of average global CBF.[303,304,476] This overestimation has previously been found to be approximately 10 % when using the Kety-Schmidt technique in desaturation phase,[327] and the CBF values obtained in our setup are consistent with a similar overes-

timation.[537] The second assumption is challenged by the fact that the internal jugular veins also drain extracranial tissues, which may comprise up to 20 % of the total tissue mass that they drain.[496] However, due to the low perfusion rate of the extracranial tissues (estimated to be 5 ml 100 g^{-1} min^{-1} in humans,[183] and thus < 10 % of CBF), the admixture of blood of extracranial origin in the internal jugular veins is < 3 %.[327] Blood is nonetheless typically only obtained from one internal jugular vein, and since the cerebral venous drainage is lateralised, so that blood from the cortex typically drains into the right internal jugular vein, whereas blood from the deep grey and white matter, the brainstem and cerebellum is typically drained by the left internal jugular vein,[61,171,296,496,514] it may be questioned whether this provides a valid estimate of mixed venous blood from the whole brain. In early studies, the interindividual variability of $CMRO_2$ estimates based on the Kety-Schmidt technique was found to be much lower when based on bilateral internal jugular venous blood samples, which was taken to suggest that part of the interindividual variation could be due to variations in the anatomy of the cerebral venous drainage.[303,305] However, a later study found no relation the interindividual $CMRO_2$ variability and the cerebral venous drainage imaged by magnetic resonance - angiography.[327] Although unilateral internal jugular venous blood and mixed cerebral venous blood are not identical due to the lateralisation of cerebral venous drainage, the impact of this on CBF and $CMRO_2$ estimates obtained by the Kety-Schmidt technique are thus probably negligible compared to other random errors of a technical nature.[327,537]

In terms of transcranial Doppler, it may be problematic that the insonation angle and cross-sectional area of the insonated vessel, in this case MCA, are unknown. Indeed, the correlation between absolute MCAv and global CBF is poor, and changes in the cyclic mean of MCAv may only provide a valid estimate of changes in global CBF if both the insonation angle and the cross-sectional area are constant.[62,588] While the former may be achieved by the use of a fixa-

tion rack, it remains controversial whether the MCA may dilate or constrict during various physiological challenges, including changes in PaO_2, $PaCO_2$, and blood pressure.[9,192,231,281] Hence, a 10 % increase in the MCA diameter (approximately 0.04 cm) may lead to a 20 % increase in the cross-sectional area of the vessel, and any concomitant change in CBF will be correspondingly underestimated by the change in MCAv.

PaO_2-induced changes in cerebrovascular tone involve the cerebral arterial vessels both up- and downstream of the penetrating arteriole at the brain surface.[110,284,573] Although the majority of the cerebrovascular response takes place in small arteries and arterioles, the tone of the large basal arteries such as the MCA is also sensitive to oxygen.[110,284,573] Indirect observations based on the power of the backscattered Doppler signal, which should theoretically be proportional to the cross-sectional area of the insonated vessel,[23] initially suggested that the diameter of the MCA does change after 20 minutes of inspiratory hypoxia at a PaO_2 of approximately 7 kPa.[438] However, later comparisons of volumetric flow changes in the internal carotid artery (ICA) with flow velocity changes in the ipsilateral MCA, which should be similar as the MCA is the main recipient of blood from the ICA,[601] indicate that MCAv underestimates CBF slightly during severe short-term hypoxia (step-wise reduction to $PaO_2 < 4.7$ kPa at three 15-minute steps) and overestimates it during short-term hyperoxia ($PaO_2 > 40$ kPa for 15 minutes) due to dilation and constriction of the MCA, respectively.[589] Similar findings have been made after six hours at an F_IO_2 of 12 %,[247] and definitive proof of a hypoxia-induced increase the MCA diameter (of approximately 15 %) was recently made by high-resolution magnetic resonance imaging in healthy volunteers after breathing at an F_IO_2 of 12 % for three hours.[591]

While hypocapnia mainly constricts the vessels downstream of the penetrating arteriole at the brain surface, hypercapnia both dilates up- and downstream vessels.[259,574] In terms of the large basal arteries,

including the MCA, studies on patients undergoing cerebral angiography from 1960s and 1970s suggested that they do respond actively to changes in $PaCO_2$,[76,241] but it nevertheless appears to be a widely held view that this is not the case.[9,231] A commonly used citation to support this notion is a classical paper by Giller *et al.* on patients undergoing craniotomy, in which the MCA was observed directly during manipulations in $PaCO_2$.[193] However, they did in fact observe approximate 2 % change in MCA diameter over a 1.3 kPa range of $PaCO_2$ values. The method for assessing the MCA diameter certainly provides a rather crude estimate of MCA diameter, but the very small reported change nonetheless superseded other studies where indirect methods pointed towards notable $PaCO_2$-induced changes.[314,438,560] When two, now widely cited, magnetic resonance imaging-based studies on healthy, conscious volunteers reported that MCA diameter neither changes during hypo- or hypercapnia,[486,561] this was considered a final validation of transcranial Doppler for assessing $CVCO_2R$. The resolution (1.5 T) was, however, relatively poor in these studies, so that the pixel size was equal to 20 % of average MCA diameter, thus rendering it impossible to detect small yet significant changes in MCA diameter.[231] Indeed, recent magnetic resonance imaging-based studies with higher resolution (3 and 7 T) have provided conclusive evidence of dilatation and constriction of the MCA during hyper- and hypocapnia, respectively.[107,108,565] In accordance with findings based on comparisons of volumetric flow in ICA and MCAv,[589] these studies furthermore indicate that MCA diameter remains unchanged within ± 1 kPa change in $PaCO_2$ from baseline.

The validity of transcranial Doppler for assessing static and dynamic cerebral autoregulation depends on MCA constancy during steady state and acute blood pressure changes, respectively. The associated changes in cerebrovascular resistance involve cerebral arterial vessels at both the macro-, meso-, and microvascular level,[40,41,167,224,324,325,517] and although they are primarily mediated by

the small arterial vessels downstream of the penetrating arteriole at the brain surface, notable changes in the large basal arteries may also occur. Hence, an early study on patients undergoing cerebral angiography, where a steady state increase in blood pressure was induced by infusion of a combined α- and β-adrenergic agonist, demonstrated that MCA diameter is reduced by approximately 15 % upon an increase in systolic blood pressure of approximately 50 mmHg, which was much less than in downstream vessels.[330] In the aforementioned study by Giller *et al.* the MCA diameter was found not to change systematically over a 30 mmHg range of MAP values which were achieved by nitroprusside and phenylephrine infusions.[193] However, studies comparing volumetric flow in the ICA to MCAv do in accordance with the cerebral angiography-based findings, suggest that the MCA does vasoconstrict and -dilate slightly during steady state increases and decreases in MAP, respectively.[309,317] Few studies have focused on MCA diameter during acute changes in MAP. In the classical first study of dynamic cerebral autoregulation by Aaslid *et al.*, a thigh-cuff deflation-induced MAP reduction in healthy volunteers was not found to trigger any concomitant changes in MCA diameter, as the power of the backscattered Doppler signal remained constant. In other studies on anaesthetised patients, volumetric ICA flow and MCAv have been found to correlate both during thigh-cuff deflation-induced and spontaneous changes in MAP.[313,373] As of now, no studies have yet used high-resolution techniques, such as magnetic resonance imaging, to assess MCA constancy, to either steady state or acute changes in MAP.

Together, the available studies thus highlight that transcranial Doppler likely underestimates both CVO_2R and $CVCO_2R$ during hypoxia and hypercapnia, respectively, while overestimating the same parameters during hyperoxia and hypocapnia. However, rather severe changes in arterial blood gases, which exceed those induced in the studies of the present thesis, are likely required before physiologically

relevant bias is introduced to the cerebral haemodynamic estimates. In terms of blood pressure changes, transcranial Doppler may likewise tend to underestimate the CBF change to a reduction and overestimate it to an increase in MAP. This is notably evident in relation to steady-state changes in MAP, where static autoregulatory function may consequently seem less effective during increases than decreases in MAP. In the present thesis, static cerebral autoregulation was only assessed upon an increase in MAP; since static cerebral autoregulation during stage III and V was evaluated by comparing the autoregulatory plateau to baseline values obtained by identical experimental procedures in healthy volunteers, a concomitant reduction in MCA diameter is unlikely to affect the overall conclusions based on our results. In terms of dynamic cerebral autoregulation, there are currently not sufficient experimental data available to determine whether any noteworthy changes in MCA diameter occur upon acute changes in MAP; if present, such diameter changes could be critical to the interpretation of our findings, as well as to the hundreds of papers on transcranial Doppler-based dynamic cerebral autoregulatory assessments that have been published since the first paper on the topic by Aaslid *et al.* from 1989.[613]

Another major limitation of both the Kety-Schmidt technique and transcranial Doppler ultrasound is that neither method provides information on compartmental or regional changes in cerebral haemodynamic function. Hence, the Kety-Schmidt technique provides whole-brain values only, and transcranial Doppler-based estimates specifically reflect changes in the cerebrovasculature downstream of the insonated vessel (provided that the diameter of the insonated vessel is constant as outlined above). In case of the MCA, which received approximately 20% of total CBF,[601] this includes large regions of the ipsilateral cerebral hemisphere, including both grey and white matter.[536,606] Both CVO_2R and $CVCO_2R$ are greater in grey than in white matter,[229,541] while dynamic cerebral autoregulation is faster in

white than in grey matter,[238] but compartmental changes between grey and white matter will go undetected by any of these methods. Furthermore, differences between different regions of the brain may also go unnoticed. In the healthy state, CVO_2R and $CVCO_2R$ are generally greater in the posterior parts of the brain, including the basal nuclei and brainstem.[60,93,229,395,589] while the lower limit of autoregulation is set at lower CPP values in the brainstem than in the cerebral cortex.[360,469] None of the studies in the present thesis provide data to elucidate whether any of these cerebral haemodynamic variables are affected differently in cerebral cortex, basal nuclei, and brainstem in sepsis.

As our estimates of the BBB transport of LNAAs are also based on CBF-values measured by the Kety-Schmidt technique combined with arterial-to-jugular venous differences of LNAAs, compartmental or regional differences in BBB-function are not detected by this method either. This may be particularly problematic in the case of differences between grey and white matter, since both blood flow, LNAA metabolism, and PS_1-values are higher in the former to such an extent that white matter changes may be unintentionally disregarded.[248,327,502] However, previous studies have shown that whole-brain BBB transport parameters for LNAAs closely follow those in grey matter,[499,502] and the changes in catecholaminergic neurotransmission, which according to the false neurotransmitter hypothesis are thought to be triggered by changes in the BBB transport of LNAAs, principally take place in grey matter. Hence, our whole-brain based model may still be relevant to elucidate such changes. Another limitation that may potentially bias our estimates of the BBB transport of LNAAs is that we exclusively focused on carrier-mediated saturable LNAA transport, and thus did not take non-saturable transport, for example by simple diffusion, into account. A recent human-experimental study by Bongiovanni *et al.* concluded that the BBB transport of LNAAs in humans is non-saturable, and thus mainly occurs by diffusion,[71] which

would render our mathematical model of LNAA transport flawed. However, Bongiovanni *et al.* used kinetic constants from isolated human capillaries obtained from cadavers up to 30 hours post-mortem,[222] while we used constants obtained *in vivo* in rats.[504] We have previously found that *in vivo* constants from rats provide the most physiologically reliable BBB-transport estimates in humans when compared to other previously published constants.[117] The conclusions by Bongiovanni *et al.* are thus likely biased by the K_M-values obtained post-mortem, which are up to 40-fold lower than values obtained *in vivo*. The discrepancy between *in vivo* and post mortem-based values most likely reflects the effects of inevitable biological decay processes when conducting studies in cadavers, as well as the changes in luminal vs. abluminal BBB transport of LNAA which are triggered when a cerebral capillary is isolated experimentally.[499] It may thus be justified to consider the BBB transport of LNAAs to be mainly saturable, but it must nonetheless be kept in mind that any changes in non-saturable transport is not accounted for in our BBB model.[VIII,117]

A main finding in the present thesis is that dynamic cerebral autoregulatory responses become slower during advanced sepsis. This is, however based on comparisons between healthy, young male volunteers and a group of patients that were older, included females, suffered from various comorbidities, and furthermore received different sedatives.[53] The question is therefore whether it is at all feasible to compare dynamic cerebral autoregulation in these two very different groups. However, age *per se* does not appear to affect estimates of dynamic cerebral autoregulation.[42] In terms of gender, only two of the patients were females, and in any event this does not likely contribute to the observed slower dynamic autoregulation in patients, as females have previously been reported to exhibit slightly more effective dynamic cerebral autoregulation than males.[130] None of the included patients suffered from any comorbidities that are known to affect dynamic cerebral autoregulation (e.g. cerebrovascular

disease,[126,127,447,584] dementia,[103,201] or diabetes mellitus[277,371]). Furthermore, patients were mainly sedated by propofol, remifentanil, and midazolam, none of which have been found to slow dynamic cerebral autoregulatory responses in humans.[163,380,543]

In addition to the abovementioned technical limitations relating to our cerebral haemodynamic assessments, it may also be questioned whether it is at all appropriate to use LPS infusion to study the cerebral pathophysiology during the early stages of sepsis without concurrently assessing neurocognitive function. Apart from flu-like symptoms, previous studies largely agree that volunteers experience slight depressive symptoms and increased anxiety in the first hours after a LPS bolus injection at a pyrogenic dose,[208,291,292,294,356] but the findings in terms of neurocognitive function up to eight hours post-LPS are conflicting, as both improved,[207,208] reduced,[294] and unaffected[72,356] performance has been reported. We did not assess changes in cerebral haemodynamic function translated into changes in neurocognitive function in our studies for two principal reasons. Firstly, our aim was not to investigate the pathophysiology of sepsis-associated encephalopathy *per se*, but rather to elucidate whether acute systemic inflammation invokes changes in cerebral haemodynamic function that may predispose to or protect from cerebral ischaemia. Although encephalopathy may arguably represent a clinical manifestation of cerebral ischaemia in the clinical setting, we did not expect to trigger cerebral ischaemia in our experimental setup; if so, our experiments would be highly unethical. Secondly, even if changes in neurocognitive performance could be detected, it would be difficult to infer this was caused by the perception of illness or by the actual systemic inflammatory response, since flu-like symptoms may be associated with changes in neurocognitive function regardless of whether these symptoms are caused by febrile illness or not.[95]

Chapter 11. Conclusion

In the studies that form the basis of the present thesis, we developed a continuous LPS-infusion model in humans that replicates several critical aspects of the systemic inflammatory response during the very early stages of sepsis. Using this model, we found that cerebral vasoconstrictive influences, which include hyperventilation, the fever response, and an enhanced cerebrovascular release of ET-1 lead to a state of cerebral hypoperfusion relative to cerebral oxidative metabolism during the acute systemic inflammatory response. This renders the cerebrovasculature more agile, in the sense that dynamic cerebral autoregulatory responses buffer the effects of acute MAP changes on CBF more effectively. Although it remains speculative, the *raison dêtre* for this phenomenon may be to prevent the concurrent failure of autonomic cardiovascular control, which encompasses less effective arterial baroreflex responses, from causing cerebral ischaemia. As the septic condition progresses into more advanced stages, as evaluated by studies on critically ill patients admitted to the ICU with sepsis, this potentially neuroprotective mechanism fails, as our findings indicate that the cerebrovasculature instead becomes slower. Although this may be related to the presence of anaemic hypoxaemia and $PaCO_2$ levels in patients, the exact mechanisms remain to be elucidated, and we have not yet identified any interventions that may restore dynamic cerebral autoregulation in this context. In any event, the presence of a slowly reacting cerebrovasculature combined with impaired autonomic regulation of cardiovascular function, whether it sets in during the very early or later stages of sepsis, may provide a mechanistic basis for the widespread cerebral ischaemic changes observed in almost all severely septic patients, and which are believed to contribute both to encephalopathy, long-term cognitive deficits in sepsis-survivors, and to the development and progression of shock.

Appendix 1. Overview of Studies A–F

Study A was conducted from February to May 2005 (ethical approval no. KF-11032/02). In a randomised, cross-over, single-blind fashion, ten healthy males aged 24 (mean, SD 5) years were studied on three separate study days on which they received (i) an intravenous bolus injection of *Escherichia coli* lipopolysaccharide (0.3 ng kg^{-1}) followed by an intravenous four-hour continuous infusion of saline; (ii) an intravenous saline bolus followed by a four-hour continuous infusion of lipopolysaccharide (total dose, 0.3 ng kg^{-1}); and (iii) an intravenous bolus injection of saline followed by an intravenous four-hour continuous infusion of saline. Blood from an antecubital vein was obtained hourly for eight hours after the initial bolus injection (*Figure A1.1*). Data from this study are included in one publication.[I]

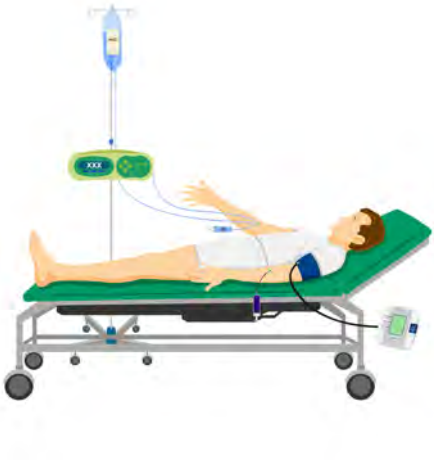


Figure A1.1. Experimental setup, Study A.
xxx: lipopolysaccharide or saline (double-blinded).

Study B was conducted in May 2006 (ethical approval no. KF-01-290011). Twelve healthy male volunteers aged 26 (mean; SD 4) years were included. Global cerebral blood flow, cerebral oxidative metabolism and transcerebral exchange were evaluated by the Kety-Schmidt technique and paired arterial-jugular venous blood samples (*Figure A1.2*). Measurements were done at baseline and 1 hour after the cessation of an intravenous four-hour continuous infusion of LPS (total dose 0.3 ng kg^{-1}). Data from this study are included in four published papers.[II,VII,VIII,55]

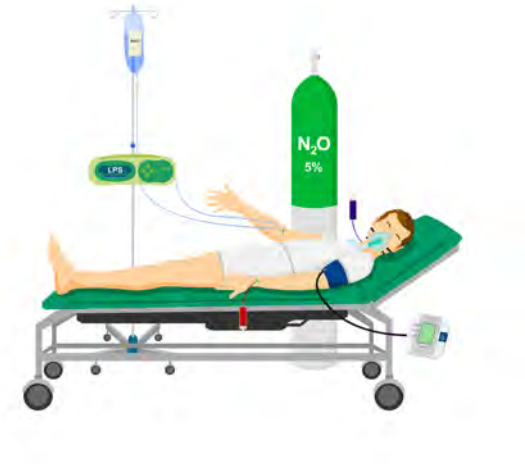


Figure A1.2. Experimental setup, Study B.
LPS: lipopolysaccharide.

Study C was conducted from November 2009 to June 2011 (ethical approval no. HA-2009-020 with amendments). Sixteen mechanically ventilated patients aged 59 (mean, SD 12) years (2 females) admitted to a tertiary ICU with severe sepsis or septic shock were included. Cerebral haemodynamic function was evaluated by transcranial Doppler ultrasound at baseline (n = 16), during noradrenaline infusion (n = 9), mechanical hyperventilation (n = 7), and in response to thigh cuff deflation (n = 6) (*Figure A1.3*). Patient characteristics are summarised in *Table A1.1*. Data from this study are included in six different papers. Since different patients are included in different papers depending on the available measures, an overview of the patients in relation to published papers is provided in *Table A1.2*.

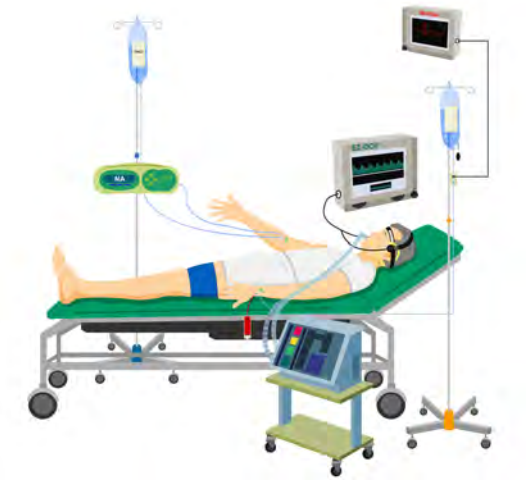


Figure A1.3. Experimental setup, Study C.
NA: noradrenaline.

	Study C	Study F
N	16	65
Age (years)	59 (12)	63 (11)
Males (%)	88	64
APACHE II	22 (18–30)	25 (11–31)
SOFA	10 (7–13)	9 (7–12)
Infectious focus		
Abdomen (%)	13	16
Blood (%)	19	9
Soft tissue (%)	38	23
Lung (%)	25	38
Unknown or other (%)	6	14
Total ICU stay (days)	6 (5–14)	8 (4–15)
30-day mortality (%)	25	25

Table A1.1. Patient characteristics.
APACHE II: acute physiology and chronic health evaluation II (score); ICU: intensive care unit; SOFA: sequential organ failure assessment (score). Age is presented as mean (SD), while the remaining variables data are presented as % of total patients within the given study or as median (IQR).

Patient	Papers
1	VI,51,53,391
2	VI,51,53,391,546
3	51,53,391
4	51,53,391
5	VI,51,53,391,546
6	V,51,53,391,546
7	VI,51,53,391,546
8	V,VI,51,53,391,546
9	V,53,391,546
10	V,53,391
11	V,53,391
12	V,53,391
13	53,391
14	53,391
15	V,53,391
16	VI,53,391

Table A1.2. Publications based on Study C.

Study D was conducted in September 2010 (ethical approval no. H2-2010-04). Ten healthy male volunteers aged 23 (mean, SD 2) were included. Cerebral haemodynamic function was evaluated by transcranial Doppler ultrasound and paired arterial-jugular venous blood samples during inspiratory normoxia (F_IO_2: 21 %), hyperoxia (F_IO_2: 40 %), and hypoxia (F_IO_2: 12 %) (*Figure A1.4*). CO_2 was added to the inspired air *ad hoc* to achieve an end-tidal PCO_2 ($P_{ET}CO_2$) of 5.5–5.8 kPa during these interventions. Measurements were done at baseline and after intravenous four-hour continuous infusion of LPS (total dose 2.0 ng kg^{-1}). Data from this study are included in two published papers.[52,102]

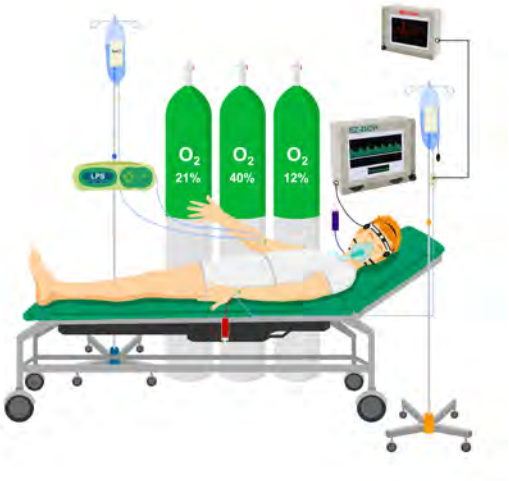


Figure A1.4. Experimental setup, Study D.
LPS: lipopolysaccharide.

Study E was conducted in November and December 2010 (ethical approval no. HA-2009-020 with amendments). Nine healthy male volunteers aged 23 (mean, SD 2) were included. Cerebral haemodynamic function was evaluated by transcranial Doppler ultrasound and paired arterial-jugular venous blood samples (*Figure A1.5*). Blood pressure was manipulated by thigh-cuff deflation and noradrenaline administration at baseline and after four-hour infusion of LPS (total dose 2.0 ng kg^{-1}). Data from this study are included in five published papers.[III,VI,53,391,392]

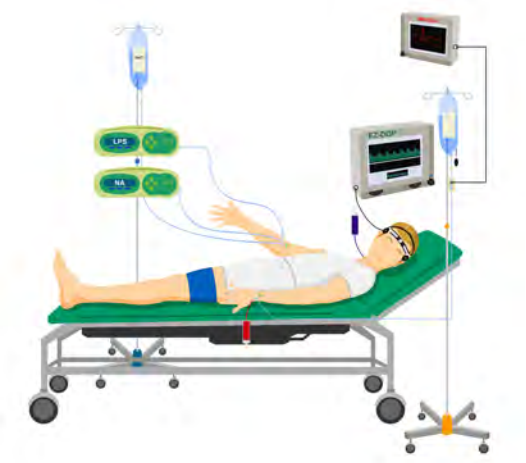


Figure A1.5. Experimental setup, Study E.
LPS: lipopolysaccharide; NA: noradrenaline.

Study F is a retrospective analysis of blood pressure recordings from 65 mechanically ventilated patients aged 63 (mean, SD 11) years (21 females) admitted to a tertiary ICU with severe sepsis or septic shock between November 2009 and October 2012 (*Figure A1.6*). Patient characteristics are summarised in *Table A1.1* The study forms the basis of one published paper.[IV]

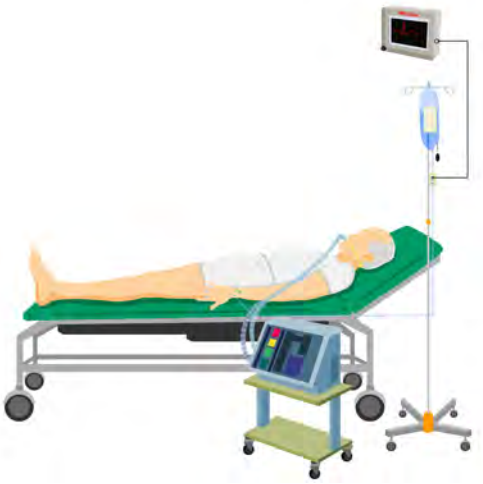


Figure A1.6. Setup, Study F.

Appendix 2. Methods for assessing dynamic cerebral autoregulation

Background

Between the 1960s and early 1980s, several scientists argued that the cerebrovascular response to a steady state change in MAP (or CPP) is initiated within eight seconds, and complete within a minute.[206,282,375,525,595] The high temporal solution offered by the transcranial Doppler ultrasound technique subsequently rendered Aaslid *et al.* capable of documenting that the cerebrovascular response to a sudden drop in MAP induced by thigh-cuff deflation is in fact much faster than this, as it was initiated within two and complete within fifteen seconds.[613] The title of their paper published in 1989 was "Cerebral Autoregulation Dynamics in Humans," and this led to the now widely used term *dynamic cerebral autoregulation*.

Although both linear and non-linear models of dynamic autoregulation have been developed,[194,400,472] only linear models are considered here, and the focus of the present *Appendix* is the specific methods that have previously been used by us or others to evaluate dynamic cerebral autoregulation in experimental or clinical sepsis by transcranial Doppler ultrasound. These encompass the classical *rate of regulation (RoR)*,[VI] the *time correlation method*,[59,418,480,481,529] and *transfer function analysis*.[III,V,52,53,84,349] The two former of these quantify dynamic cerebral autoregulation in the *time domain*, which indicates that a MAP-induced change in MCAv (as a proxy for CBF) is considered a function of time, and thus estimates the *response time* of the cerebrovasculature. In contrast, transfer function analysis quantifies dynamic cerebral autoregulation in the *frequency domain*, which implies that the ability of the cerebrovasculature to respond to MAP oscillations at different frequencies is specifically considered. In the latter scheme, dynamic cerebral autoregulation is thus considered a 'filter' that functions to dampen the impact of MAP oscillations on

CBF. Of note, assessments of dynamic cerebral autoregulation in the time and frequency domain have previously been found to agree poorly, and it remains to be established whether this is due to physiological or methodological matters,[556] and at present, there is no established gold standard for assessing dynamic autoregulation.

Rate of regulation (RoR)

The cerebrovascular response to an acute and transient reduction in blood pressure triggered by the rapid deflation of bilateral thigh-cuffs that have been inflated to supra-systolic pressures for ≥ 2 minutes is evaluated by RoR[k]:

$$RoR = \frac{\frac{\Delta CVC}{\Delta time}}{\Delta MAP}$$

where ΔCVC is the change in the cerebrovascular conductance (see *Appendix 4*) during the time period from 1.0 to 3.5 seconds (Δtime) after thigh-cuff deflation, and ΔMAP is the corresponding change in MAP. According to this definition, a RoR of 0.2 sec^{-1} signifies a per-second adjustment of 20 % of the CVC change necessary to fully compensate for the change in MAP, and an increase in RoR thus indicates improved dynamic autoregulation, while a reduction indicates the opposite.

This 1.0-3.5 second time interval was originally put forth by Aaslid *et al.*[613] on the basis of two assertions: (1) the baroreflex response does not set in until after this time period, such that only cerebral autoregulation and not the baroreflex is involved in the restoration of CBF during this time interval, and (2) the change in CVC is relatively linear during this period, allowing a slope of the response to be derived. However, the claim that the baroreflex does not respond within the first 3.5 seconds after thigh-cuff deflation is not accurate. The cardio-acceleratory response to an acute blood pressure reduction sets

[k] Sometimes the *rate of recovery* is used instead, which uses the cerebrovascular resistance index instead of CVC.

in within 0.5 seconds of baroreceptor unloading, and RoR thus reflects the integrated response of the arterial baroreflex and dynamic cerebral autoregulation.[382] Moreover, the onset of both the blood pressure reduction and the dynamic autoregulatory response exhibit substantial interindividual variation, and may thus take place outside the 1.0–2.5 second interval. A modified RoR (mRoR) has therefore been introduced, in which the 2.5 seconds following the post-deflation CVC nadir is used, as this presumably represents the point where the dynamic cerebral autoregulatory response sets off.[312]

Time correlation method

The time correlation method assesses the dependency of changes in MCAv on spontaneous changes in MAP by providing the so-called mean flow index (Mxa),[116,310,607] which measures the linear correlation between 30 consecutive 5–10 second averages of MCAv and MAP.[1] It is essentially a Pearson correlation coefficient and ranges from −1.0 (negative correlation) to +1.0 (positive correlation). A positive value ≥ 0.15 is generally interpreted as disturbed autoregulation, while lower values, including negative values, are interpreted as signs of intact autoregulation.[112,113] The calculation may be repeated with a moving time window, and Mxa may thus be used to continuously monitor dynamic cerebral autoregulation.[113] In contrast to the frequency domain methods, the time domain method does not require stationarity, and is claimed to be equally valid for detecting impaired autoregulation when linearity is not fulfilled.[112] Hypercapnia-induced changes in dynamic cerebral autoregulation detected by this method have been found to correlate reasonably well with the corresponding changes in RoR.[421]

[1] When ICP is concurrently measured, and the index is based on CPP it is typically termed Mx; when MAP is used, as in the studies on septic patients reviewed in the present thesis,[59,418,480,481,529] it is usually termed Mxa.[310,608] Mxa is normally slightly higher than Mx, but the difference decreases with the degree of impairment of dynamic cerebral autoregulation.[608]

Transfer function analysis

Cole A. Giller was the first to describe the relation between oscillatory patterns of blood pressure and MCAv in the frequency domain.[191] The approach was refined during the subsequent years, and in 1998, Rong Zhang *et al.* published a now classical paper,[604] in which a transfer function analysis-based approach for studying the dynamics of tuberoglomerular feedback in the kidney[234] was successfully used to describe the frequency-dependent behaviour of dynamic cerebral autoregulation.

In transfer function analysis, two temporal signals that include an input (in this case MAP) and an output (in this case MCAv) are transformed to the frequency domain by a fast Fourier transformation. This yields the autospectrum[m] of the input signal, $S_{xy}(f)$, the autospectrum of the output signal, $S_{yy}(f)$, and the cross-spectrum[n] between in- and output, $S_{xx}(f)$. Under the assumption of linearity, sinusoids at the input will be transformed into sinusoids of the same frequency at the output. The transfer function, $H(f)$, between sinusoid waveforms (oscillations) at the in- and output is defined:

$$H(f) = \frac{S_{xy}(f)}{S_{xx}(f)}$$

This is subsequently quantified by three parameters: *gain*, *phase*, and *coherence*.

G*ain* (or transfer magnitude), $|H(f)|$, of the transfer function quantifies the dampening of the amplitude of an input waveform at a given frequency as it is transferred to the output (*Figure A2.1*), and is

[m] An autospectrum shows a variable as a function of its own frequency (see *Figure A7.1*, *Appendix 7*). If a continuous blood pressure recording from a resting subject is subjected to spectral analysis, the autospectrum shows the spectral power of blood pressure ('variability') at different frequencies, that is, how much blood pressure oscillations at a given frequency contribute to the overall variability in blood pressure.

[n] A cross-spectrum is obtained by spectral analysis of the cross-correlation between in- and output, where the cross-correlation is a measure of the similarity between the two.

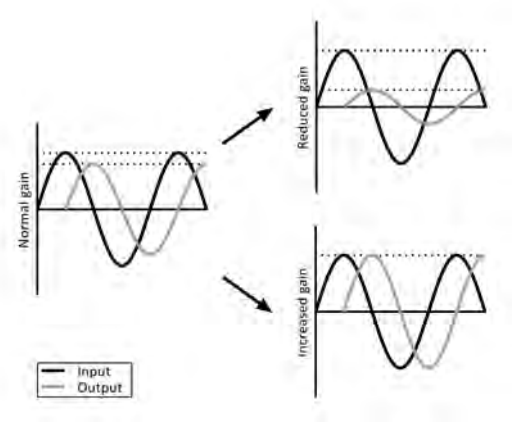


Figure A2.1. Gain.
Gain denotes the dampening (or amplification) of a waveform's amplitude as it is transferred from an input to an output signal.

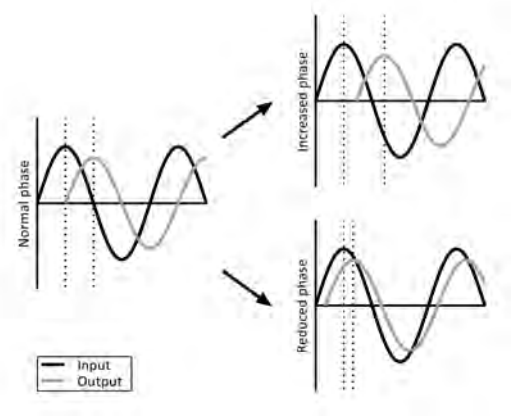


Figure A2.2. Phase.
Phase denotes the displacement of a waveform as it is transferred from an input to an output signal.

obtained from the real part $|H_R(f)|$ and imaginary part $|H_I(f)|$ of the complex transfer function:

$$|H(f)| = \{[H_R(f)]^2 + [H_I(f)]^2\}$$

In the context of cerebral autoregulation, gain describes the ability of the cerebrovasculature to buffer the impact of oscillations in CPP at specific frequencies on CBF (evaluated by MAP and MCAv) through adjustments in cerebrovascular resistance. An increase in gain will thus indicate less efficacious dynamic cerebral autoregulation and *vice versa*.

Phase, $|\Phi(f)|$, describes the displacement of an output waveform relative to an input waveform with the same period (***Figure A2.2***), and is obtained from real part $|H_R(f)|$ and imaginary part $|H_I(f)|$ of the complex transfer function:

$$|\Phi(f)| = \arctan\left[\frac{H_I(f)}{H_R(f)}\right]$$

The phase shift can be expressed in degrees from 0 to 360, or in radians from 0 to 2π, thus ranging from no phase shift to a phase shift of one full period. In relation to dynamic cerebral autoregulation, changes MCAv recover faster than the changes in MAP when dynamic cerebral autoregulation is intact. [136,293] This causes a displacement of the waveforms in such a manner that MCAv oscillations appear to lead the corresponding MAP oscillations.[136,293] This shift renders the phase value mathematically negative, but the tradition in the literature on dynamic cerebral autoregulation is nonetheless to define it as a 'phase lead' and provide it as a positive number.[42] According to this definition, a positive phase value indicates the presence of autoregulation, whereas as phase value ≤ 0 indicates absence of autoregulation.[233,293,556,604] In terms of physiological interpretation,

phase may be considered to reflect the time delay of the autoregulatory response, with higher values indicating a faster response time and *vice versa* for lower values.[42,136,233,293,556,604] Accordingly, phase (in the 0.07–0.20 Hz range, see below) is the transfer function analysis-based parameter that shows the best correlation with RoR in healthy volunteers.[556]

The third parameter of the transfer function is *coherence* (or the mean magnitude-squared coherence function), $\mathrm{MSC}(f)$, which describes the linearity between input and output:

$$\mathrm{MSC}(f) = \frac{\left|\mathrm{S}_{xy}(f)\right|^2}{\left[\mathrm{S}_{xx}(f)\mathrm{S}_{yy}(f)\right]}$$

Coherence is thus like a correlation coefficient of linear regression, ranging from zero to one, reflecting the fraction of the spectral power at the output that can be linearly explained by the spectral power at the input. Coherence approaching unity in a specific frequency range indicates that the signals are linearly related, whereas coherence approximating zero may reflect 1) that the relationship between the signals is nonlinear or that they are entirely unrelated, 2) the presence of substantial noise in the signals, or 3) that the spectral power of the input is trivial.[191,194,399,402,604] In relation to assessments of dynamic cerebral autoregulation, coherence is typically used to ensure sufficient linearity between MAP and MCAv for the gain and phase estimates to be valid. Most researchers therefore exclude gain and phase values when the corresponding coherence is < 0.4 or < 0.5.[42,137,604]

From the very early transfer function analysis-based studies on dynamic cerebral autoregulation, three frequency ranges were noted in healthy volunteers[o,604,605]:

[o] These are typically referred to as the high-, low-, and very low-frequency range. The terminology is thus different than for the corresponding frequency ranges in blood pressure, which are designated high, middle, and low frequency ranges, respectively (see *Appendix 3*).

- 0.02–0.07 Hz (approximately one cycle per minute), where the relationship between MAP and MCAv tends to be nonlinear, thus yielding low coherence values;

- 0.07–0.20 Hz (six cycles per minute), characterised by increasing coherence, increasing gain, and decreasing phase;

- 0.20–0.30 Hz (twelve to eighteen cycles per minute), with a high coherence, relatively large gain, and minimal phase lead.

From this, it is clear that dynamic cerebral autoregulation has the characteristics of a high-pass filter that becomes increasingly ineffective at higher frequency MAP oscillations,[137,315,604] presumably because the adaptive changes in cerebrovascular resistance are not fast enough to counteract MAP oscillations at higher frequencies.[104] Due to the nonlinear relationship between MAP and MCAv at frequencies below 0.07 Hz, and the characteristically ineffective dynamic autoregulation at frequencies at frequencies above 0.20 Hz, the 0.07–0.20 Hz range is often considered best at providing valid estimates of dynamic cerebral autoregulation, notwithstanding that there currently is no international consensus in relation to this.[104] In the review present thesis, I exclusively report transfer function parameters from the 0.07–0.20 Hz range.

There are two major limitations of transfer function analysis for assessing dynamic cerebral autoregulation that deserve mention here. Firstly, transfer function analysis provides no information as to whether dynamic cerebral autoregulatory responses differ in response to increases or decreases in MAP. Indeed, autoregulatory responses appears to be more effective and faster upon decreases than increases in MAP in healthy conditions.[282,555] Secondly, transfer function analysis is typically based on the cerebrovascular responses to spontaneous oscillations in MAP. It is possible that the small magnitude and

changeability of spontaneous MAP pressure oscillations are not sufficient to show more subtle deficiencies in dynamic cerebral autoregulation. Indeed, previous studies have reported changes in dynamic autoregulation upon induced, but not spontaneous oscillations in MAP.[30,218] Accordingly, the sensitivity of transfer function analysis may thus be enhanced by dynamically forcing systematic changes in MAP, for example by squat-stand manoeuvres or repeated thigh-cuff inflation-deflations.[218,266]

Appendix 3. Spontaneous cardiovascular oscillations

Background

In the 18th century, Stephen Hales and Albrecht von Haller were the first to document the presence of spontaneous and systematic cardiovascular oscillations in vertebrates.[216,217] When instruments that permitted continuous recordings of heart rate and blood pressure, including the application of spectral analysis for assessing these variables (*Figure A3.1*), were later developed, it was evident that these oscillations encompass high-, middle-, and low-frequency oscillations.[34,36,176,230,332,334,344,405,406,549] These oscillations are thought result from a combination of external perturbations and the homeostatic control mechanisms that attempt to oppose their effects.[38,331,388,393,394,406]

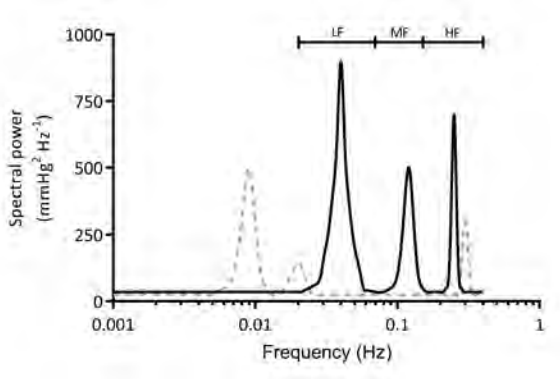


Figure A3.1. Spectral analysis of blood pressure.
Idealised autospectrum of blood pressure in a healthy subject (solid line) with spectral peaks at 0.14–0.35 Hz, 0.07–0.14 Hz, and 0.02–0.07 Hz. These peaks correspond to high- (HF), middle- (MF), and low-frequency (LF) blood pressure oscillations, respectively. Each peak reflects to which extent oscillations at the given frequency contribute to the overall variability (spectral power) in blood pressure. The dashed line is the autospectrum of blood pressure in a representative septic patient (stage V) from *paper IV*, where the MF and LF spectral peaks were found to be suppressed to lower frequencies than in the healthy state (*Chapter 8*). The curves have been smoothened and noise removed for clarity.

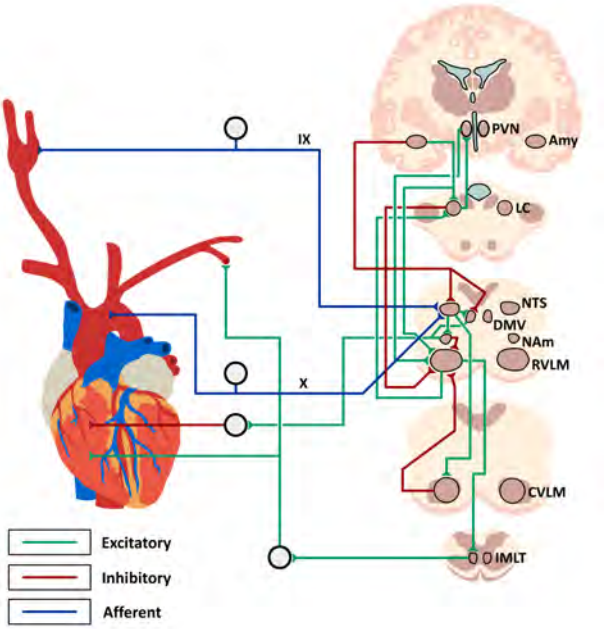

IX
X
PVN
Amy
LC
NTS
DMV
NAm
RVLM
CVLM
IMLT
Excitatory
Inhibitory
Afferent

Figure A3.2. Main pathways involved in the autonomic regulation of cardiovascular function.

The figure is based on data from a number of publications.[11,56,57,64,123,124,128,129,209,210,212,307,316,347,372,446,448–451,465,466,470,471,474,475,524,533,563,570]

Briefly, the rostral ventrolateral medulla (RVLM) is typically considered the principal 'vasomotor centre' of the brainstem.[209,448,465,466] It contains a collection of glutamatergic neurons, most of which also synthesise adrenaline.[522,523]

A small subpopulation of the RVLM neurons are non-adrenergic and exhibit intrinsic pacemaker properties.[522,523] These are thought to synchronise sympathetic vasomotor outflow from the central nervous system by modulating the activity of the glutamatergic cells.[211,213] The RVLM sends excitatory projections caudally to the intermediolateral tract (IMLT), which contains sympathetic preganglionic neurons.[11,123] An increase in the activity of the RVLM causes an increase in sympathetic output to the cardiovascular system, and thus an increase in heart rate and systemic vascular resistance.[209,448,465,466] This is modulated by feedback from the cardiopulmonary and arterial baroreceptors, which send projections back to the nucleus of the solitary tract (NTS).[307,335,337,451] An increase in arterial blood pressure will thus enhance impulses from the arterial baroreceptors to the NTS; the NTS in turn sends excitatory signals to the dorsal nucleus of the vagal nerve (DMV) and nucleus ambiguous (NAm), which then enhance the output of the vagal nerve to the heart and thus reduce heart rate[372,460]; furthermore, NAm sends direct inhibitory impulses to the RVLM and thus reduces the sympathetic output to the cardiovascular system, in effect reducing both heart rate and systemic vascular resistance.[347] The main pathway for arterial baroreceptor activation to reduce blood pressure is, however, by increasing the activity in the caudal ventrolateral medulla (CVLM), which continuously restrains the RVLM output.[122]

While being tonically inhibited from the CVLM, the RVLM is coupled to overall homeostasis of the body through numerous excitatory inputs, from areas involved in body fluid homeostasis, thermoregulation, immune function, and behaviour, some of which are shown here.

IX: ninth cranial nerve (glossopharyngeal nerve); X: tenth cranial nerve (vagal nerve); Amy: central nucleus of the amygdala; LC: locus coeruleus; PVN: paraventricular nucleus.

The homeostatic control is based on a complex interplay between autonomic nuclei within the central nervous system and baroreflex feedback (*Figure A3.2*). The mechanisms of spontaneous high-, middle-, and low-frequency oscillations will briefly be reviewed below.

High-frequency oscillations

High-frequency oscillations are synchronous with respiratory activity and occur at 0.14–0.35 Hz in humans. They involve a reduction in arterial blood pressure during inspiration and an increase during expiration. These changes encompass two components: 1) changes in systemic vascular resistance caused by impulses from the respiratory centre to the vasomotor centre in the medulla oblongata (Traube-Hering waves),[276,332] and 2) the mechanical effects of the respiration on the heart.[118,158,547] The latter of these is believed to be the main contributor to the high-frequency blood pressure oscillations, as left ventricular preload is reduced during inspiration, and restored during expiration, resulting in concomitant changes in stroke volume.[118,158,215,457,458,547]

The high-frequency oscillations in heart rate, so-called respiratory sinus arrhythmia, involve an increase in heart rate during inspiration and a reduction during expiration, mainly due to a central feed forward mechanism.[160] Respiratory sinus arrhythmia functions to buffer the impact of the respiration-induced changes in stroke volume on cardiac output, but oscillations in cardiac output and thus in arterial blood pressure nonetheless ensue.[156,158]

Middle-frequency oscillations

Middle-frequency oscillations occur at 0.07–0.14 Hz in healthy humans. The oscillations in arterial blood pressure are also known as *Mayer waves*, named after the German physiologist Sigmund Mayer.[p]

[p] Paradoxically, the blood pressure waves originally demonstrated by Sigmund Mayer in cats are not middle-frequency oscillations as they occurred at a frequency of 0.05 Hz. The middle-frequency oscillations typically occur at approximately 0.30 Hz in cats, and the oscillations demonstrated by Mayer are thus likely low-frequency

They result from fluctuations in systemic vascular resistance due to changes in sympathetic output to the vascular beds of several regional circulations, including the kidney, mesentery and skeletal muscles.[261,276,611] Heart rate also exhibits middle-frequency oscillations, which appear to buffer the blood pressure oscillations in the upright position, and to enforce them in the supine position.[159]

There are two main theories regarding the mechanism of middle-frequency oscillations: the *central pacemaker theory* and the *baroreflex loop theory*. According to the central pacemaker theory, the middle-frequency oscillations are caused by a central rhythm generated by autonomic nuclei within the brainstem.[212,611] Indeed, a group of non-adrenergic, so-called pre-sympathetic vasomotor neurons within the rostral ventrolateral medulla show such intrinsic pacemaker-like activity.[522,523] The rostral ventrolateral medulla is part of a complex network of autonomic nuclei in the brainstem which may function as a pacemaker that drives oscillations in the sympathetic output to the cardiovascular system, and which is continually under the influence of baroreceptor feedback as well as input from other areas of the brain, that are involved in blood pressure regulation, fluid homeostasis, immune responses, and behaviour (*Figure A7.2*).

As of now, definitive evidence linking the activity of a central pacemaker in the brainstem to the generation of middle-frequency oscillations is nevertheless lacking.[333] According to the *baroreflex loop theory*, a given change in blood pressure is sensed by the arterial baroreceptors, and heart rate and systemic vascular resistance are consequently adjusted with a slight time delay in an attempt to buffer the initial change. Since this response is slightly shifted in time, it causes its own opposite change in blood pressure, which is then again sensed by the baroreceptor system. According to mathematical modelling, this

oscillations.[333,344] Since middle-frequency blood pressure oscillations nevertheless traditionally are designated Mayer waves, this may have led to misclassification of spontaneous blood pressure oscillations in some studies, particularly in relation to interspecies comparisons.

could cause systematic blood pressure waves, consistent with middle-frequency oscillations.[147,214,333] The link between these oscillations and baroreflex function is nevertheless ambiguous, since middle-frequency oscillations are reduced, but not abolished, by sino-aortic denervation in animals.[357] Furthermore, different interventions that increase (nitro-glycerine infusion), decrease (moderate physical exercise) or do not affect arterial baroreflex sensitivity (myocardial ischaemia) in humans have all been reported to increase the amplitude of these oscillations.[333] More recently, the baroreflex loop theory was modified as the middle-frequency oscillations were proposed to represent transient oscillatory responses to hemodynamic perturbations rather than true feedback oscillations.[147,261] Consequently, their amplitude is suggested to reflects a composite of the strength of the triggering perturbation and arterial baroreflex function.[147,261]

Since middle-frequency oscillations in blood pressure depend critically on the sympathetic output to the cardiovascular system, their amplitude, as evaluated by their spectral power, and thus their contribution to the overall variability in blood pressure, has often been used as a measure of sympathetic activity. Accordingly, an increase in sympathetic output to the cardiovascular system induced by either orthostatic stress[185,295,358] or pharmacologically induced hypotension[75] increases the spectral power of middle-frequency blood pressure oscillations. This relationship is, however, not clear cut, since other interventions that are likewise associated with an increased sympathetic output, such as arterial hypertension and heart failure show no change and a reduction, respectively, in the spectral power of middle-frequency blood pressure oscillations.[74,363] Concomitant changes in baroreflex function and/or endothelial function may act as confounding factors in this context. In terms of the latter, changes in vascular shear stress due to the blood pressure oscillations is thought to cause cyclic variations in nitric oxide release from the endothelium, which consequently reduces the amplitude of the blood pressure oscilla-

tions.[367,368,511] The middle-frequency blood pressure variability *per se* is thus not a robust quantitative index of sympathetic output to the cardiovascular system.[157,333]

A classical notion is that the specific frequency of the middle-frequency blood pressure oscillations depends on the output from a central pacemaker in the brainstem, a view that was mainly based on animal studies showing a preterminal frequency reduction during asphyxia-induced shock.[611] Notwithstanding that the specific frequency also depends on the time delay in the baroreflex arch, the size and geometry of the vascular tree,[261] a change in the output from a central pacemaker may indeed cause a shift of the frequency, so that an assessment of the predefined 0.07–0.14 Hz range may misestimate the actual middle-frequency spectral power. Along with changes in baroreflex function, this may, at least to some extent, contribute to the discrepant findings regarding the relationship between sympathetic output to the cardiovascular system and middle-frequency oscillations, as outlined above.

Low-frequency oscillations

In healthy humans, low-frequency oscillations occur at 0.03–0.07 Hz. Like middle-frequency oscillations, the low-frequency blood pressure oscillations appear to be caused mainly by fluctuations in systemic vascular resistance,[406] while the concomitant changes in heart rate largely depend on baroreflex influences.[147] Even though the latter may affect the amplitude of the oscillations, baroreflex influences do not appear to be critical to the generation of these oscillations as such. Hence, the oscillations in blood pressure persist despite aberration of the arterial baroreflex,[q] and the same central pacemaker mechanism

[q] Preiss and Polosa[440] found that spontaneous blood pressure oscillations were present at approximately 0.10 Hz in cats, and these were accompanied by similar oscillations in preganglionic sympathetic neuronal activity. The oscillations in preganglionic sympathetic neuronal activity remained when the blood pressure oscillations were abolished, either mechanically or by means of α-adrenergic receptor blockade. Montano *et al.*[357] observed that spontaneous blood pressure oscillations at a similar

that has been suggested for middle-frequency oscillations is likely involved.[440] Accordingly, the specific frequency of these oscillations at a given time may potentially reflect the output from the network of central autonomic nuclei to the cardiovascular system through the sympathetic nervous system.

Apart from baroreflex influences and changes in vascular shear stress,[333] the amplitude of low-frequency blood pressure oscillations appears to be dampened by cyclic changes in the release of vasopressin from the posterior pituitary gland[295,354] and similar cyclic changes in the renin-angiotensin system.[10,83,204,262] Furthermore, the activation of pathways related to regional thermoregulation may increase their amplitude.[10,145,279,416]

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frequency, also in cats, remained after section of baroreflex afferents. Together, these studies indicate that spontaneous blood pressure oscillations at approximately 0.10 Hz in cats occur independently of baroreflex influences, and both studies concluded that their findings were consistent with the presence of a central pacemaker. However, both studies wrongly classified the spontaneous oscillations as Mayer waves, and thus middle-frequency oscillations, although oscillations that occur at 0.10 Hz in cats are in fact low-frequency oscillations.

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