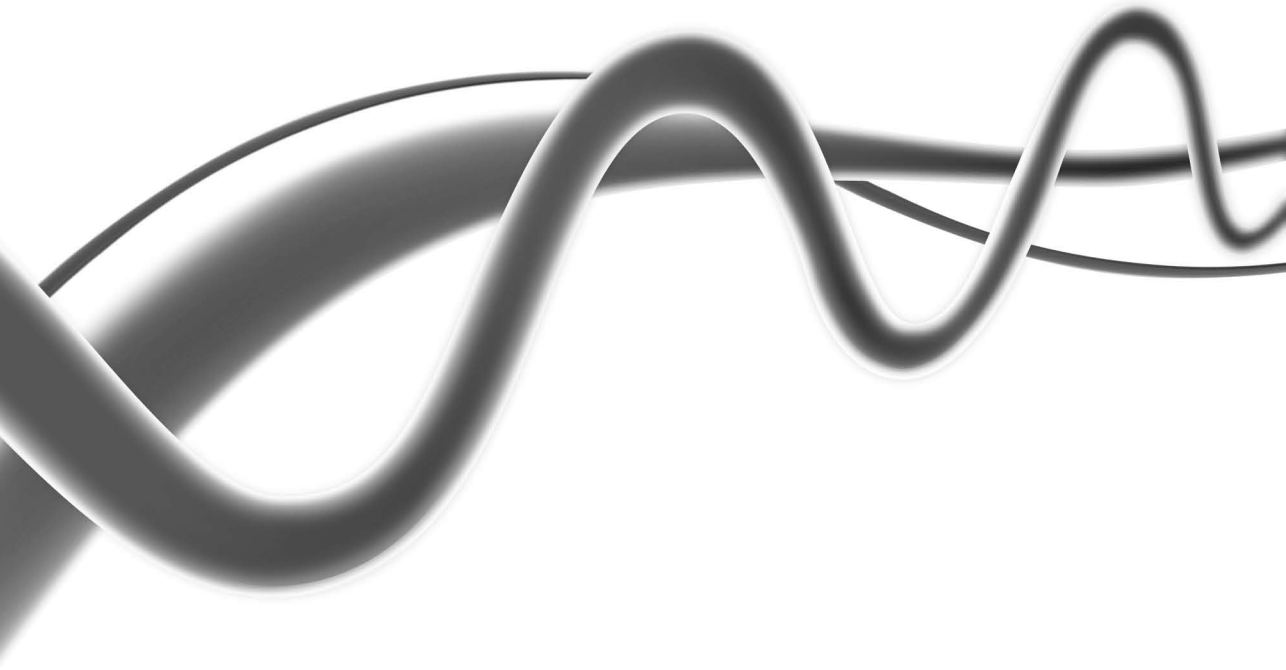
Cerebral hemodynamics in aging: the interplay between blood pressure, cerebral perfusion, and dementia

J.A.H.R. Claassen

Cerebral hemodynamics in aging:

the interplay between blood pressure, cerebral perfusion, and dementia

J.A.H.R. Claassen



the interplay between blood pressure, cerebral perfusion, and dementia

J.A.H.R. Claassen

Design cover and lay out by In Zicht Grafisch Ontwerp, Arnhem
Printed and bound by PrintPartners Ipskamp, Enschede

ISBN 978-90-9023498-4

The research presented in this thesis was performed at the department of Geriatric Medicine, Radboud University Nijmegen Medical Center, and at the Institute for Exercise and Environmental Medicine, Presbyterian Hospital Dallas and University of Texas Southwestern Medical Center at Dallas, TX USA.

Financial support for the publication of this thesis was kindly provided by
Janssen-Cilag BV; FMS, Finapres Medical Systems BV; AstraZeneca; Merck Sharp & Dohme BV; Novartis Pharma BV; Procter & Gamble; Servier Nederland Farma BV; Lundbeck BV; Wyeth.

Cerebral hemodynamics in aging:

the interplay between blood pressure, cerebral perfusion, and dementia

Een wetenschappelijke proeve
op het gebied van de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus, prof. mr. S.C.J.J. Kortmann,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 25 november 2008
om 10.30 uur precies

door

Jurgen Alain Henri Reinier Claassen
geboren op 20 april 1969,
te Maastricht

Promotores:

Prof. dr. M.G.M. Olde Rikkert

Prof. dr. B.D. Levine (UT Southwestern Medical Center at Dallas)

Copromotores:

Dr. R. Zhang (UT Southwestern Medical Center at Dallas)

Dr. R.W.M.M. Jansen

Manuscriptcommissie:

Prof. dr. J.W.M. Lenders (voorzitter)

Prof. dr. M.T.E. Hopman

Prof. dr. Ph. Scheltens (VU medisch centrum)

Prof. dr. F. Nobili (Università degli Studi di Genova)

Dr. J.W. Pasman

Het verschijnen van dit proefschrift werd mede mogelijk gemaakt door de steun van de Nederlandse Hartstichting en Internationale Stichting Alzheimer Onderzoek.

Cerebral hemodynamics in aging:

the interplay between blood pressure, cerebral perfusion, and dementia

An academic essay in the Medical Sciences

Doctoral thesis

to obtain the degree of doctor of philosophy
from Radboud University Nijmegen
on the authority of the Rector Magnificus, prof. dr. S.C.J.J. Kortmann,
according to the decision of the Council of Deans
to be defended in public on Tuesday, November 25, 2008
at 10.30 hours

by

Jurgen Alain Henri Reinier Claassen
born in Maastricht, The Netherlands,
on April 20, 1969

Doctoral supervisors:

Prof. dr. M.G.M. Olde Rikkert

Prof. dr. B.D. Levine (University of Texas Southwestern Medical Center at Dallas)

Co-supervisors:

Dr. R. Zhang (University of Texas Southwestern Medical Center at Dallas)

Dr. R.W.M.M. Jansen

Doctoral Thesis Committee:

Prof. dr. J.W.M. Lenders (Chair)

Prof. dr. M.T.E. Hopman

Prof. dr. Ph. Scheltens

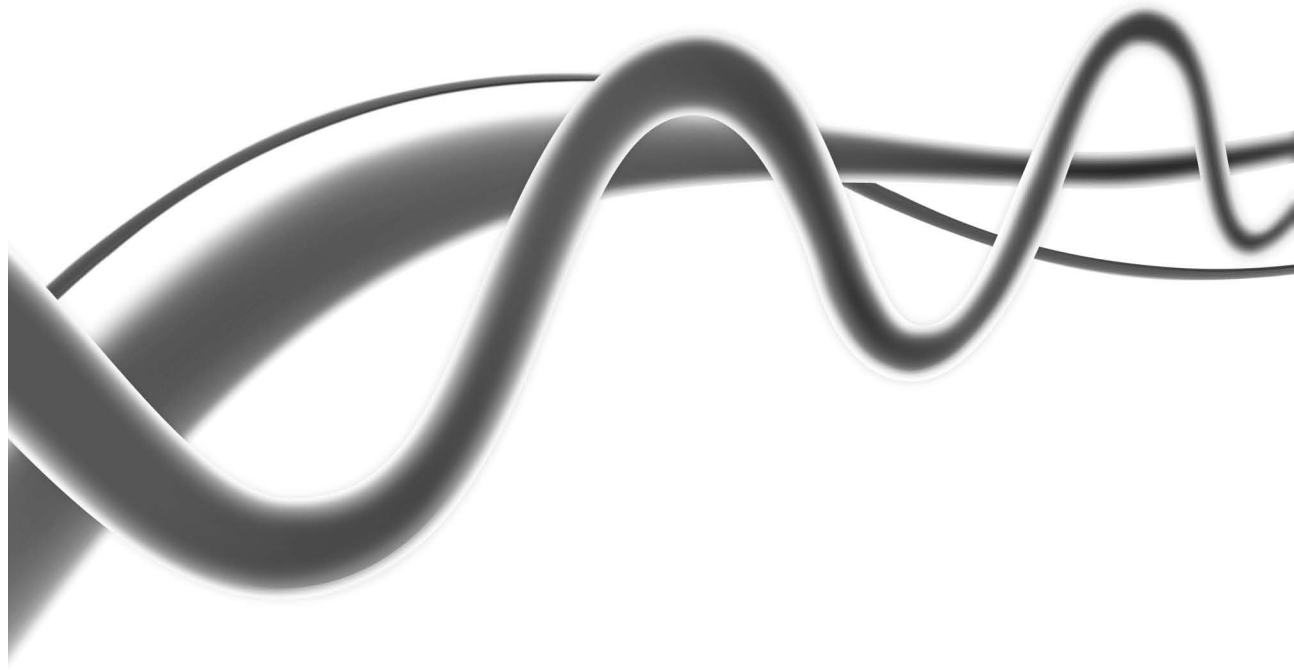
Prof. dr. F. Nobili (University of Genoa)

Dr. J.W. Pasman

Contents

Part 1

Introduction and clinical and methodological background



Chapter 1

Introduction and outline

The case report in paragraph 1.1 has been published as:
Claassen JA, Jansen RW. Carotid sinus syndrome: looking sideways is sufficient cause for syncope.
J Am Geriatr Soc 2006;54(1):188-9.

1.1 Case report

Carotid sinus syndrome (CSS) is found in up to one-third of older patients presenting with unexplained falls.[1] CSS is diagnosed when carotid sinus massage elicits a drop in systolic blood pressure of more than 50 mmHg, asystole of longer than 3 seconds, or both. This procedure is safe in older patients.[2] How CSS may lead to syncope outside test settings is still a matter of debate.[3] Wearing a tight collar, shaving, or turning the head are suggested to be eliciting factors. The present case clearly illustrates how merely looking sideways may be a sufficient cause for syncope.

A healthy 70-year-old woman participated in research in our department. She had been treated for hypertension in the past but was currently not taking any medication. She had never smoked and consumed alcohol with moderation. There were no abnormalities on physical examination, and her electrocardiogram (ECG) showed normal sinus rhythm. Carotid ultrasonography gave no evidence for atherosclerotic plaques or stenosis. It was noted that the left internal carotid artery had a marked tortuous trajectory, although flow patterns over both carotid arteries and both middle cerebral arteries (using transcranial Doppler) were symmetrically normal.

She underwent the following test protocol in the morning, after an overnight fast, in a quiet, cool room, wearing a loose-fitting shirt. Beat-to beat blood pressure (BP) and single-lead ECG were recorded using the Finometer (Finapres Medical Systems, Amsterdam, the Netherlands).

After 10 minutes of supine rest, mean BP ± standard deviation during the last minute was 178 ± 7 mmHg systolic, 79 ± 4 mmHg diastolic; heart rate was 62 ± 2 beats per minute (bpm). Upon standing, BP dropped to 119 mmHg systolic; mean BP during the 5-second nadir was 124 ± 4 mmHg systolic, 63 ± 3 diastolic, and heart rate was 83 ± 1 bpm. The subject was asymptomatic, and BP recovered quickly. After 1 minute of stable BP (165 ± 4 mmHg systolic, 86 ± 2 diastolic, heart rate 77 ± 2 bpm), the subject was asked to look to her right. BP decreased to 124 mmHg systolic, with mean BP during the 5-second nadir 127 ± 4 mmHg systolic, 67 ± 1 diastolic, with heart rate 74 ± 2 bpm. After 1 minute of stable BP (167 ± 5 mmHg systolic, 87 ± 3 mmHg diastolic, heart rate 82 ± 2 bpm), she was asked to look to her left. BP now decreased to 70 mmHg systolic, and heart rate briefly

dropped to 40 bpm immediately after turning the head. Mean BP during the 10-second nadir was: 73 ± 3 mmHg systolic, 42 ± 2 mmHg diastolic, and heart rate was 70 ± 4 bpm. At this time, she complained of transient fatigue and light-headedness. After hemodynamic stabilization, carotid sinus massage was performed in the supine position. On the right side, BP fell 40 mmHg, after brief bradycardia of 40 bpm. On the left side, BP decreased 33 mmHg after brief bradycardia of 45 bpm.

This case illustrates three important points. First, evidence is presented that a turn of the head to look sideways can contribute to syncope in a patient with CSS. In this subject, systolic BP fell 94 mmHg. This is sufficient to diagnose CSS of the vasodepressor type. A possible explanation for the greater sensitivity of the carotid sinus on the left side than on the right is the observed variant anatomy of the internal carotid artery.

Second, this case represents a healthy, community dwelling subject, without a history of falls or syncope, in whom CSS was diagnosed by chance. A population study in healthy volunteers found CSS in four of 32 participants.[4] Apparently, CSS is asymptomatic in certain affected individuals.

The third point illustrates that despite severe reductions in BP during standing, head turning, and carotid sinus massage, this subject was only symptomatic when her systolic BP fell almost 100 mmHg. This means that adequate or even improved dynamic cerebral autoregulation was able to prevent cerebral hypoperfusion and thus syncope, explaining her complete lack of symptoms in everyday life.

References

1. Kenny RA, Richardson DA. Carotid sinus syndrome and falls in older adults. *Am J Geriatr Cardiol 2001;10:97-99.*
2. Munro NC, McIntosh S, Lawson J, et al. Incidence of complications after carotid sinus massage in older patients with syncope. *J Am Geriatr Soc 1994;42:1248-1251.*
3. Healey J, Connolly SJ, Morillo CA. The management of patients with carotid sinus syndrome: is pacing the answer? *Clin Auton Res 2004;14 Suppl 1:80-86.*
4. McGlinchey PG, Armstrong L, Spence MS. Effect of carotid sinus massage and tilt-table testing in a normal, healthy older population (The Healthy Ageing Study). *Am J Cardiol 2002;90:1015-1017.*

1.2 Blood pressure and cerebral blood flow

Hypertension

Hypertension affects nearly a third of the general population.[1] The prevalence of hypertension increases dramatically with age, such that as much as 77% of subjects over age 70 are hypertensive.[2] The majority (≈60%) of hypertensive patients have isolated systolic hypertension. Hypertension is an important risk factor for cardiovascular disease. For example, hypertension is the strongest risk factor for stroke, with a more than doubling of stroke mortality risk for each 20 mmHg increase in systolic blood pressure, departing from 115 mmHg.[1] This association is found in all age groups, importantly however, the absolute risk is higher with advanced age.

The benefits of antihypertensive treatment have been well established, and although data in the very old (those over 80 and especially those over 85 years old) are scarce, these benefits clearly extend to elderly subjects over age 70. Despite these facts, only a third of hypertensive patients over age 70 receive proper treatment and have adequately controlled blood pressure; another third receive treatment but have inadequate control of hypertension; the final third is untreated.[2] Looking specifically at secondary prevention, e.g. hypertensive patients with a history of stroke, the number of patients receiving antihypertensive treatment is higher (70-80%). However, also in this group only a third of patients have adequate control of BP, defined as a systolic BP ≤ 140 mmHg.[2]

An important contribution to these low treatment rates is the widespread concern that the benefits of antihypertensive treatment in elderly patients are offset by negative effects of blood pressure (BP) lowering on morbidity and quality of life.[3,4] Specifically, it is feared that lowering of BP to targets set by current guidelines (ESH/ESC 2007, JNC-7) may cause cerebral hypoperfusion, resulting in ischemia, cognitive decline and/or falls. Hypothetically, cerebrovascular remodeling due to longstanding hypertension could impair vasodilatation, and together with diffuse small-vessel disease and age-related increases in vascular stiffness, this may pose an increased risk for cerebral hypoperfusion when blood pressure is lowered in elderly patients. These observations indicate the need for further studies that investigate the effects of hypertension and antihypertensive treatment on cerebral hemodynamics.

As a step in this direction, we have investigated the effects of rapid (within 1-2 weeks) blood pressure reduction, as well as of prolonged blood pressure lowering treatment (3 months) on cerebral hemodynamics in middle-aged subjects. Specifically, we have studied effects on dynamic cerebral autoregulation[5] (see chapter 2 for detailed information on cerebral autoregulation) and vasomotor reactivity (chapter 5 and 7).

Blood pressure instability

The case report (1.1), describing an elderly subject with carotid sinus hypersensitivity, is just one example of a wide array of common and less common causes of blood pressure instability in the elderly.

Orthostatic hypotension and post-prandial hypotension are commonly found in geriatric patients[6], and carotid sinus hypersensitivity has emerged as an important factor contributing to falls in the elderly.[7] As often in geriatric medicine, the underlying causes remain unknown, or are multifactorial.[8] For example, factors contributing to blood pressure instability may include volume deletion, cardiovascular (side) effects of medication, cardiac disease, or hypertension. In addition, an underlying condition may be autonomic dysfunction, associated with diabetes, Parkinson's disease or related lewy body diseases, frontotemporal dementia and possibly Alzheimer disease.[9]

It is interesting to note that in orthostatic hypotension/ post-prandial hypotension as well as in carotid sinus hypersensitivity, all attention is focused on blood pressure measurements. Arbitrarily defined cut-off values for reductions in blood pressure determine whether or not a "diagnosis" is made - with the exception of carotid sinus syndrome, where cardio-inhibition (asystole) is also taken into account. Still, also in the cardio-inhibitory carotid sinus syndrome the duration of asystole is arbitrary. The clinical relevance of these and other disorders of blood pressure regulation lies in their symptoms, for example dizziness, or falls -with (syncope) or without loss of consciousness, which often causes severe decline in health related quality of life. The symptoms mentioned are fully explained by impaired cerebral perfusion.[10] Mild symptoms (dizziness/light headedness) represent a mild or short-lasting reduction in cerebral blood flow, and severe symptoms (syncope) represent a substantial and/or prolonged reduction in cerebral blood flow.

In clinical practice and thinking, however, these symptoms are attributed directly to the observed reduction in blood pressure. This means that it is assumed -often inexplicitly- that cerebral blood flow varies proportionally to perfusion pressure (blood pressure).

Pressure-flow relationship of the cerebral circulation

According to Ohm's law, flow F is determined by pressure P and resistance R as F=P/R. As long as resistance is not changed, flow varies in proportion with pressure.

However, the brain is capable of adapting cerebrovascular resistance in order to compensate or reduce the effects of pressure on brain blood flow. This process is known as cerebral autoregulation. Consequently, it is incorrect to assume that changes in cerebral blood flow always parallel changes in blood pressure, and therefore that measurements of changes in blood pressure can suffice to predict the clinical symptoms associated with disorders of blood pressure regulation. This is clearly illustrated by the case report in the beginning of this chapter. It logically follows that measurements of cerebral blood flow will better predict these symptoms, and would make a useful addition to measurements of blood pressure alone. A preceding dissertation from our group, "Hypotensive syndromes and

cerebral oxygenation in elderly patients" (Mehagnoul-Schipper, 2003), made this transition by adding measurements of cerebral hemodynamics to research into disorders of blood pressure regulation.

Chapter 2 of this thesis provides an extensive review of the concept of cerebral autoregulation. It includes an overview of the historical developments of techniques to measure cerebral perfusion. From this it is clear that in the past, easily performed measurements of blood pressure were used as a surrogate marker of changes in flow, because the techniques available at that time to measure cerebral blood flow were not clinically applicable. This has changed with the development of transcranial Doppler ultrasonography by Rune Aaslid in the 1980's. As detailed in chapter 2, this technique has enabled us to perform non-invasive and non-obtrusive measurements of changes in brain blood flow that have now been incorporated in clinical practice.[11]

Application in clinical geriatrics

Chapter 2 introduces examples of diseases and conditions that are prevalent in the aging population and that have in common that knowledge of cerebral hemodynamics is important to further our understanding of these conditions and to develop or improve therapeutic options. It also becomes clear that at present only limited information is available on cerebral autoregulation in aging, especially in those older than 75 years.

Together, the findings laid out in this chapter indicate the importance of identifying methods that can provide information on the dynamic pressure-flow relationship of the cerebral circulation, and that at the same time remain well suited for use in elderly subjects and geriatric patients.

1.3 Dementia and cerebral blood flow

So far, we have considered how the cerebral circulation is affected by changes in blood pressure. Now, we will take a different approach by looking at how disorders of the brain itself, or more specifically, dementia syndromes, lead to alterations in perfusion.

The interaction between dementia and cerebral blood flow is very complex, and has only begun to be unraveled. A brief introduction to those aspects that are relevant for this thesis will follow.

Vascular innervation

In chapter 3, using the example of Alzheimer's disease, the evidence is reviewed that there is an "intrinsic" brain vascular innervation that can alter cerebral perfusion. For example, cholinergic neurons, originating from the nucleus basalis of Meynert –located at the base of the brain- have both direct and indirect (through nitrergic interneurons) contact with cortical blood vessels.[12] Stimulation of these neurons causes vasodilatation, and inhibition leads to vasoconstriction.

Alzheimer's disease is characterized by a profound loss of cholinergic innervation. As explained in chapter 3, this may in part explain the reduction in cerebral blood flow in this disease. Also, this suggests that treatment aimed at restoring cholinergic balance may influence cerebral perfusion. Of interest, loss of cholinergic function is also found in vascular dementia, dementia with Lewy bodies, and Parkinson's dementia.[12] The chapter concludes with suggestions for measurement techniques to further study this topic.

Vascular disease and dementia

A simplified description of current views on dementia would see Alzheimer's disease as a neurodegenerative disease characterized by amyloid-beta deposition in plaques and hyperphosphorilated tau in tangles. It is distinguished from vascular dementia, characterized by cerebrovascular lesions secondary to vascular disease. Current consensus criteria (NINCDS-ADRDA, 1984) are such that the diagnosis of Alzheimer's disease is made by exclusion; importantly, "vascular" criteria are among the excluded conditions.

However, large epidemiological studies such as the Rotterdam study have demonstrated an association between vascular risk factors and Alzheimer's disease. For example, the presence of mid-life hypertension,[13] or carotid artery disease,[14] increase the risk of developing Alzheimer's disease dementia in later life (relative risk up to 2.5). However, these studies were not designed to identify the nature of this association.[15] Regardless, these findings are difficult to reconcile with

a view of Alzheimer's disease that excludes a possible contribution of vascular pathology, and call for further research to address the interaction of Alzheimer's disease and cerebrovascular pathology. In this way, this epidemiological research generates questions and hypotheses that can be further explored by basic science.[16]

1.4 Aims

This thesis addresses two primary questions. First, we aimed to investigate how dysregulation of blood pressure, which is often seen in elderly subjects with and without cognitive decline, is related to changes in cerebrovascular perfusion, and how this relationship can be reliably and validly measured. As such this research is a continuation of the research presented in the dissertation "hypotensive syndromes and cerebral oxygenation in elderly patients" by Mehagnoul-Schipper, 2003. In the latter thesis, Near Infra-Red Spectroscopy (NIRS) was introduced as a method to study the effect of hypotensive syndromes on cerebral oxygenation. In the present thesis, the emphasis was shifted to the role of transcranial Doppler ultrasonography (TCD) to measure changes in cerebral hemodynamics, with the ultimate goal to use combined measurements of Finapres, TCD and NIRS.

The second question approaches this matter from a different angle. With the hypothesis that Alzheimer's disease may be associated with alterations in blood pressure regulation and cerebral perfusion, our aim was to investigate whether such changes can be measured with the proposed techniques of Finapres and TCD.

1.5 Outline of this thesis

These two general aims have been translated in a series of experiments and background studies, which are presented in the subsequent chapters of this thesis.

Chapter 2 provides a further clinical background for the evaluation of cerebral hemodynamics in the elderly population, and describes the application of transcranial Doppler ultrasonography (TCD) to measure cerebral blood flow-velocity, a technique that will be used throughout this thesis. The concept of cerebral autoregulation is explained and the currently available methods to test and quantify cerebral autoregulation are described. Specifically, the technique of transfer function analysis is explained in detail, as this technique is further explored in this thesis.

Chapter 3 provides background information about the interaction of dementia and Cerebral Blood Flow (CBF). This review indicates the need for methods to measure cerebral hemodynamics in patients with Alzheimer's disease, on the one hand to identify the effects of the disease on cerebral perfusion, and on the other hand the possible effects of treatment on cerebral perfusion.

Chapter 4 continues research into the application of NIRS in the elderly. NIRS can be used for example to measure cerebral oxygenation. Several validation studies have been performed, however, studies in aging were lacking. In this chapter, the reproducibility of NIRS in an aging population will be examined.

Chapter 5 introduces vasomotor reactivity as a method to investigate cerebral hemodynamics. Brain vasculature is extremely sensitive to changes in CO_2, and this has led to the developments of tests to measure the reduction in cerebral blood flow during hypocapnia, and the increase in flow during hypercapnia. In this chapter, a new method is reported that investigates dynamic changes in the CO_2-CBF relationship, and takes into account the confounding effects of changes in blood pressure.

Chapter 6 proposes a new method to measure dynamic cerebral autoregulation. Specifically, transfer function analysis of spontaneous oscillations (see chapter 2) has certain limitations. This chapter introduces a method of repeated squat-stand maneuvers to induce oscillatory changes in blood pressure and cerebral blood flow, aimed at enhancing transfer function analysis.

Chapter 7 applies the method that was introduced in chapter 5 to investigate the effects of blood pressure lowering on cerebral vasomotor reactivity in patients with hypertension.

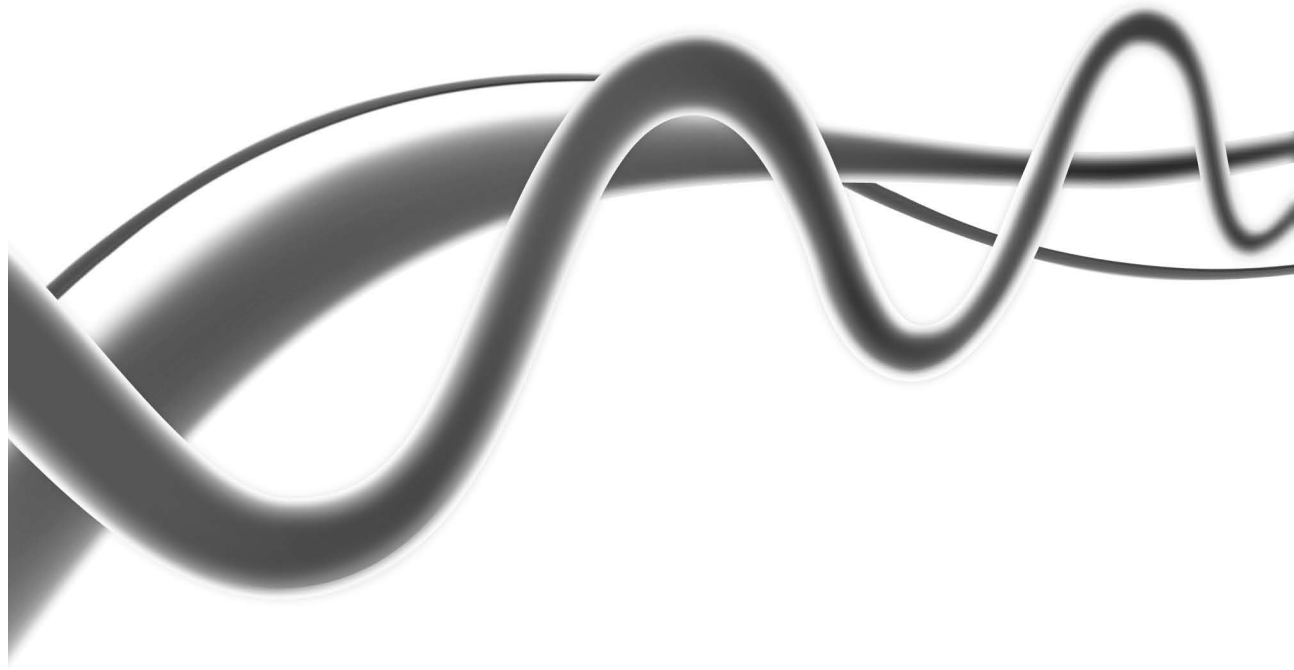
Chapter 8 applies the method described in chapter 6, and to a lesser extent the method described chapter 5, to investigate cerebral hemodynamics in patients with early Alzheimer's disease.

Chapter 9 provides a summary and discussion of the findings in this thesis.

References

1. Zhang H, Thijs L, Staessen JA. Blood pressure lowering for primary and secondary prevention of stroke. *Hypertension 2006; 48(2):187-195.*
2. Wong ND, Lopez VA, L'Italien G, Chen R, Kline SE, Franklin SS. Inadequate control of hypertension in US adults with cardiovascular disease comorbidities in 2003-2004. *Arch Intern Med 2007; 167(22):2431-2436.*
3. Oates DJ, Berlowitz DR, Glickman ME, Silliman RA, Borzecki AM. Blood pressure and survival in the oldest old. *J Am Geriatr Soc 2007; 55(3):383-388.*
4. Birns J, Markus H, Kalra L. Blood pressure reduction for vascular risk: is there a price to be paid? *Stroke 2005; 36(6):1308-1313.*
5. Zhang R, Witkowski S, Fu Q, Claassen JA, Levine BD. Cerebral hemodynamics after short- and long-term reduction in blood pressure in mild and moderate hypertension. *Hypertension 2007; 49(5):1149-1155.*
6. Vloet LC, Pel-Little RE, Jansen PA, Jansen RW. High prevalence of postprandial and orthostatic hypotension among geriatric patients admitted to Dutch hospitals. *J Gerontol A Biol Sci Med Sci 2005; 60(10):1271-1277.*
7. Davies AJ, Steen N, Kenny RA. Carotid sinus hypersensitivity is common in older patients presenting to an accident and emergency department with unexplained falls. *Age Ageing 2001; 30(4):289-293.*
8. Davison J, Bond J, Dawson P, Steen IN, Kenny RA. Patients with recurrent falls attending Accident & Emergency benefit from multifactorial intervention--a randomised controlled trial. *Age Ageing 2005; 34(2):162-168.*
9. Allan LM, Ballard CG, Allen J et al. Autonomic dysfunction in dementia. *J Neurol Neurosurg Psychiatry 2007; 78(7):671-677.*
10. Claassen JA. Cerebral perfusion in neurogenic orthostatic hypotension. *Lancet Neurol 2008;7(7):573-574.*
11. van Beek AH, Claassen JA, Rikkert MG, Jansen RW. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab 2008; 28(6):1071-1085.*
12. Claassen JA, Jansen RW. Cholinergically mediated augmentation of cerebral perfusion in Alzheimer's disease and related cognitive disorders: the cholinergic-vascular hypothesis. *J Gerontol A Biol Sci Med Sci 2006; 61(3):267-271.*
13. Launer LJ, Ross GW, Petrovitch H et al. Midlife blood pressure and dementia: the Honolulu-Asia aging study. *Neurobiol Aging 2000; 21(1):49-55.*

14. van Oijen M, de Jong FJ, Witteman JC, Hofman A, Koudstaal PJ, Breteler MM. Atherosclerosis and risk for dementia. *Ann Neurol 2007; 61(5):403-410.*
15. Claassen JA. Alzheimer's disease: trade-off for increased survival with atherosclerosis? *Ann Neurol 2007;DOI 10.1002/ana.21285.*
16. Claassen JA. Epidemiology: friend or foe? *BMJ 2004; 329:467.*



Chapter 2

Cerebral autoregulation:
an overview of current concepts and methodology
with special focus on the elderly

Arenda HEA van Beek
Jurgen AHR Claassen
Marcel GM Olde Rikkert
René WMM Jansen

Journal of Cerebral Blood Flow and Metabolism 2008;28:1071-1085.

Abstract

Cerebral autoregulation (CA) refers to the properties of the brain vascular bed to maintain cerebral perfusion despite changes in blood pressure (BP). Whereas classic studies have assessed CA during changes in BP that have a gradual onset, dynamic studies quantify the fast modifications in cerebral blood flow (CBF) in relation to rapid alterations in BP. There is a lack of standardization in the assessment of dynamic CA. This review provides an overview of the methods that have been applied, with special focus on the elderly. We will discuss the relative merits and shortcomings of these methods with regard to the aged population. Furthermore, we summarize the effects of variability in BP on CBF in older people. Of the various dynamic assessments of CA, a single sit-tostand procedure is a feasible and physiologic method in the elderly. The collection of spontaneous beat-to-beat changes in BP and CBF allows estimation of CA using the technique of transfer function analysis. A thorough search of the literature yielded eight studies that have measured dynamic CA in the elderly aged < 75 years. Regardless of the methods used, it was concluded from these studies that CA was preserved in this population.

Introduction

The brain has a high metabolic demand and therefore requires adequate nutritional flow. To maintain stable cerebral blood flow (CBF), the brain's vasculature must respond to changes in arterial blood pressure (BP) or intracranial pressure. Thus, cerebral blood vessels have an inherent ability to keep CBF constant over a wide range of systemic BP levels, by means of myogenic, neurogenic, or metabolic mechanisms.[1,2] In response to a variation in perfusion pressure (mean arterial pressure–intracranial pressure), an adaptation in cerebrovascular resistance (CVR) will cause CBF to return to its baseline. This hemodynamic process is known as cerebral autoregulation (CA).[1,3]

The term 'autoregulation' within the cerebral circulation was introduced by Lassen[4]. His review covered more than 200 clinical studies assessing CBF using the inert gas method (which measures arterial-venous gas difference), developed by Kety and Schmidt, and the indicator dilution method (which measures the venous dilution of an intraarterially injected indicator), developed by Gibbs, Maxwell, and Gibbs.[4] Notably, the more recently developed positron emission tomography scanning technique enabling, for example, measurement of cerebral oxygen metabolism, is founded on these principles.

Lassen's review included the now classic studies regarding static CA (sCA), which describes the response of CBF, averaged over a longer period of time, to long-term changes in BP with gradual onset. By plotting these changes in CBF and BP, it was found that CBF remains constant over a wide range of BP changes – the 'plateau phase,' with the upper and lower limits forming the boundaries of CA. It should be noted, however, that this classic single curve was a composite of many studies, and critical analysis of this work brings into question its validity.[1] Frequently, a slight slope exists over the pressure range where the supposed plateau had been plotted.[5] Predictions from a physiologic computer model are that in between the lower and upper boundaries of this plateau phase, CBF actually changes from 80% to 120% of baseline.[6] Nonetheless, between these upper and lower limits, autoregulation of CBF is effective. It is important to realize that these boundaries are not fixed.[1] Chronic hypertension, for example, shifts these limits of autoregulation toward higher BP levels. This adaptation protects the brain against hypertension,

but may also render it more vulnerable to hypoperfusion during episodes of hypotension.[1]

The classic studies of CA are performed in the semi-steady state. The changes in BP occur gradually and the measured CBF values reflect the results of adaptation of CVR to the new, stable BP value. In other words, these studies assess the outcome of CA rather than the process of CA itself. In terms of maintaining sufficient nutritional blood flow, these semi-steady state studies provide no information as to whether a period of hypoperfusion precedes the eventual return to stable perfusion. Furthermore, the clinical applicability of sCA estimation is limited, because it requires pharmacological interventions to establish a sustained period of hypertension and hypotension.

More recent technological developments, such as transcranial Doppler ultrasonography (TCD) and servo-controlled finger photoplethysmography (Finapres), have offered possibilities to investigate the dynamics of the pressure–flow relationship of the cerebral circulation. In contrast to the classic studies, dynamic studies of CA quantify the fast modifications in CBF velocity (CBFV) in a major cerebral artery in relation to rapid alterations in BP within the upper and lower limits (plateau phase) of sCA. This approach allows differentiation of the CA response to fluctuations in beat-to-beat BP of different magnitudes and durations. Also in contrast to the classic studies, dynamic studies can identify episodes of reduced flow in this major perfusing artery in the period needed for CBF to return to baseline after hypotension. However, these dynamic studies are not able, at present, to measure flow at the brain tissue level. For studies of dynamic CA (dCA), a pharmacological intervention is not required, and the method is entirely noninvasive, which makes it clinically suitable. Recently, dCA has been shown to be impaired in several conditions that are highly prevalent in the elderly, such as ischemic stroke[7] and carotid stenosis[8]. Therefore, the vulnerable brain in these patients is insufficiently protected against the effects of rapid BP changes. Furthermore, postprandial hypotension or orthostatic hypotension, both common hypotensive syndromes in elderly patients[9], may lead to cerebral symptoms like syncope, falls, and dizziness.[10,11] Whether these hypotensive syndromes result in symptoms and clinical signs and thus have clinical consequences is largely determined by the process of CA.[12,13]

In hypertension, dCA has been addressed only in those who were < 75 years old. In mild-to-moderate hypertension, dCA is unimpaired[14], and reduction of BP to achieve normotension is not associated with cerebral hypoperfusion in middle-aged subjects[15] and elderly subjects < 75 years.[16] Although the beneficial effects of antihypertensive treatment in preventing cardiac disease and stroke are well known[17], whether it is similarly safe to treat hypertension in the elderly cannot be answered at present.[18]

In contrast with studies of sCA, no uniform method exists to measure, analyze, and report dCA. In fact, the various methods applied differ in the type of BP stimuli (spontaneous or induced BP fluctuations) and in the model for analysis and interpretation.

Studies describing dCA in the elderly and in diseases affecting older people have used different methods to evaluate the dynamic autoregulatory capacity of the brain. The important clinical question whether aging affects CA can therefore only be attempted to be answered after careful interpretation of these different methodologies.

In this review, we will first provide an overview of measurements of the various parameters that have been applied to assess dCA. Also, we will discuss the different methods to provoke cerebral hemodynamics and allow dynamic assessments of CA. Later, we present an overview of dynamic studies of CA in the elderly and will discuss the interaction between CA and disease.

Review of Methods

Measurements

To evaluate the dynamic process of the autoregulatory capacity of the human brain, assessment of BP and CBF with high temporal resolution is essential, and photoplethysmography (Finapres) and TCD enable these measurements, respectively.[19-21] During all measurements of cerebral hemodynamics, changes in end-tidal CO_2 should be closely monitored. We will address the measurements of these parameters in more detail below.

Cerebral blood flow: The inert gas method and the indicator dilution methods measure global CBF as blood flow in volume per unit weight per unit time (for example, mL per 100 g per min). These methods are rather invasive and time-consuming, and the indicator dilution methods have a low temporal resolution.

In contrast, TCD is noninvasive, easy to use, and offers a very high temporal resolution (< 0.1 secs) to monitor changes in CBF.[19] Transcranial Doppler ultrasonography measures flow-velocity, in centimeter per second, in one of the supplying arteries of the brain, usually the middle cerebral artery (MCA). The flow-velocity in the MCA measured by TCD is determined by both the flow in the MCA and by its diameter, or rather its cross-sectional area. Therefore, changes in flowvelocity recorded with TCD represent changes in flow, provided that the diameter of the insonated vessel remains constant. It has been shown in (neuro) surgical patients that the diameter of the MCA does not significantly increase or decrease during, respectively, a mean decrease or increase in BP of 30 (SD 16) mm Hg.[22,23] Furthermore, in healthy subjects, lower body negative pressure at 20 or 40 mm Hg induced decreases in flow-velocity in the MCA, whereas diameter changes did not occur during these tests.[24] These hemodynamic changes are similar to those encountered in dynamic tests of autoregulation, as will be illustrated later. In this review, consistent with the literature on this subject, flow-velocity in the MCA as recorded with TCD is denoted as CBFV, and it is assumed that the changes in CBFV reflect changes in CBF. However, in contrast with other methods (see above), this representation of CBF does not include brain tissue weight. As a further caveat, these measurements only reflect changes in flow for the perfused territory. It is conceivable that the perfused territory of the insonated vessel (mostly the MCA) might change under pathologic conditions, such as focal cerebral ischemia or vasogenic edema, and under nonpathologic conditions, such as hyper- or hypocapnia and extreme hypoxia.

Some TCD devices include a provision to determine an index of the cross-sectional area of the vessel being insonated, according to the backscattered power. In surgical patients, the vessel area increased after inhalation of isoflurane, whereas CBFV remained unchanged.[25] A similar observation was made during combined hypoxia and hypercapnia, whereas during either hypoxia or hypercapnia alone, no change in vessel diameter was noted.[26] These data suggest that stability of vessel diameter cannot be assumed under all conditions.

Arterial blood pressure: Blood pressure can be measured noninvasively and beat to beat with a servo-controlled finger photoplethysmography as in the Finapres device.[21] The obtained BP recordings using this device correspond well to invasive BP recordings in the brachial artery under various conditions, including during assessment of dCA.[20]

The combination of Finapres and TCD has made it possible to study the dynamic process of CA.

During BP recordings with Finapres, the finger is held at heart level. In autoregulation research using Finapres, changes in mean BP measured at heart level are used to estimate changes in cerebral perfusion pressure both in the supine position and during standing or head-up tilt. However, it has been argued that finger BP does not represent cerebral perfusion pressure because of the hydrostatic gradient between finger and brain when the subject is sitting or standing. Conversely, if the heart–brain circulation is considered to represent a closed-loop system, the subject's body position should not matter. This is based on the assumption that the intracranial and cerebral venous pressures are relatively low and constant and that, during standing or sitting, hydrostatic components of intracranial and cerebral venous pressure at head level are reduced commensurate with arterial pressure as for a closed circulatory system.[27] However, controversies regarding these assumptions must be recognized.[28] Currently, there are no experimental data that either unequivocally support or disprove this theory.[29]

Carbon dioxide: Monitoring of CO_2, mostly as endtidal CO_2, is essential in studies on cerebral perfusion and autoregulation. CO_2 is a very potent cerebral vasodilator, and hypercapnia may cause a 3% to 5% increase in CBF per mm Hg rise in endtidal CO_2.[30,31] Hypocapnia induces vasoconstriction and can reduce CBF by 2% to 3% per mm Hg reduction in end-tidal CO_2. Thus, changes in respiratory pattern during tests of autoregulation may greatly confound the measurements of CBF. Moreover, CO_2 causes sympathetic activation and may cause a substantial increase in BP.[31]

Methods to Induce Hemodynamic Changes

Essential for the evaluation of CA dynamics is to observe the response of cerebral perfusion to an alteration in BP. There are several potential methods to induce oscillations or rapid changes in BP, of which the thigh cuff method was the first to be introduced.

Step decreases in blood pressure: Aaslid et al.[32] introduced the thigh cuff method, which induces step decreases in BP by rapid deflation of cuffs around both thighs after an inflation lasting at least 2 mins. A different approach to reduce BP is lower body negative pressure. If the atmospheric pressure surrounding the lower body (which is enclosed in a sealed box) is reduced, this reduces the extravascular pressure, which causes dilatation of veins and a lowered vascular resistance, increasing the flow to the lower limbs.[33]

Of interest, even without a change in mean BP, CBFV decreases during lower body negative pressure.[15,34] The exact mechanism that is responsible for this reduction in CBFV during lower body negative pressure remains uncertain; recently it was shown that it is not likely due to cerebral vasoconstriction caused by sympathetic activation.[15]

Both the thigh cuff and the lower body negative pressure techniques have been developed to allow assessment of dCA in the supine position. This is of relevance in patients who are confined to bed, for example, in head trauma patients in an intensive care unit. The thigh cuff method can be painful[35] and is not a method of choice in aged subjects, although pain can be reduced if large-sized cuffs are used (R Aaslid, personal communication). Lower body negative pressure is also an uncomfortable procedure that is not well tolerated and cannot be applied in obese subjects. Therefore, both techniques are not ideally suited for the assessment of dCA in elderly patients. As the majority of these patients can be tested in an upright position, alternative methods to induce oscillations in BP can be used more efficiently.

A simple and feasible method used to assess dCA is the Valsalva maneuver, which causes characteristic changes in BP and CBFV.[36] In this procedure, the decrease in BP is due to elevation of intrathoracic and intraabdominal pressure induced by straining. With the increase in intrathoracic pressure, the venous return decreases, causing a reduction in cardiac output and hence a decrease in BP, which in turn leads to baroreflexmediated vasoconstriction.[37] The increase in intrathoracic pressure also causes an increase in intracranial pressure[38], leading to a considerably stronger reduction in the cerebral perfusion pressure than indicated by the BP decrease. In addition, an increase in end-tidal CO_2 pressure during the Valsalva maneuver has been observed[39] and the effect on cerebral vessels of a change in arterial CO_2 pressure has to be taken into account. These observations suggest that the Valsalva maneuver is too complex a method to quantify the effects of changes in BP on CBFV.

Placing a hand in ice water (cold pressor test), as well as isometric exercise such as sustained handgrip, result in increased BP by stimulating sympathetic efferent pathways. Both methods have been used for the assessment of dCA.[40-42] However, the CBFV response to these tests is very heterogeneous.

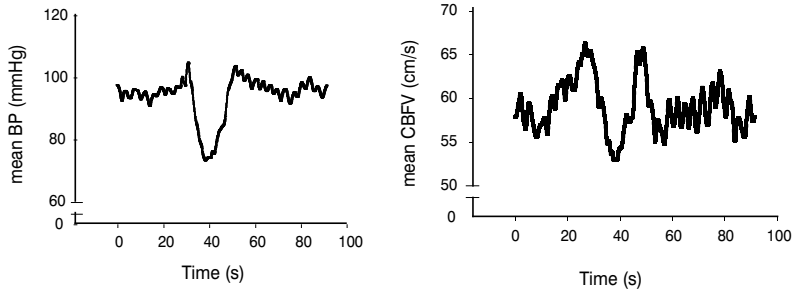
A different stimulator of the sympathetic nervous system is passive head-up tilt.[43] Cerebral blood flow velocity decreases during head-up tilt, and this test has been used to assess dCA in patients with syncope.[44] However, during head-upright tilt in healthy subjects, a slight but significant decrease in both end-tidal CO_2 pressure and CBFV has been observed, without a change in the rate of breathing.[45] The change in end-tidal CO_2 is thought to be due to the changes in the ventilation–perfusion relationship during tilting.

The role of the sympathetic nerve system in the regulation of CBF remains controversial. The cerebrovascular bed is well innervated by both sympathetic and parasympathetic nerve fibers[46], but most studies in animals have found that baseline CBF does not change after autonomic denervation.[47] On the contrary, Zhang et al.[48] found that dCA was altered markedly after ganglion blockade, suggesting that autonomic neural control of the cerebral circulation is involved in beat-to-beat regulation of CBFV. Therefore, possible direct effects on cerebral vessels of sympathetic activation have to be taken into account when using these tests to induce an increase in BP and assess dCA.

A more straightforward technique used to assess dCA is the sit-to-stand procedure, which induces a depressor change in BP and CBFV. The reduction in BP (of

approximately 25%), resulting from an immediate and marked decrease in total peripheral resistance upon standing, is presumably caused by cardiopulmonary baroreflex mechanisms.[49] This characteristic decrease in BP, named initial orthostatic hypotension, is absent in passive tilt testing.[50] After this initial decrease in total peripheral resistance, the arterial baroreceptors respond to the hypotension and the cardiopulmonary receptors react to the decline in preload, leading to an increase in total peripheral resistance. In Figure 1, we present a typical BP and CBFV response in a healthy elderly subject during a sit-to-stand maneuver. Standing up causes a small decrease in end-tidal CO_2, which cannot, however, explain the immediate decrease in CBFV upon standing.[51]

Figure 1 Blood pressure (BP) and cerebral blood flow velocity (CBFV) response during a sit to stand maneuver



Continuous beat-to-beat blood pressure (BP) and cerebral blood flow-velocity (CBFV) measurements during a sit to stand maneuver in a healthy elderly subject. Beat-to-beat data were resampled at 2 Hz to create an equal time base. The subject starts standing after 30 s.

When changing position from sitting to standing, the Doppler probe remains in the same spatial in relation to the heart and Finapres finger cuff throughout the procedure in contrast to passive head-up tilt, and thus avoiding the issue of a possible hydrostatic effect on cerebral perfusion pressure. The sit-to-stand

procedure is a useful and feasible method to test dCA in elderly subjects. An additional benefit of this method is that it represents a physiologic challenge that occurs in daily life.

Oscillations in blood pressure: The techniques described above provide a single-step challenge to cerebral hemodynamics. In contrast, periodic squatting[52], oscillatory lower body negative pressure[53,54], oscillatory thigh cuff inflation and deflation[55], and periodic breathing[56] give rise to almost sinusoidal oscillations in BP. When BP is forced to oscillate at a determined frequency, CBFV follows these induced oscillations. These induced periodic variations in BP and CBFV can be used for the assessment of dCA.

A disadvantage of the periodic breathing approach is that it may lead to a larger tidal volume and induce hypocapnia. Instructing patients to breathe slowly and with small tidal volumes can prevent hypocapnia. However, older subjects and subjects with cognitive impairment may not be able to adequately perform this technique. The same holds true for the technique of periodic squatting. The disadvantages of lower body negative pressure and thigh cuffs have been described above. A single sit-to-stand maneuver is well tolerated in elderly subjects. Therefore, repeated sit-stand maneuvers may be easier to perform and better tolerated in the elderly population. However, this procedure has never been tested to produce sinusoidal oscillations in BP. It could be useful to test a well-developed, repeated, sit-to-stand protocol in experiments in the elderly population.

Fortunately, it is also possible to estimate dCA without inducing changes in BP. Blood pressure oscillations ranging from 0.02 to 0.4 Hz occur in humans during daytime and nighttime without an externally triggered BP manipulation, independent of stimuli of daily life.[57] If spectral analysis is applied to this BP variability, three frequency ranges are noticeable: one around the respiratory frequency showing peaks at 12 cycles per minute (the high-frequency region, respiratory waves, around 0.2 to 0.4 Hz); one around 6 cycles per minute related to variations in vasomotor tone (mid-frequency region, Mayer waves at 0.1 Hz); and the lowest frequency region, of which the cause is speculative, around 1 cycle per minute (low-frequency region, 0.02 to 0.07 Hz; alternatively named the very low frequency).[58] Similarly, CBFV measured by TCD is not constant over time. Lindegaard et al.[59] found

individual ranges of spontaneous fluctuations between ±15% and ±35% of the mean flow in patients after carotid endarterectomy. Diehl et al (1991) found oscillations up to 30% of the mean CBFV with low-frequency oscillations between 0.4 and 9 cycles per min. In conclusion, spontaneous fluctuations of BP and CBFV may be useful for the assessment of dCA.[60] This mechanism limits the need for the above-mentioned methods to induce alterations in BP and seems to be the simplest method for the assessment of dCA in a clinical setting.

Baseline (resting) measurements of BP are the most physiologic situation to assess dCA. However, they depend on the magnitude of the spontaneous oscillations. This magnitude, in turn, depends on the variability of BP, which is not always high enough to allow estimations of dCA. Furthermore, spontaneous oscillations in the low-frequency range appear inconsistently. These reasons may explain the lack of coherence between BP and CBFV (we will further explain and discuss the coherence below) in approximately 10% of all spontaneous oscillation recordings.[61] In this situation, augmented oscillations are expected to increase coherence between BP and CBFV, and it will be necessary to induce these oscillations using repeated maneuvers as described above.

Quantifications of Dynamic Cerebral Autoregulation

After a change in BP, the relationship between CBF and BP has to be quantified. Here, we will discuss the methods that are most commonly used in clinical situations. These methods approach the relationship between BP and CBF as if it is linear. It should be noted that more complex mathematical models have been developed that allow for nonlinear dynamics.[62]

In Table 1, we provide an overview of the different quantifications discussed below.

Cerebrovascular resistance: Linear system analysis facilitates the examination of the transfer of BP oscillations to CBF as a measure of autoregulation. It quantifies the extent to which the BP input signal is reflected in the CBFV output signal. The regulator between the input and output is the resistance of the small cerebral vessels distal to the site of insonation. According to Ohm's law, this resistance is defined as mean BP/CBF. Thus, CVR is defined by mean BP divided by mean CBF:

$$CVR = \frac{BPmean}{CBFmean}$$

The use of CBF to calculate CVR takes into account the brain weight, whereas studies that use TCD and measure flow-velocity lack this tissue weight. These studies calculate the CVR index (CVRi), which is defined by the mean BP divided by mean CBFV:

$$CVRi = \frac{BPmean}{CBFVmean}$$

Studies that use CBFV ideally would have to take into account the cross-sectional area factor between CBF and CBFV in addition to brain tissue weight in their estimates of CVR. Furthermore, it should be emphasized that the use of the ratio between BP and CBF (velocity) to represent CVR (index) corresponds to a model where the cerebrovascular circulation is characterized by a single parameter. This calculation infers from Ohm's law and is based on a number of hypotheses, and resistance is not measured directly.

Gosling's pulsatility index (PI) is sometimes used to reflect CVR.63 This index is defined as the difference between systolic and diastolic extremes of CBFV divided by the mean CBFV:

$$PI = \frac{CBFVsys - CBFVdias}{CBFVmean}$$

The PI changes with alterations in CVRi in the following manner. CVRi and the PI are both inversely correlated to CBFV in steady state (e.g., reduction in CVRi causes an increase in CBFV and a reduction in PI). Under these stable conditions, a quantitative relationship indeed exists between CVR and PI. However, during neurally mediated syncope, CVRi decreases, whereas the PI increases.[44] Therefore, the PI cannot serve as an adequate parameter to assess dCA.

Another estimate for vasomotor resistance is the critical closing pressure, determined from the systolic–diastolic pressure–flow relationship within each heartbeat. The critical closing pressure of the cerebral circulation indicates the value of BP at which CBF is projected to approach zero.[64] This value is obtained by linear extrapolation of the BP and CBFV values in the systolic to diastolic range that were

obtained within each cardiac cycle. The slope of this 'intrabeat' BP–CBFV curve is nonlinear, however, and therefore it remains uncertain if the true critical closing pressure can be accurately predicted by linear extrapolation. The critical closing pressure has not been validated in disease states or in elderly subjects, and further research will be necessary to determine if it could be a useful tool (for a review, we refer to Panerai[64]).

Table 1 Overview of the various dynamic quantifications of cerebral autoregulation in the time domain.

CA measurement	Abbreviation	Unit / Value	Definition
CerebroVascular Resistance	CVR	mmHg per mL per min	$\frac{\text{BPmean}}{\text{CBFmean}}$
CerebroVascular Resistance Index	CVRi	mmHg per cm per sec	$\frac{\text{BPmean}}{\text{CBFVmean}}$
Pulsatility Index	PI	--	$\frac{\text{CBFVsys - CBFVdias}}{\text{CBFVmean}}$
Critical Closing Pressure	CrCP	mmHg	The extrapolated value of BP at which CBF approaches zero
Rate of Recovery	RoR	CBFV per sec	$\frac{\Delta CVR / \Delta T}{\Delta BP}$
Autoregulatory Index	ARI	0 – 9	9 models for CA: 0 indicates no autoregualion 9 indicates very fast regulation
Correlation Coefficient	Mx	0 – 1	Pearson's correlation coefficient between BP and CBFV

BP, blood pressure; CBFV, cerebralblood flow velocity

Time Domain versus Frequency Domain

A linear system can be described in the time domain or in the frequency domain. We will first describe the techniques used in the time domain.

The time domain is used to describe the analysis of physical signals with respect to time. The signal's value is known at various discrete time points and a time domain graph shows how a signal changes over time.

Rate of recovery: The time of CBFV recovery after a single pressor or depressor stimulus is taken as a measure for the efficiency of dCA. One of the quantifications used in this approach is the rate of recovery. The rate of recovery is defined as the normalized changes in CVRi per second during a BP decrease[32]:

$$\text{Rate of Recovery} = \frac{\Delta CVR / \Delta T}{\Delta BP}$$

This rate uses CVRi in the calculation, but, as mentioned above, this does not take into account the cross-sectional area factor between CBF and CBFV and lacks brain weight.

Furthermore, this method has not been tested specifically in an elderly population.

Autoregulatory index: Using the alterations in CVRi in relation to the change in BP during thigh cuff maneuvers, Tiecks et al.[65] introduced the autoregulatory index. For an observed decrease in BP, a hypothetical CBFV curve without autoregulation is calculated. Eight other different computer models of possible flow-velocity curves are calculated as well, each assuming a higher degree of dCA. An autoregulatory index of 0 indicates that CBFV passively follows BP (in other words, the percentage change in CBFV is equal to that of BP); a value of 9 indicates that CBFV recovers much faster than BP.

Panerai et al.[66] showed that this model can also be used to grade autoregulation using baseline recordings of BP and CBFV, estimating the impulse response function. This function expresses the relationship between BP and CBFV after a very short, impulse-like disturbance in BP.

The autoregulation index has been applied in elderly subjects with hypertension, stroke, and carotid sinus syndrome (see section on CA in aging and comorbidity).

Correlation index: In patients with head injury, Czosnyka et al.[67] calculated Pearson's correlation coefficients between consecutive samples of averaged cerebral perfusion pressure and flow velocity for every 3-min period determining a correlation index known as 'Mx.' Lang et al.[68] showed that this index is valid if BP is used instead of cerebral perfusion pressure in these patients. This obviates the need for the invasive measurement techniques to determine intracranial pressure. Presently, however, the Mx has not been validated in nonbrain trauma patients, and the proposed cutoff values were not valid in healthy subjects.[69]

The Frequency Domain

In the frequency domain, physical signals are described with respect to frequency.

In Figure 2 we illustrate two signals that show clear repetitive patterns over time. In fact, they can be described as two sinusoidal waveforms (oscillations) with a given period, T, and amplitudes A and B. The frequency in hertz (Hz) is calculated from the period:

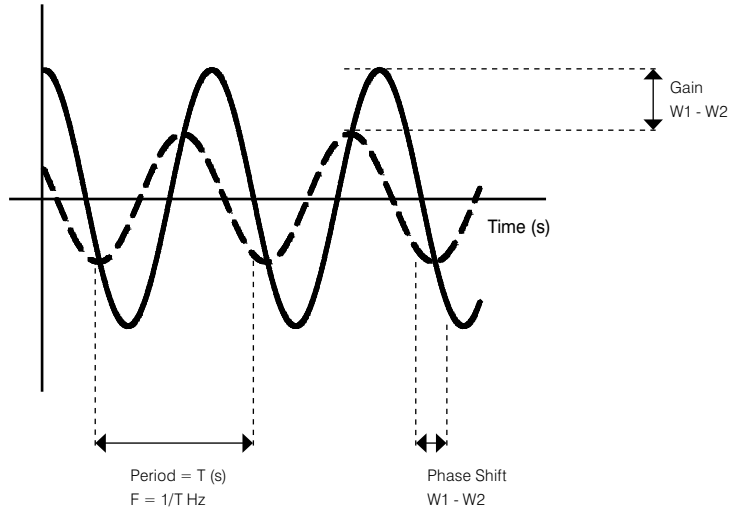
$$F = \frac{1}{T}$$

Giller was the first to use frequency domain analysis to describe the relation between oscillatory patterns of BP and CBFV.[70]

In Figure 3A, we present an example of the spontaneous oscillations in BP and CBFV recorded in a healthy old subject over a period of 5 mins of rest in sitting position. The signals are composed of several different oscillations with different periods (and thus frequencies) and amplitudes. Spectral analysis makes it possible to create the frequency domain graph that shows the frequency spectrum of these different oscillations that are hidden in the signal. In Figure 3B, we illustrate the power spectra of the two signals presented in Figure 3A. A power spectrum is a frequency graph that describes how much of the signal lies within a given frequency.

In the frequency domain analysis, the regulation of CBF in response to changes in BP can be described using transfer function analysis. Transfer function analysis makes it possible to examine the transfer of the BP oscillations to CBF as a measure of

Figure 2 Transfer function analysis

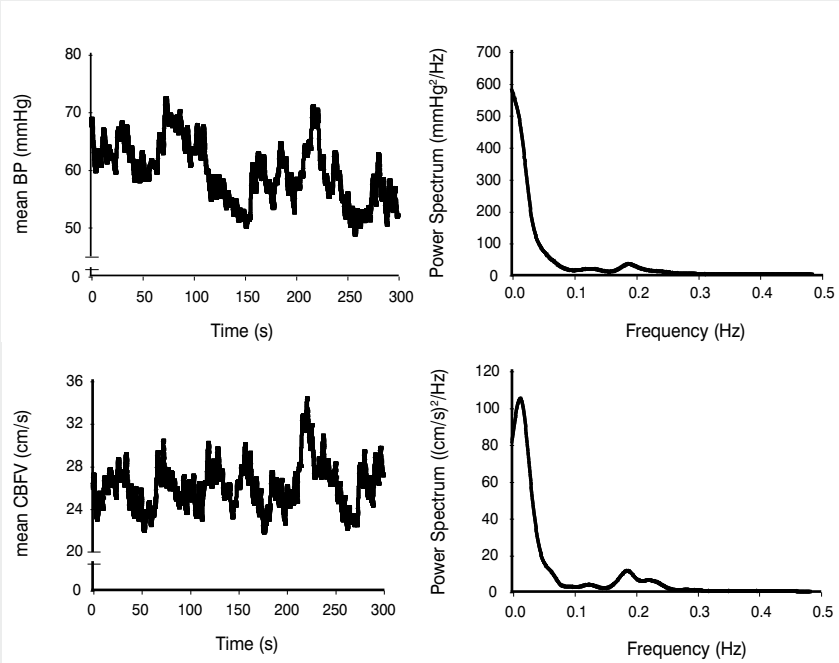


Graphical explanation of the concept of transfer function analysis. Two sinusoidal waveforms with the same period (T) are presented. Waveform frequency (F) is defined as 1/T. The solid line represents waveform 1 (W1), with a larger amplitude than the dashed line, representing waveform 2 (W2). The gain is the damping effect between W1 and W2, and the phase shift represents the time delay between W1 and W2. Provided that W1 is the input and W2 is the output of the transfer function, the regulator in this system attenuates the amplitude of the input leading to a lower amplitude (gain) of W2. The phase shift of W2 characterizes the displacement in time of this waveform relative to W1.

autoregulation. It quantifies the extent to which the input signal, BP, is reflected in the output signal, CBF, with CA as the regulator between the input (BP) and output (CBF).

The regulation of CBFV is effective in the low-frequency range of BP fluctuations, but not in the high-frequency range because of the time delay required to initiate cerebrovascular adaptations to the changes in perfusion pressure. Therefore, CA functions like a high-pass filter, allowing rapid BP changes to be transmitted to CBFV, whereas slow BP changes are filtered. Cerebral blood flow velocity in relation to BP can therefore be expressed by transfer function analysis using a high-pass filter model in the frequency range of 0.07 to 0.30 Hz.[71,72]

Figure 3 A power spectrum is a frequency graph that describes how much of the signal lies within a given frequency.



(**A**) Spontaneous oscillations of blood pressure (BP) and cerebral blood flow velocity (CBFV). Five minutes of continuous BP and CBFV measurements in a healthy elderly subject in sitting position, showing spontaneous oscillations in both signals. (**B**) Power spectra of BP and CBFV. The power spectra of BP and CBFV data presented in panel (A), obtained after spectral analysis. The power spectra describe how much of the BP and CBFV oscillations lie within a given frequency. Note the different scales on the y axis illustrating the difference of the magnitude of the oscillations in BP and CBFV.

Gain, phase, and coherence: The transfer function between the oscillations in BP and CBFV in this autoregulatory system is characterized by three parameters: gain (or magnitude), phase shift, and coherence.

The gain, or transfer magnitude, quantifies the damping effect between the input and output of the transfer function. Provided that in Figure 2, waveform 1 is the input and waveform 2 is the output of the transfer function, the regulator in this system attenuates the amplitude of the input (A), yielding a low value for gain.

In the transfer function between BP and CBFV, the gain represents the damping effect of CA on the magnitude of the BP oscillations. The gain of this transfer function marks the efficiency of the regulator, which is the response of CA. A low gain indicates an efficient autoregulation, whereas an increase in gain represents a diminished efficiency of the dynamic process of CA. According to the high-pass filter model of CA, the gain rises with increasing frequencies of BP oscillations.[54]

A specific problem in the interpretation of gain is that absolute measures of gain (estimated from transfer function analysis of absolute changes in CBFV and BP) are influenced by baseline values of CBFV and BP. Especially for CBFV, interindividual differences can be substantial and reflect the differences in MCA diameter rather than baseline differences in flow. Therefore, normalization of gain has been proposed. This can be achieved by substituting gain by the product of gain and CVR, named normalized gain. If the CVRi is determined from averaged baseline values for BP and CBFV, normalized gain is mathematically similar to gain calculated from relative changes in BP and CBFV.[73,74]

The second parameter of the transfer function we will discuss is the phase shift. Synchronous waveforms are 'in phase.' The phase shift of a waveform therefore represents the displacement of this waveform relative to another waveform with the same period (Figure 2). The phase shift can be expressed in degrees from 0° to 360°, or in radians from 0 to 2 π, thus ranging from no phase shift to a phase shift of one full period. The phase shift can be recalculated to a time difference between two signals: a 90° phase shift between two signals with 10-sec periods equals a time delay of 2.5 secs between these signals.

The oscillations of CBFV do not occur simultaneous to those of BP. Changes in CBFV recover faster than the changes in BP, which causes a displacement of CBFV relative to BP in such a manner that CBFV oscillations appear to lead BP oscillations. This is interpreted as intact dCA.[75] In relation to BP, this leftward shift is mathematically negative. However, in literature, the phase shift between BP and CBFV is often given as a positive number and is defined as a 'phase lead'. A positive phase indicates an intact CA, whereas in patients with a complete loss of CA, the phase shift between CBFV and BP is expected to be close to 0°. In a clinical

population of patients with carotid stenosis or occlusion, phase shifts were significantly lower than in normal subjects and could sometimes approach 0°.[56,76]

Phase shifts can be considered surrogate measures for the time delay of the autoregulatory response. When we translate the phase shift to the time domain, a phase shift of 0 means no time delay between oscillations of BP and CBFV.

The measurement of phase shifts and gain is applicable to all stationary models that induce oscillatory BP and CBFV changes, and these parameters have an apparent relationship to the rate of autoregulation. During induced oscillations in the three frequencies regions (the high, > 0.2 Hz; low, 0.07 to 0.20 Hz; and very low frequencies, < 0.07 Hz), the gain values increased and the phase shifts decreased with increasing frequency.[54] Analysis of spontaneous oscillations yielded the same results.[71,72] These results all confirm the high-pass filter model of dynamic CA. In assessing dCA, gain should be calculated in combination with phase shifts. For the calculation of phase shifts during an short, nonstationary BP/CBFV response, multimodal pressure–flow analysis has been introduced as a mathematical tool.[77]

The third parameter of the transfer function the coherence function tests the linearity of the relation between input and output. Coherence approaching unity in a specific frequency range suggests a linear relationship in this domain, whereas coherence approximating zero may indicate no relationship between the signals. However, a low coherence could also indicate severe extraneous noise in the signals.[70,78] Therefore, for the calculation of phase shifts and gain values, thresholds of coherence of >0.4 or >0.5 have been used by most researchers.[71,72] Conversely, low coherence can also be an expression of the nonlinear qualities of dCA, and the validity of using these cutoff and maximum points is debatable.[79] A solution to this limitation is to induce oscillatory changes in BP and CBFV. Several maneuvers (as described above) induce oscillations. These enlarge the power of the oscillations and thereby the coherence of the transfer function.[54] These maneuvers can be performed in the different frequency regions. Another possible solution is nonlinear analysis and the use of more complex mathematical models.

In accordance with the high-pass filter model of dCA, in healthy older people, values of coherence and gain are the highest in the high-frequency region of BP oscillations

and the lowest in the very low frequency region, whereas phase shifts are the highest in the very low frequency region and the lowest in the high-frequency region.[14,71,80]

Dynamic Cerebral Autoregulation in Elderly Subjects

Healthy Elderly

To include all the literature regarding the dynamic process of CA in healthy elderly subjects, we performed a literature search with the following search terms: cerebrovascular circulation, cerebrovascular disorders, and aged. Furthermore, we used free text words, aging, ageing, elderly and the found eight studies that have investigated dCA in healthy elderly subjects.[81-83] These studies compared the autoregulatory capacity of healthy young subjects with healthy aged people. An overview of these studies is presented In Table 2. The age range was 50 to 75 years. All studies measured BP and assessed CBFV in the MCA, using TCD; one study also measured CBFV in the posterior cerebral artery (PCA).[84] Unfortunately, important methodological differences exist among these studies in the measurements and assessments of dCA.

Different investigators measured spontaneous oscillations of BP and CBFV during sitting and standing, and calculated transfer functions (resulting in values of gain, phase shifts, and coherence) in elderly people.[14,80] Yam et al.[69] also made use of spontaneous BP and CBFV recordings in moderately aged people, but these investigators calculated the correlation index between BP and CBFV. Sorond *et al.*[84] measured BP and CBFV in elderly subjects during a sit-to-stand procedure and evaluated the rate of recovery of CBFV. Carey et al.[81,82] used lower body negative pressure, the Valsalva maneuver, spontaneous oscillations, the thigh cuff test and head-up tilt to induce BP changes, and for each test they assessed the autoregulatory index. These investigators measured dCA in 25 aged subjects, including five subjects > 75 years. Heckmann et al.[85] determined dCA during ergomotor exercise in older subjects and calculated the PI. Franke et al.[83] assessed the PI in two different groups of elderly people; one group of fit elderly subjects and another group of frail elderly subjects. They measured CBFV continuously, but assessed BP only once every minute. To determine dCA, BP should ideally be measured continuously.

Table 2 Dynamic cerebral autoregulation in healthy, elderly people.

Authors	Study sample	Measurement	Assessment dynamic CA	Conclusion
Lipsitz et al.[14]	N=10, age 24 +/- 1 years N=10, age 72 +/- 3 years N=10, age 72 +/- 2 years	Continuous cerebral blood flow velocity (CBFV) and blood pressure (BP) measurements during standing and steady-state sitting and standing	Transfer function analysis	Elderly normotensive and previously treated hypertensive elderly subjects retain dynamic cerebral autoregulation (CA)
Carey et al.[81]	N=27, 29 +/- 5 years N=27, 68 +/- 5 years	Changes CBFV and BP were measured during: lower body negative pressure release, valsalva maneuvre, spontaneous rises, thigh cuff test, spontaneous falls	Autoregulatory index values of each subject for each dynamic (de)pressor stimulus; step response analysis	Dynamic CA is unaffected by aging
Narayanan[80]	N=10, age 24 +/- 1 years N=10, age 72 +/- 3 years	CBFV and BP recordings of oscillations during sitting and standing	Transfer function analysis	Dynamic CA in healthy elderly subjects is intact in the low frequency range
Carey et al.[82]	N= 25, 28 +/- 8 years N=25, 69 +/-10 years (five very elderly, >75 years)	Changes in CBFV and BP were measured during head-up tilt	Autoregulatory index	Dynamic CA during orthostatic stress is unaffected by aging
Heckmann et al.[85]	N=18, 29,4 +/- 4,7 years N=18, 66,5 +/- 5.8 years	Continuous monitoring of CBFV and BP during ergomotor exercise	Pulsatility index	Cerebral autoregulatory capacity is retained but delayed in elderly subjects
Sorond et al.[84]	N=13, 30 (7) years N=13, 74 (4) years	Continuous CBFV and BP measurements in response to standing from a sitting position	Rate of recovery	Older participants have a similar decline in CBFV in both territories and a significantly larger CBFV decline in the posterior cerebral artery compared to the middle cerebral artery.
Yam et al.[69]	N=16, age 28 +/- 5 years N=16, age 54 +/- 8 years	CBFV and BP recordings of spontaneous oscillations	Correlation index	No correlation between age and correlation index
Franke et al.[83]	N=10, 72 +/- 1 years N=10, 73 +/- 2 years N=10, 23 +/-1 years N=10, 23 +/-1 years	CBFV continuous measurements; BP assessment every minute	Cerebrovascular resistance, pulsatility index	Older subjects autoregulate CBFV as well as younger subjects regardless of fitness

Regardless of the method by which it was assessed, all studies concluded that in healthy aged subjects, dCA is preserved during induced decreases in BP and during spontaneous oscillations in BP in the low-frequency range (0.07 to 0.20 Hz). In the high-frequency range (> 0.15 Hz), dCA is less efficient in both young and older people.[14] This is in agreement with the concept that the relationship between CBFV and BP can be expressed by transfer function analysis using a high-pass filter model (see above). The studies that calculated changes in the CVRi and the PI also concluded that cerebral autoregulatory capacity is retained, regardless of physical fitness.[83,85] However, these investigators noted that the change of the PI in healthy elderly is delayed during ergomotor exercise compared with young subjects.[85] The CVRi and the PI only have a quantitative relationship under stable conditions, and therefore these parameters are difficult to interpret during exercise.

Most investigators studied dCA in the MCA, but the PCA can also be insonated with the TCD[19], and dCA can be investigated in the posterior circulation. Sorond et al.[84] observed that CBFV in the PCA declined to a greater extent than in the MCA. In both young and aged humans, the characteristics of dCA in the PCA differ from that in the MCA. The regulation of CBFV in the PCA to spontaneous BP oscillations acts as a high-pass filter similar to the MCA.[86] The phase shifts in the PCA suggest that the latency of CA is sufficient, as it is in the MCA territory, but in all frequency ranges, the gain values in the PCA were significantly higher compared with the values in the MCA. These higher magnitudes do not necessarily indicate an inefficient autoregulation in the PCA, considering the similar phase shifts. This dissimilarity in gain more likely is reflective of the different resonance properties of the two vascular beds and of the combination of different flow and different vessel diameters; flowvelocity in the MCA is approximately 1.5 times higher than in the PCA.[84,86] In summary, no evidence was found for an effect of aging on dCA in the age ranges studied (50 to 75 years). Whether dCA is preserved at more advanced age (> 75 years) is at present unknown

CA in Aging and Comorbidity

With TCD, CBFV can be measured during gradual and prolonged changes in BP, cf. the classic method to determine CA.[87] Under normal conditions, the upper and lower limits of CA are found at 60 and 150 mm Hg mean arterial pressure, respectively. The important difference between the measurement of classic and

dynamic CA is that the classic measurements are performed in the semisteady state and that the use of vasoactive medication is necessary to drive these prolonged changes in BP. In contrast to this approach, dCA measures flow changes in response to BP fluctuations that are mostly within the limits of autoregulation.

In one study in normal human subjects, dynamic measurement of CA yielded results similar to static testing under conditions of intact autoregulation and following pharmacologically induced impairment of autoregulation.[65] However, very few studies have combined estimations of sCA and dCA. Therefore, further research should combine sCA and dCA and attempt to describe the behavior of dCA around the lower and upper limits of BP as identified by the classic sCA curve.

However, for clinical applications, dynamic measurements of CA are probably the method of choice. This practice is noninvasive and less prolonged, it could be performed at bedside, and pharmacological interventions are not necessary.

Diseases such as acute ischemic stroke may bilaterally impair sCA leaving the brain tissue unprotected against the potentially harmful effect of BP changes.[1] Stroke is more common in older subjects, and 72 to 96 h after an acute stroke, dCA is bilaterally impaired, rendering CBFV dependent on BP.[7,88] Reinhard et al.[61,76,89] found no major disturbance of dCA after 4 to 48 h after stroke. However, after 72 to 171 h, they found a trend toward poorer autoregulatory values. Therefore, it could be argued that in poststroke patients, BP should be well controlled to stabilize CBF and to prevent cerebral hyperperfusion or ischemia. However, which levels of BP are acceptable in the acute stroke phase, and to which level BP can or should be lowered, is still uncertain.[90]

Further research addressing the dynamic pressure–flow relationship in acute stroke may help to shed light on this important issue.

For hypertensive patients, estimations of CA offer a simple method to evaluate not only the effect of hypertension but also the effect of antihypertensive treatment on cerebral perfusion. Because of an upward shift of the lower limit of the sCA curve toward higher pressure, and with this, a possible impaired tolerance to a decline in BP, a conservative approach in elderly patients with hypertension has been

suggested.[91] However, classic studies of sCA have shown that this rightward shift with hypertension undergoes a leftward shift with treatment[92]. Indeed, with dCA, middle-aged and elderly (< 75 years) hypertensive patients were found to retain the ability to maintain CBFV in the face of acute BP changes, assessed by the autoregulatory index during different maneuvers.[93] Using the autoregulatory index during the Valsalva maneuver, Novak et al.[77] did not find any difference between healthy normotensive and hypertensive middle-aged subjects. However, when they used the multimodal pressure–flow analysis, they observed a smaller phase shift pointing toward less efficient autoregulation in the hypertensive group. In patients with controlled hypertension, the cerebral autoregulatory response was preserved.[94] In newly diagnosed middle-aged patients with untreated mild to moderate hypertension, dCA was normal. Shortand long-term reduction of BP did not compromise dCA.[15] Furthermore, in uncontrolled hypertensive patients with a mean age of 72 years, 6 months of successful, aggressive treatment with antihypertensive therapy increased CBFV significantly compared with controlled hypertensive patients and healthy controls.[16] This therapy did not impair dCA assessed with an active sit-to-stand procedure.

Autoregulation studies can contribute valuable clinical information regarding CBF control by identifying or confirming patients at high risk for impaired cerebral perfusion. Transient cerebral hypoperfusion can lead to syncope, which is a common medical problem in the elderly.[95] In addition, many older patients are vulnerable to cerebral hypoperfusion after orthostatic BP decreases.[10] The combined mechanisms of (baroreflex mediated) BP regulation and CA limit the reduction of the cerebral perfusion induced by the postural position. However, if, despite these protective mechanisms, cerebral perfusion becomes impaired, symptoms of orthostatic hypotension such as dizziness and syncope occur.[96] For recurrent falls among elderly subjects, orthostatic hypotension is an independent risk factor, but orthostatic hypotension alone may not be sufficient to produce symptoms like falls in most elderly patients.[97] In contrast, decreases in standing BP that are smaller than the formal definition of orthostatic hypotension (a decrease of 20mm Hg or more) may exceed the threshold for CA in some patients and therefore be sufficient to cause cerebral hypoperfusion. In many patients with orthostatic hypotension, BP remains within the autoregulatory range. Because of CA, the effect of a given change in BP on cerebral perfusion cannot be predicted.

Conclusion

Classic studies regarding CA are performed in the semi-steady state, and they measure CBF after a persistent change in BP with a gradual onset. In contrast, recent studies have investigated the fast modifications of CBF in relation to rapid changes in BP. Technological developments, such as TCD and servo-controlled finger photoplethysmography, have offered the possibilities to investigate more thoroughly the dynamics of the process of CA. This dynamic approach is the most promising approach for the clinical evaluation of CA. These measurements are less time consuming, entirely noninvasive, and could be performed at bedside. However, no uniform method exists to assess the dynamic cerebral autoregulatory capacity of the human brain. Researchers have introduced several techniques to challenge CA, ranging from a single episode of induced hypotension to induced oscillatory changes in BP, and additionally have used spontaneous BP oscillations to quantify dCA. The quantifications used vary from calculated indices using regression analysis to rate of CVR changes and to transfer function analysis. We have provided an overview of the various methods applied to study CA dynamically.

Subsequently, we have reviewed the literature regarding dCA in healthy elderly subjects. Regardless of the assessment used, dCA is preserved in healthy elderly subjects < 75 years. Owing to this intact dCA, a lowering of BP should not influence CBF. However, in diseases affecting the elderly, symptoms of cerebral hypoperfusion are frequent. Testing the dynamic cerebral autoregulatory capacity could be a promising tool to determine the risk of hypoperfusion and ischemia of the brain. To determine dCA during orthostatic stress, the sit-to-stand procedure is a feasible method in elderly patients. Baseline measurements of spontaneous fluctuations in BP and CBFV are elegant measurements to assess dCA and ask little of the patient. If the magnitude of the spontaneous oscillations in BP is not high enough to assess dCA (i.e., coherence is < 0.4), it may be necessary to induce oscillations with any of the methods described above. In both situations, CBFV in relation to BP can then be expressed by transfer function analysis using a high pass-filter model resulting in values of gain, phase, and coherence. More clinical studies are necessary to investigate the effect of a truly old age (80 years and older) and comorbidity on the dynamic pressure–flow relationship. At present, technological developments have given good prospects to study this relationship in the elderly population.

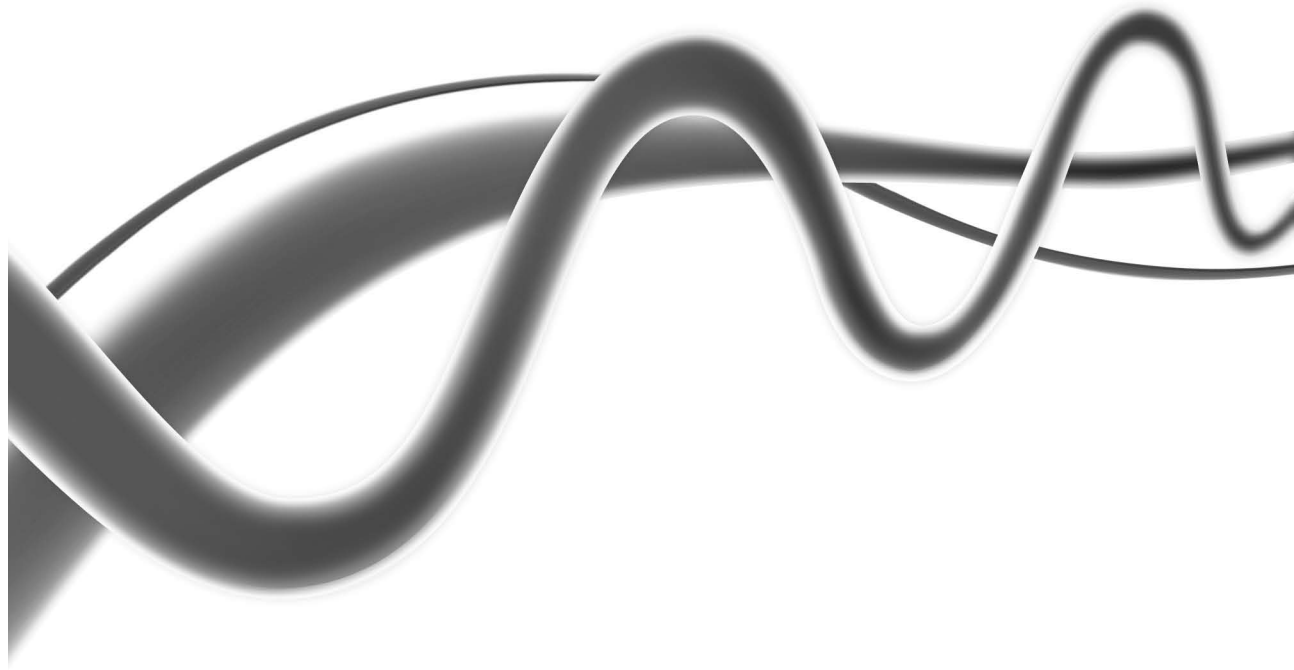
References

1. Paulson OB, Strandgaard S, Edvinsson L. Cerebral autoregulation. *Cerebrovasc Brain Metab Rev 1990;2:161–92.*
2. Chillon J-M, Baumbach GL. Autoregulation: arterial and intracranial pressure. In: Edvinsson L, Krause DN (eds). Cerebral blood flow and metabolism. *Philadelphia: Lippincott Williams & Wilkins, 2002: 395–412.*
3. Panerai RB. Assessment of cerebral pressure autoregulation in humans—a review of measurement methods. *Physiol Meas 1998;19:305–38.*
4. Lassen NA. Cerebral blood flow and oxygen consumption in man. *Physiol Rev 1959;39:183–238.*
5. Rosenblum WI. Autoregulatory plateau: does it exist? *J Cereb Blood Flow Metab 1995;15:174–7.*
6. Banaji M, Tachtsidis I, Delpy D, Baigent S. A physiological model of cerebral blood flow control. *Math Biosci 2005;194:125–73.*
7. Eames PJ, Blake MJ, Dawson SL, Panerai RB, Potter JF. Dynamic cerebral autoregulation and beat to beat blood pressure control are impaired in acute ischaemic stroke. *J Neurol Neurosurg Psychiatry 2002;72:467–72.*
8. White RP, Markus HS. Impaired dynamic cerebral autoregulation in carotid artery stenosis. *Stroke 1997;28:1340–4.*
9. Jansen RW, Lipsitz LA. Postprandial hypotension: epidemiology, pathophysiology, and clinical management. *Ann Intern Med 1995;122:286–95.*
10. Mehagnoul Schipper DJ, Vloet LC, Colier WN, Hoefnagels WH, Jansen RW. Cerebral oxygenation declines in healthy elderly subjects in response to assuming the upright position. *Stroke 2000;31:1615–20.*
11. Vloet LC, Pel-Little RE, Jansen PA, Jansen RW. High prevalence of postprandial and orthostatic hypotension among geriatric patients admitted to Dutch hospitals. *J Gerontol A Biol Sci Med Sci 2005;60:1271–7.*
12. Krajewski A, Freeman R, Ruthazer R, Kelley M, Lipsitz LA. Transcranial Doppler assessment of the cerebral circulation during postprandial hypotension in the elderly. *J Am Geriatr Soc 1993;41:19–24.*
13. Novak V, Novak P, Spies JM, Low PA. Autoregulation of cerebral blood flow in orthostatic hypotension. *Stroke 1998;29:104–11.*
14. Lipsitz LA, Mukai S, Hamner J, Gagnon M, Babikian V. Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke 2000;31:1897–903.*
15. Zhang R, Witkowski S, Fu Q, Claassen JA, Levine BD. Cerebral hemodynamics after short- and longterm reduction in blood pressure in mild and moderate hypertension. *Hypertension 2007;49(5):1149–55.*
16. Lipsitz LA, Gagnon M, Vyas M, Iloputaife I, Kiely DK, Sorond F, Serrador J, Cheng DM, Babikian V, Cupples LA. Antihypertensive therapy increases cerebral blood flow and carotid distensibility in hypertensive elderly subjects. *Hypertension 2005;45:216–21.*
17. Collins R, Peto R, MacMahon S, Hebert P, Fiebach NH, Eberlein KA, Godwin J, Qizilbash N, Taylor JO, Hennekens CH. Blood pressure, stroke, and coronary heart disease. Part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet 1990;335:827–38.*
18. Birns J, Markus H, Kalra L. Blood pressure reduction for vascular risk: is there a price to be paid? *Stroke 2005;36:1308–13.*
19. Aaslid R, Markwalder TM, Nornes H. Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg 1982;57:769–74.*
20. Imholz BP, Wieling W, van Montfrans GA, Wesseling KH. Fifteen years experience with finger arterial pressure monitoring: assessment of the technology. *Cardiovasc Res 1998;38:605–16.*
21. Parati G, Ongaro G, Bilo G, Glavina F, Castiglioni P, Di RM, Mancia G. Non-invasive beat-to-beat blood pressure monitoring: new developments. *Blood Press Monit 2003;8:31–6.*
22. Giller CA, Bowman G, Dyer H, Mootz L, Krippner W. Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery 1993; 32:737–41.*
23. Newell DW, Aaslid R, Lam A, Mayberg TS, Winn HR. Comparison of flow and velocity during dynamic autoregulation testing in humans. *Stroke 1994;25:793–7.*
24. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke 2000;31:1672–8.*
25. Schregel W, Schaefermeyer H, Sihle-Wissel M, Klein R. Transcranial Doppler sonography during isoflurane/N2O anaesthesia and surgery: flow velocity, 'vessel area' and 'volume flow'. *Can J Anaesth 1994;41:607–12.*
26. Poulin MJ, Robbins PA. Indexes of flow and crosssectional area of the middle cerebral artery using Doppler ultrasound during hypoxia and hypercapnia in humans. *Stroke 1996;27:2244–50.*
27. Hicks JW, Badeer HS. Gravity and the circulation: 'open' versus 'closed' systems. *Am J Physiol 1992;262:*

28. Dawson EA, Secher NH, Dalsgaard MK, Ogoh S, Yoshiga CC, Gonzalez-Alonso J, Steensberg A, Raven PB. Standing up to the challenge of standing: a siphon does not support cerebral blood flow in humans. *Am J Physiol Regul Integr Comp Physiol 2004;287:R911–4.*
29. Hicks JW, Munis JR. The siphon controversy counterpoint: the brain need not be 'baffling'. *Am J Physiol Regul Integr Comp Physiol 2005;289:R629–32.*
30. Hurn PD, Traystman RJ. Changes in arterial gas tension. In: Edvinsson L, Krause DN (eds). Cerebral blood flow and metabolism. *Philadelphia: Lippincott Williams & Wilkins, 2002: 384–94.*
31. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol 2007;102:870–7.*
32. Aaslid R, Lindegaard KF, Sorteberg W, Nornes H. Cerebral autoregulation dynamics in humans. *Stroke 1989;20:45–52.*
33. Schmidt JF,Waldemar G, Vorstrup S, Andersen AR, Gjerris F, Paulson OB. Computerized analysis of cerebral blood flow autoregulation in humans: validation of a method for pharmacologic studies. *J Cardiovasc Pharmacol 1990;15:983–8.*
34. Levine BD, Giller CA, Lane LD, Buckey JC, Blomqvist CG. Cerebral versus systemic hemodynamics during graded orthostatic stress in humans. *Circulation 1994;90:298–306.*
35. Crews JC, Cahall MA. An investigation of the neurophysiologic mechanisms of tourniquet-related pain: changes in spontaneous activity and receptive field size in spinal dorsal horn neurons. *Reg Anesth Pain Med 1999;24:102–9.*
36. Tiecks FP, Lam AM, Matta BF, Strebel S, Douville C, Newell DW. Effects of the Valsalva maneuver on cerebral circulation in healthy adults. A transcranial Doppler Study. *Stroke 1995;26:1386–92.*
37. Remmen JJ, Aengevaeren WR, Verheugt FW, Jansen RW. Normal values of pulmonary capillary wedge pressure and the blood pressure response to the Valsalva manoeuvre in healthy elderly subjects. *Clin Physiol Funct Imaging 2005;25:318–26.*
38. Williams B. Simultaneous cerebral and spinal fluid pressure recordings. I. Technique, physiology, and normal results. *Acta Neurochir (Wien) 1981;58:167–85.*
39. Hetzel A, Reinhard M, Guschlbauer B, Braune S. Challenging cerebral autoregulation in patients with preganglionic autonomic failure. *Clin Auton Res 2003;13: 27–35.*
40. Micieli G, Tassorelli C, Bosone D, Cavallini A, Viotti E, Nappi G. Intracerebral vascular changes induced by cold pressor test: a model of sympathetic activation. *Neurol Res 1994;16:163–7.*
41. Roatta S, Micieli G, Bosone D, Losano G, Bini R, Cavallini A, Passatore M. Effect of generalised sympathetic activation by cold pressor test on cerebral haemodynamics in healthy humans. *J Auton Nerv Syst 1998;71:159–66.*
42. Giller CA, Giller AM, Cooper CR, Hatab MR. Evaluation of the cerebral hemodynamic response to rhythmic handgrip. *J Appl Physiol 2000;88:2205–13.*
43. Oblak JP, Zaletel M, Zvan B, Kiauta T, Pogacnik T. The effect of age on cerebrovascular reactivity to cold pressor test and head-up tilt. *Acta Neurol Scand 2002;106:30–3.*
44. Schondorf R, Benoit J, Wein T. Cerebrovascular and cardiovascular measurements during neurally mediated syncope induced by head-up tilt. *Stroke 1997;28:1564–8.*
45. Cencetti S, Bandinelli G, Lagi A. Effect of PCO2 changes induced by head-upright tilt on transcranial Doppler recordings. *Stroke 1997;28:1195–7.*
46. Edvinsson L. Neurogenic mechanisms in the cerebrovascular bed. Autonomic nerves, amine receptors and their effects on cerebral blood flow. *Acta Physiol Scand Suppl 1975;427:1–35.*
47. Heistad DD, Marcus ML. Evidence that neural mechanisms do not have important effects on cerebral blood flow. *Circ Res 1978;42:295–302.*
48. Zhang R, Zuckerman JH, Iwasaki K, Wilson TE, Crandall CG, Levine BD. Autonomic neural control of dynamic cerebral autoregulation in humans. *Circulation 2002;106:1814–20.*
49. Sprangers RL, Wesseling KH, Imholz AL, Imholz BP, Wieling W. Initial blood pressure fall on stand up and exercise explained by changes in total peripheral resistance. *J Appl Physiol 1991;70:523–30.*
50. Wieling W, Krediet CT, van DN, Linzer M, Tschakovsky ME. Initial orthostatic hypotension: review of a forgotten condition. *Clin Sci (Lond) 2007;112:157–65.*
51. Immink RV, Secher NH, Roos CM, Pott F, Madsen PL, Van Lieshout JJ. The postural reduction in middle cerebral artery blood velocity is not explained by $PaCO_2$. *Eur J Appl Physiol 2006;96:609–14.*
52. Birch AA, Dirnhuber MJ, Hartley-Davies R, Iannotti F, Neil-Dwyer G. Assessment of autoregulation by means of periodic changes in blood pressure. *Stroke 1995; 26:834–7.*
53. Birch AA, Neil-Dwyer G, Murrills AJ. The repeatability of cerebral autoregulation assessment using sinusoidal lower body negative pressure. *Physiol Meas 2002;23:73–83.*
54. Hamner JW, Cohen MA, Mukai S, Lipsitz LA, Taylor JA. Spectral indices of human cerebral blood flow control: responses to augmented blood pressure oscillations. *J Physiol 2004;559:965–73.*
55. Aaslid R, Blaha M, Sviri G, Douville CM, Newell DW. Asymmetric dynamic cerebral autoregulatory response to cyclic stimuli. *Stroke 2007;38:1465–9.*

56. Diehl RR, Linden D, Lucke D, Berlit P. Phase relationship between cerebral blood flow velocity and blood pressure. A clinical test of autoregulation. *Stroke 1995;26:1801–4.*
57. Parati G, Castiglioni P, Di RM, Omboni S, Pedotti A, Mancia G. Sequential spectral analysis of 24-h blood pressure and pulse interval in humans. *Hypertension 1990;16:414–21.*
58. Parati G, Saul JP, Di RM, Mancia G. Spectral analysis of blood pressure and heart rate variability in evaluating cardiovascular regulation. A critical appraisal. *Hypertension 1995;25:1276–86.*
59. Lindegaard KF, Lundar T, Wiberg J, Sjoberg D, Aaslid R, Nornes H. Variations in middle cerebral artery blood flow investigated with noninvasive transcranial blood velocity measurements. *Stroke 1987;18:1025–30.*
60. Diehl RR, Diehl B, Sitzer M, Hennerici M. Spontaneous oscillations in cerebral blood flow velocity in normal humans and in patients with carotid artery disease. *Neurosci Lett 1991;127:5–8.*
61. Reinhard M, Muller T, Guschlbauer B, Timmer J, Hetzel A. Transfer function analysis for clinical evaluation of dynamic cerebral autoregulation—a comparison between spontaneous and respiratory-induced oscillations. *Physiol Meas 2003;24:27–43.*
62. Mitsis GD, Zhang R, Levine BD, Marmarelis VZ. Modeling of nonlinear physiological systems with fast and slow dynamics. II. Application to cerebral autoregulation. *Ann Biomed Eng 2002;30:555–65.*
63. Gosling RG, Dunbar G, King DH, Newman DL, Side CD, Woodcock JP, Fitzgerald DE, Keates JS, MacMillan D. The quantitative analysis of occlusive peripheral arterial disease by a non-intrusive ultrasonic technique. *Angiology 1971;22:52–5.*
64. Panerai RB. The critical closing pressure of the cerebral circulation. *Med Eng Phys 2003;25:621–32.*
65. Tiecks FP, Lam AM, Aaslid R, Newell DW. Comparison of static and dynamic cerebral autoregulation measurements. *Stroke 1995A;26:1014–9.*
66. Panerai RB, White RP, Markus HS, Evans DH. Grading of cerebral dynamic autoregulation from spontaneous fluctuations in arterial blood pressure. *Stroke 1998;29:2341–6.*
67. Czosnyka M, Smielewski P, Kirkpatrick P, Menon DK, Pickard JD. Monitoring of cerebral autoregulation in head-injured patients. *Stroke 1996;27:1829–34.*
68. Lang EW, Lagopoulos J, Griffith J, Yip K, Mudaliar Y, Mehdorn HM, Dorsch NW. Noninvasive cerebrovascular autoregulation assessment in traumatic brain injury: validation and utility. *J Neurotrauma 2003;20:69–75.*
69. Yam AT, Lang EW, Lagopoulos J, Yip K, Griffith J, Mudaliar Y, Dorsch NW. Cerebral autoregulation and ageing. *J Clin Neurosci 2005;12:643–6.*
70. Giller CA. The frequency-dependent behavior of cerebral autoregulation. *Neurosurgery 1990;27:362–8.*
71. Zhang R, Zuckerman JH, Giller CA, Levine BD. Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol 1998;274:H233–41.*
72. Diehl RR, Linden D, Lucke D, Berlit P. Spontaneous blood pressure oscillations and cerebral autoregulation. *Clin Auton Res 1998;8:7–12.*
73. Holstein-Rathlou NH, Wagner AJ, Marsh DJ. Tubuloglomerular feedback dynamics and renal blood flow autoregulation in rats. *Am J Physiol 1991;260:F53–68.*
74. Blaber AP, Bondar RL, Stein F, Dunphy PT, Moradshahi P, Kassam MS, Freeman R. Transfer function analysis of cerebral autoregulation dynamics in autonomic failure patients. *Stroke 1997;28:1686–92.*
75. Kuo TB, Chern CM, Yang CC, Hsu HY, Wong WJ, Sheng WY, Hu HH. Mechanisms underlying phase lag between systemic arterial blood pressure and cerebral blood flow velocity. *Cerebrovasc Dis 2003;16:402–9.*
76. Reinhard M, Muller T, Guschlbauer B, Timmer J, Hetzel A. Dynamic cerebral autoregulation and collateral flow patterns in patients with severe carotid stenosis or occlusion. *Ultrasound Med Biol 2003A;29:1105–13.*
77. Novak V, Yang AC, Lepicovsky L, Goldberger AL, Lipsitz LA, Peng CK. Multimodal pressure-flow method to assess dynamics of cerebral autoregulation in stroke and hypertension. *Biomed Eng Online 2004;3:39.*
78. Marmarelis VZ. Coherence and apparent transfer function measurements for nonlinear physiological systems. *Ann Biomed Eng 1988;16:143–57.*
79. Panerai RB, Eames PJ, Potter JF. Multiple coherence of cerebral blood flow velocity in humans. *Am J Physiol Heart Circ Physiol 2006;291:H251–9.*
80. Narayanan K, Collins JJ, Hamner J, Mukai S, Lipsitz LA. Predicting cerebral blood flow response to orthostatic stress from resting dynamics: effects of healthy aging. *Am J Physiol Regul Integr Comp Physiol 2001;281:R716–22.*
81. Carey BJ, Eames PJ, Blake MJ, Panerai RB, Potter JF. Dynamic cerebral autoregulation is unaffected by aging. *Stroke 2000;31:2895–900.*
82. Carey BJ, Panerai RB, Potter JF. Effect of aging on dynamic cerebral autoregulation during head-up tilt. *Stroke 2003;34:1871–5.*
83. Franke WD, Allbee KA, Spencer SE. Cerebral blood flow responses to severe orthostatic stress in fit and unfit young and older adults. *Gerontology 2006;52:282–9.*
84. Sorond FA, Khavari R, Serrador JM, Lipsitz LA. Regional cerebral autoregulation during orthostatic stress: age-related differences. *J Gerontol A Biol Sci Med Sci 2005;60:1484–7.*

85. Heckmann JG, Brown CM, Cheregi M, Hilz MJ, Neundorfer B. Delayed cerebrovascular autoregulatory response to ergometer exercise in normotensive elderly humans. *Cerebrovasc Dis 2003;16:423–9.*
86. Haubrich C, Wendt A, Diehl RR, Klotzsch C. Dynamic autoregulation testing in the posterior cerebral artery. *Stroke 2004;35:848–52.*
87. Larsen FS, Olsen KS, Hansen BA, Paulson OB, Knudsen GM. Transcranial Doppler is valid for determination of the lower limit of cerebral blood flow autoregulation. *Stroke 1994;25:1985–8.*
88. Dawson SL, Blake MJ, Panerai RB, Potter JF. Dynamic but not static cerebral autoregulation is impaired in acute ischaemic stroke. *Cerebrovasc Dis 2000;10:126–32.*
89. Reinhard M, Roth M, Guschlbauer B, Harloff A, Timmer J, Czosnyka M, Hetzel A. Dynamic cerebral autoregulation in acute ischemic stroke assessed from spontaneous blood pressure fluctuations. *Stroke 2005;36:1684–89.*
90. Spengos K, Tsivgoulis G, Zakopoulos N. Blood pressure management in acute stroke: a long-standing debate. *Eur Neurol 2006;55:123–35.*
91. Strandgaard S. Cerebral blood flow in the elderly: impact of hypertension and antihypertensive treatment. *Cardiovasc Drugs Ther 1991;4(Suppl 6):1217–21.*
92. Barry DI, Lassen NA. Cerebral blood flow autoregulation in hypertension and effects of antihypertensive drugs. *J Hypertens Suppl 1984;2:S519–26.*
93. Eames PJ, Blake MJ, Panerai RB, Potter JF. Cerebral autoregulation indices are unimpaired by hypertension in middle aged and older people. *Am J Hypertens 2003;16:746–53.*
94. Fu CH, Yang CC, Kuo TB. Effects of different classes of antihypertensive drugs on cerebral hemodynamics in elderly hypertensive patients. *Am J Hypertens 2005;18:1621–5.*
95. Rubenstein LZ, Josephson KR. The epidemiology of falls and syncope. *Clin Geriatr Med 2002;18:141–58.*
96. Carey BJ, Manktelow BN, Panerai RB, Potter JF. Cerebral autoregulatory responses to head-up tilt in normal subjects and patients with recurrent vasovagal syncope. *Circulation 2001;104:898–902.*
97. Ooi WL, Hossain M, Lipsitz LA. The association between orthostatic hypotension and recurrent falls in nursing home residents. *Am J Med 2000;108:106–11.*



Chapter 3

Cholinergically mediated augmentation of cerebral perfusion in Alzheimer's disease and related cognitive disorders: The Cholinergic–Vascular Hypothesis

Jurgen A.H.R. Claassen
René W.M.M. Jansen

Journal of Gerontology: Medical Sciences 2006; 61A(3): 267–271.

Abstract

The treatment of Alzheimer's disease (AD) with cholinesterase inhibitors (ChEIs) is based on the cholinergic hypothesis. This hypothesis fails to account for the global nature of the clinical effects of ChEIs, for the replication of these effects in other dementias, and for the strong and unpredictable intraindividual variation in response to treatment. These findings may be better explained by the premise that ChEIs primarily act by augmenting cerebral perfusion: the cholinergic–vascular hypothesis. This article will review the evidence from preclinical and clinical investigations on the vascular role of the cholinergic neural system. The clinical relevance of this hypothesis is discussed with respect to its interactions with the vascular and amyloid hypotheses of AD. Implications for treatment are indicated. Finally, we propose that the role of the cholinergic system in neurovascular regulation and functional hyperemia elucidates how the cholinergic deficit in AD contributes to the clinical and pathological features of this disease.

The treatment of Alzheimer's disease (AD) with cholinesterase inhibitors (ChEIs) is based on the cholinergic hypothesis[1–3]. Thirty years ago, post mortem studies revealed a severe loss of cholinergic innervation in brains of AD patients[1,2]. The severity of this cholinergic deficit was later found to correlate with the level of cognitive impairment[4,5]. Furthermore, it was observed that the loss of cholinergic innervation in AD occurs most prominently in the hippocampus and temporal cortex; this loss could account for the clinical presentation with a predominant and severe loss of memory[6,7]. Although the concept of a cholinergic deficit as a monocausal model for AD has long since been abandoned, there is solid evidence for the important role of the cholinergic system in the processes of memory, attention, and behavior[6,8].

ChEIs (donepezil, galantamine, and rivastigmine) reduce the synaptic breakdown of acetylcholine (Ach) and thus partially correct the cholinergic deficit. Early expectations were that these drugs would produce a significant improvement in memory. In practice, however, treatment effects are far more global and consist of modest improvements in cognition, attention, executive function (including activities of daily living), and global rating scales[9–11]. In addition, the response to treatment varies markedly among patients, and it is currently unpredictable whether an individual patient will benefit from therapy. These findings cannot be fully explained from the cholinergic hypothesis.

Moreover, the observed treatment effects are not specific for AD. Very similar outcomes can be found in patients with vascular cognitive impairment, dementia with Parkinson's disease, and dementia with Lewy bodies[12–15]. It should be noted that this nonspecificity of treatment effects may in part reflect the current lack of precision in diagnosing and separating ''pure'' forms of AD and other dementias. In contrast, these data could point toward a different mode of action of ChEIs than is suggested by the cholinergic hypothesis. A further argument for this explanation is the finding that healthy individuals receiving ChEIs show increased attention and reaction speed and, in airline pilots, improved scores on flight simulator tasks[16].

There is substantial evidence for the hypothesis that ChEIs primarily act by improving cerebral blood flow (CBF). We will refer to this premise as the cholinergic–vascular hypothesis. A cholinergic augmentation of CBF could account for the

global nature of the observed clinical improvement. It also serves to explain why this benefit is not limited to AD. Finally, it may clarify the intraindividual variation in response to treatment. These aspects will be further addressed in this article. The clinical relevance of this hypothesis is found in the recent recognition of the importance of vascular disease in the etiology of AD, in the frequent occurrence of cerebrovascular comorbidity in AD, and in the growing interest in the use of ChEIs for the spectrum of vascular cognitive impairment.

The cholinergic–vascular hypothesis is based on the property of the brain's cholinergic neurons to induce cerebral vasodilatation and to augment CBF[17,18]. These attributes of cholinergic neurons, together with the vascular effects of ChEIs, have been thoroughly investigated. This article will review the pertinent evidence for this hypothesis and discuss its implications for patient care and research. The classic cholinergic deficit hypothesis and the new cholinergic–vascular hypothesis can be mutually viable, and the interaction between these hypotheses will be addressed, with an additional comment on a possible interaction with the amyloid hypothesis.

Evidence for the cholinergic–vascular hypothesis

Cholinergic Vasodilatory Innervation of Cerebral Blood Vessels

The basal forebrain is the major source of brain cholinergic neurons. The hippocampus receives most of its input from the medial septal nucleus and the diagonal band of Broca, whereas the whole of the cerebral cortex is supplied by the nucleus basalis of Meynert (NBM)[19]. The most compelling evidence for the cholinergic–vascular hypothesis was found with the demonstration that these basal forebrain cholinergic neurons have projections to cerebral blood vessels. More precisely, in both rats and humans, arterioles in the frontoparietal cortex were found to contain perivascular cholinergic nerve terminals, and their origin could be traced back to the NBM[20,21]. When brains of AD patients were compared with those of age-matched controls, there was a loss of cholinergic innervation in cortical arterioles in AD, most prominently in the temporal lobes[21].

The neurotransmitter for cholinergic neurons is Ach, which is also a potent vasodilator and can bind to two receptor types: nicotinic and muscarinic. Basal forebrain cholinergic neurons primarily involve muscarinic receptors. Evidence that Ach can induce vasodilatation as a postsynaptic neurotransmitter has come from the identification of these muscarinic receptors in perivascular astrocytes, smooth muscle cells, and endothelial cells, in cortical arterioles [22,23].

In addition to these direct connections between NBM cholinergic neurons and cortical blood vessels, indirect connections involving nitrergic interneurons have been identified[20,21,24]. Cholinergic stimulation of these interneurons causes vasodilatation through the release of nitric oxide.

Stimulation and Inhibition of Cholinergic Neurons Modulates Cerebral Perfusion

Experiments in rats demonstrated that electrical and chemical stimulation of cholinergic neurons in the NBM results in a significant increase in CBF in several cortical areas[25–29]. It is uncertain, however, if these stimuli were truly selective to the NBM. Other groups of neurons may have been activated as well. In contrast, inhibition of cholinergic neurons can be achieved with high selectivity. Following complete destruction of the NBM by a cholinergic immunologic toxin (192 immunoglobulin G–saporin), CBF decreased globally. Most severely affected regions included the posterior parietal and temporal regions (24%–40% decrease)[30]. It is remarkable that the regional distribution of hypoperfusion corresponded to the regions of the brain that are most prominently affected in AD.

The long-term effects of cholinergic inhibition were described in one study that found deposits of amyloid-betaprotein (aß) in the cerebral vasculature 6 months after a lesion to the NBM[31]. In patients with AD, deposits of aß are found in the cortex in the form of neuritic plaques, but also around cortical vessels, especially in patients with vascular comorbidity[32,33]. These data can be interpreted as follows: The cholinergic deficit promotes perivascular aß-deposition, which could contribute to chronic brain hypoperfusion. Alternatively, vascular aß-deposition may be a result of the chronic hypoperfusion that follows a cholinergic lesion.

Cholinergic and Anticholinergic Drugs Influence CBF

The effects of stimulation and inhibition of cholinergic neurons have also been assessed in pharmacological experiments. Scopolamine, an anticholinergic drug, blocks the binding of Ach to its muscarinic receptor. In young humans, scopolamine reduced frontal cerebral perfusion by 20% [34]. An increase in CBF in various cortical regions was observed with cholinergic drugs (the ChEIs eptastigmine and physostigmine) in young and aged humans[35]. Moreover, physostigmine was able to restore CBF after it had been reduced by scopolamine[36]. In rats, rivastigmine reduced brain injury from hypoperfusion, indicating that autoregulation of CBF was improved[37].

Effects of ChEI Treatment on CBF in AD

Computed tomography using radionuclides has provided information on regional changes in CBF in Alzheimer patients. Two early studies, looking at the effects of a single ChEI dose on CBF, found an increase in posterior parietotemporal and superior frontal perfusion[38,39]. The longer-term outcome of treatment with ChEIs has been investigated extensively[40–48]. Prospective studies in untreated patients have found a strong correlation between clinical deterioration and progressive regional hypoperfusion. Consistently, patients who responded to treatment showed either improvement or stabilization of CBF[49]. In contrast, nonresponders (those patients who demonstrated progressive cognitive deterioration with neuropsychological evaluation) had a progressive decline of CBF[50]. Aside from AD, a rise in CBF after treatment with ChEIs was noted in patients with vascular dementia, dementia with Lewy bodies, and dementia of Parkinson's disease, albeit in case reports and investigations in small numbers of patients[50–53].

The Increase in CBF Is Not an Effect of Increased Metabolism

An obvious thought is that the augmentation of CBF by ChEIs is a consequence of a regional increase in cerebral metabolism, which in turn is caused by cholinergic activation of cortical neurons. The available data, however, point toward a direct vascular effect. Blocking cortical neuronal activity did not prevent the increase in blood flow induced by cholinergic agonists[54]. Electrical stimulation of the rat NBM augmented cortical CBF (up to 300% in frontal areas) without an increase in metabolic activity[28,29]. Physostigmine increased CBF in both healthy young and aged humans, without a rise in cerebral glucose consumption[35]. Other studies have also confirmed the lack of activation of glucose metabolism by physostigmine in rats and humans[55,56]. In AD patients receiving ChEIs, the effects on CBF, paralleled by clinical effects, preceded effects on glucose metabolism by months[45,57].

Effects of CBF on Cognition

Central to the cholinergic–vascular hypothesis is the assumption that an increase in CBF improves cognition. To the best of our knowledge, direct evidence for this assumption is lacking, although the available circumstantial evidence is highly suggestive. In patients with carotid stenosis and impaired cerebral perfusion, restoration of normal cerebral perfusion by carotid endarterectomy improved cognitive functioning[58,59]. In these studies, impairment in cerebrovascular reserve was used as a surrogate marker for chronic cerebral hypoperfusion, and postoperative restoration of cerebrovascular reserve was interpreted as an increase in cerebral perfusion. A recent investigation, however, found no improvement in cognition related to carotid endarterectomy[60]. The cerebral hemodynamic status of these patients was not reported in this study. Therefore, an absence of cerebral hypoperfusion prior to surgery could explain the lack of cognitive benefit, which would be consistent with the previous investigations.

Two brief reports have mentioned an improvement in cognition after pacemaker implantation in older patients with bradycardia[61,62]. In the first report, an improvement in CBF after implantation was correlated with an improvement in cognition. In the second report, the most striking effect on cognition was observed in three patients with dementia; in one of these patients, the dementia had fully reversed 6 months after implantation of the pacemaker.

The association between a reduced CBF and cognitive impairment has received much more attention. In the Rotterdam Study, a large population-based cohort study, individuals with cognitive decline were found to have lower CBF than were those individuals who had stable cognitive function in the previous years[63]. Others have found that reduced CBF correlated with reduced cognitive functioning, regardless of the underlying brain disease[64]. The mechanism for this relationship is likely to be the increasing sensitivity of neurons to ischemia or hypoperfusion with age[65]. The evidence for the causal relationship between impairment in CBF, neuronal injury, and cognitive decline has recently been reviewed elsewhere[17,66].

Clinical relevance

Interaction With the Vascular, Cholinergic, and Amyloid Hypotheses of AD

The cholinergic–vascular hypothesis implies that the cholinergic deficit in AD not only affects cholinergic innervation of cortical neurons, but also leads to a loss of cholinergic innervation of cortical blood vessels. This vascular cholinergic deficit causes (regional) cerebral hypoperfusion, which in turn contributes to cognitive decline and neurodegeneration. Consequently, treatment with ChEIs may improve clinical functioning by augmenting cerebral perfusion. This mechanism offers an explanation for the intraindividual variation in response to treatment in AD. Recent research has focused on the vascular risk factors and the signs of overt vascular disease that are observed in many patients with AD[17,66,67]. Cholinergic augmentation can lead to an increase in CBF only if the cerebral vasculature is able to respond with vasodilatation. The presence of severe microvascular deformity that is found in certain AD patients[18], as well as endothelial dysfunction from vascular disease or ischemia[68,69], could reduce or obstruct cholinergic vasodilatation. This obstruction would explain the lack of clinical response in a large subgroup of AD patients. Theoretically, these patients might benefit from the addition of medication aimed at improving vascular endothelial function, such as statins and angiotensin-converting enzyme (ACE) inhibitors.

Vasodilatation mediated by Ach is reduced by aß, and aß increases neuronal susceptibility to ischemia[70–72]. Vice versa, ischemia promotes vascular and neuronal aß deposition[66,73]. Therapeutic interventions aimed at reducing aß burden are thus likely to benefit from and be synergistic with strategies to improve cerebral perfusion [for review, see[74]].

Because hypoperfusion contributes to the neuropathology of AD, the (partial) restoration of perfusion by ChEIs may slow neurodegeneration and hence progression of disease. Because there is no reason to assume that ChEIs halt cholinergic degeneration itself, the positive effect on CBF is likely to wane with the progressive loss of cholinergic neurons. Indeed, ChEI treatment stabilizes disease for a short period of time and may slow disease progression, but it fails to halt it, as has been observed in all trials in AD and, more recently, in Mild Cognitive Impairment[9,11,75]. Combining ChEI therapy with strategies aimed at reducing cholinergic degeneration may hold promise to slow disease progression[76,77].

Neurovascular Regulation

The original cholinergic hypothesis and the new cholinergic–vascular hypothesis are not mutually exclusive. In contrast, they can coexist if we attribute a dual role to the cholinergic system: the coordination of neuronal activation and perfusion in cognitive tasks. This review has provided evidence that the cholinergic system is equipped to increase regional cerebral perfusion. Recent other reviews[6,78] have summarized the large volume of evidence that this system also controls cognitive and attentional processes. Most experiments on this topic suggest that it acts as a central control system that shifts activity between cortical areas and regulates the process of attention, a prerequisite to perform cognitive tasks. Studies that explore patterns of cognitive activation use changes in cerebral hemodynamics as a surrogate measure for neuronal activity. For instance, functional magnetic resonance imaging (fMRI) measures the blood oxygen level dependent (or BOLD) signal, which depends on changes in deoxyhemoglobin. This practice is valid because changes in blood oxygenation occur almost instantaneously with neuronal activation[79]. The striking temporal and spatial association of neuronal activation and increased blood flow fuels a speculative hypothesis on the physiological role of the cholinergic system. This system has the unique capability to activate regional cortical neurons through its cortical projections from the NBM and, at the same time, to direct blood flow to these neurons by simultaneously dilating the corresponding cortical microvessels through its vascular projections from that same NBM. This mechanism ensures that adequate nutrients (oxygen and glucose) are directly available for activated neurons. This process of coupling of CBF with neuronal activation is described as functional hyperemia. Even though this mechanism is more complex than is suggested here, it is not unthinkable that the cholinergic system has a part in it. In AD, the cholinergic deficit will impair neurovascular regulation and lead to neuronal dysfunction and cognitive decline. Cerebrovascular disease and aß deposition further contribute to this uncoupling by delaying the hemodynamic response following neuronal activation[73,74,80].

Future research

The validity and clinical relevance of this hypothesis need further confirmation in future studies. More precisely, such research could investigate whether

nonresponders (AD patients who do not benefit from ChEIs) have an impaired vasodilatory response to ChEIs in comparison with responders. If so, a next step is to evaluate whether adding vascular therapy can improve this response. Recent developments in monitoring cerebral hemodynamics, such as fMRI, transcranial Doppler sonography, and near infrared spectroscopy, facilitate the noninvasive registration of the vascular effects of cholinergic augmentation in patients with dementia. For instance, transcranial Doppler sonography measures changes in CBF velocity, and near infrared spectroscopy measures changes in cerebral cortical tissue oxygenation, both with excellent temporal resolution and a relatively low cost. Both high temporal and spatial resolutions for measuring CBF and brain tissue oxygenation are offered by fMRI[81]. Specifically, arterial spin labeling techniques allow dynamic monitoring of changes in regional CBF, whereas diffusion tensor imaging may identify subtle changes in white matter integrity, which could be used as sensitive outcome parameters to record the effects of vascular treatment[82].

Regarding the concept of neurovascular regulation, it can be hypothesized that ChEIs will augment functional hyperemia in AD. If such an effect can indeed be observed, for instance with fMRI or positron emission tomography using a cortical stimulation paradigm, this may prove a valuable parameter to measure the complex response to pharmacotherapy in AD.

References

1. Bowen DM, Smith CB, White P, Davison AN. Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain 1976;99:459–496.*
2. Davies P, Maloney AJ. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet 1976;2:1403.*
3. Coyle JT, Price DL, DeLong MR. Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 1983;219:1184–1190.
4. Francis PT, Palmer AM, Sims NR, et al. Neurochemical studies of early-onset Alzheimer's disease. Possible influence on treatment. *N Engl J Med 1985;313:7–11.*
5. Perry EK, Blessed G, Tomlinson BE, et al. Neurochemical activities in human temporal lobe related to aging and Alzheimer-type changes. *Neurobiol Aging 1981;2:251–256.*
6. Mesulam M. The cholinergic lesion of Alzheimer's disease: pivotal factor or side show? *Learn Mem 2004;11:43–49.*
7. Pappas BA, Bayley PJ, Bui BK, Hansen LA, Thal LJ. Choline acetyltransferase activity and cognitive domain scores of Alzheimer's patients. *Neurobiol Aging 2000;21:11–17.*
8. Cummings JL, Kaufer D. Neuropsychiatric aspects of Alzheimer's disease: the cholinergic hypothesis revisited. *Neurology 1996;47:876–883.*
9. Birks J, Grimley EJ, Iakovidou V, Tsolaki M. Rivastigmine for Alzheimer's disease. *Cochrane Database Syst Rev 2000;CD001191.*
10. Birks JS, Harvey R. Donepezil for dementia due to Alzheimer's disease. *Cochrane Database Syst Rev 2003;CD001190.*
11. Loy C, Schneider L. Galantamine for Alzheimer's disease. *Cochrane Database Syst Rev 2004;CD001747.*
12. Malouf R, Birks J. Donepezil for vascular cognitive impairment. *Cochrane Database Syst Rev 2004;CD004395.*
13. Emre M, Aarsland D, Albanese A, et al. Rivastigmine for dementia associated with Parkinson's disease. *N Engl J Med 2004;351: 2509–2518.*
14. Simard M, van Reekum R. The acetylcholinesterase inhibitors for treatment of cognitive and behavioral symptoms in dementia with Lewy bodies. *J Neuropsychiatry Clin Neurosci 2004;16:409–425.*
15. Aarsland D, Mosimann UP, McKeith IG. Role of cholinesterase inhibitors in Parkinson's disease and dementia with Lewy bodies. *J Geriatr Psychiatry Neurol 2004;17:164–171.*

16. Mumenthaler MS, Yesavage JA, Taylor JL, et al. Psychoactive drugs and pilot performance: a comparison of nicotine, donepezil, and alcohol effects. *Neuropsychopharmacology 2003;28:1366–1373.*
17. Farkas E, Luiten PG. Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog Neurobiol 2001;64:575–611.*
18. De La Torre JC. Hemodynamic consequences of deformed microvessels in the brain in Alzheimer's disease. *Ann N Y Acad Sci 1997; 826:75–91.*
19. Mesulam MM. The cholinergic innervation of the human cerebral cortex. *Prog Brain Res 2004;145:67–78.*
20. Vaucher E, Hamel E. Cholinergic basal forebrain neurons project to cortical microvessels in the rat: electron microscopic study with anterogradely transported Phaseolus vulgaris leucoagglutinin and choline acetyltransferase immunocytochemistry. *J Neurosci 1995;15:7427–7441.*
21. Tong XK, Hamel E. Regional cholinergic denervation of cortical microvessels and nitric oxide synthase-containing neurons in Alzheimer's disease. *Neuroscience 1999;92:163–175.*
22. Luiten PG, De Jong GI, van der Zee EA, van Dijken H. Ultrastructural localization of cholinergic muscarinic receptors in rat brain cortical capillaries. *Brain Res 1996;720:225–229.*
23. Elhusseiny A, Cohen Z, Olivier A, Stanimirovic DB, Hamel E. Functional acetylcholine muscarinic receptor subtypes in human brain microcirculation: identification and cellular localization. *J Cereb Blood Flow Metab 1999;19:794–802.*
24. Vaucher E, Linville D, Hamel E. Cholinergic basal forebrain projections to nitric oxide synthase-containing neurons in the rat cerebral cortex. Neuroscience 1997;79:827–836.
25. Sato A, Sato Y. Cerebral cortical vasodilatation in response to stimulation of cholinergic fibres originating in the nucleus basalis of Meynert. *J Auton Nerv Syst 1990;30 (Suppl):S137–S140.*
26. Barbelivien A, MacKenzie ET, Dauphin F. Regional cerebral blood flow responses to neurochemical stimulation of the substantia innominata in the anaesthetized rat. *Neurosci Lett 1995;190:81–84.*
27. Lacombe P, Sercombe R, Verrecchia C, Philipson V, MacKenzie ET, Seylaz J. Cortical blood flow increases induced by stimulation of the substantia innominata in the unanesthetized rat. *Brain Res 1989;491:1–14.*
28. Lacombe P, Sercombe R, Vaucher E, Seylaz J. Reduced cortical vasodilatory response to stimulation of the nucleus basalis of Meynert in the aged rat and evidence for a control of the cerebral circulation. *Ann N Y Acad Sci 1997;826:410–415.*

29. Vaucher E, Borredon J, Bonvento G, Seylaz J, Lacombe P. Autoradiographic evidence for flow-metabolism uncoupling during stimulation of the nucleus basalis of Meynert in the conscious rat. *J Cereb Blood Flow Metab 1997;17:686–694.*
30. Waite JJ, Holschneider DP, Scremin OU. Selective immunotoxin induced cholinergic deafferentation alters blood flow distribution in the cerebral cortex. *Brain Res 1999;818:1–11.*
31. Beach TG, Potter PE, Kuo YM, et al. Cholinergic deafferentation of the rabbit cortex: a new animal model of Abeta deposition. *Neurosci Lett 2000;283:9–12.*
32. Kalaria RN, Premkumar DR, Pax AB, Cohen DL, Lieberburg I. Production and increased detection of amyloid beta protein and amyloidogenic fragments in brain microvessels, meningeal vessels and choroid plexus in Alzheimer's disease. *Brain Res Mol Brain Res 1996;35:58–68.*
33. Premkumar DR, Cohen DL, Hedera P, Friedland RP, Kalaria RN. Apolipoprotein E-epsilon4 alleles in cerebral amyloid angiopathy and cerebrovascular pathology associated with Alzheimer's disease. *Am J Pathol 1996;148:2083–2095.*
34. Honer WG, Prohovnik I, Smith G, Lucas LR. Scopolamine reduces frontal cortex perfusion. *J Cereb Blood Flow Metab 1988;8:635–641.*
35. Blin J, Ivanoiu A, Coppens A, et al. Cholinergic neurotransmission has different effects on cerebral glucose consumption and blood flow in young normals, aged normals, and Alzheimer's disease patients. *Neuroimage 1997;6:335–343.*
36. Prohovnik I, Arnold SE, Smith G, Lucas LR. Physostigmine reversal of scopolamine-induced hypofrontality. *J Cereb Blood Flow Metab 1997;17:220–228.*
37. Sadoshima S, Ibayashi S, Fujii K, Nagao T, Sugimori H, Fujishima M. Inhibition of acetylcholinesterase modulates the autoregulation of cerebral blood flow and attenuates ischemic brain metabolism in hypertensive rats. *J Cereb Blood Flow Metab 1995;15:845–851.*
38. Geaney DP, Soper N, Shepstone BJ, Cowen PJ. Effect of central cholinergic stimulation on regional cerebral blood flow in Alzheimer disease. *Lancet 1990;335:1484–1487.*
39. Ebmeier KP, Hunter R, Curran SM, et al. Effects of a single dose of the acetylcholinesterase inhibitor velnacrine on recognition memory and regional cerebral blood flow in Alzheimer's disease. *Psychopharmacology (Berl) 1992;108:103–109.*
40. Hanyu H, Shimizu T, Tanaka Y, Takasaki M, Koizumi K, Abe K. Regional cerebral blood flow patterns and response to donepezil treatment in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord 2003;15:177–182.*

41. Lojkowska W, Ryglewicz D, Jedrzejczak T, et al. The effect of cholinesterase inhibitors on the regional blood flow in patients with Alzheimer's disease and vascular dementia. *J Neurol Sci 2003;216:119–126.*
42. Nakano S, Asada T, Matsuda H, Uno M, Takasaki M. Donepezil hydrochloride preserves regional cerebral blood flow in patients with Alzheimer's disease. *J Nucl Med 2001;42:1441–1445.*
43. Nobili F, Koulibaly M, Vitali P, et al. Brain perfusion follow-up in Alzheimer's patients during treatment with acetylcholinesterase inhibitors. *J Nucl Med 2002;43:983–990.*
44. Nobili F, Vitali P, Canfora M, et al. Effects of long-term Donepezil therapy on rCBF of Alzheimer's patients. *Clin Neurophysiol 2002;113:1241–1248.*
45. Nordberg A. PET studies and cholinergic therapy in Alzheimer's disease. *Rev Neurol (Paris) 1999;155 (Suppl 4):S53–S63.*
46. Rodriguez G, Vitali P, Canfora M, et al. Quantitative EEG and perfusional single photon emission computed tomography correlation during long-term donepezil therapy in Alzheimer's disease. *Clin Neurophysiol 2004;115:39–49.*
47. Staff RT, Gemmell HG, Shanks MF, Murray AD, Venneri A. Changes in the rCBF images of patients with Alzheimer's disease receiving Donepezil therapy. *Nucl Med Commun 2000;21:37–41.*
48. Van Heertum RL, Tikofsky RS. Positron emission tomography and single-photon emission computed tomography brain imaging in the evaluation of dementia. *Semin Nucl Med 2003;33:77–85.*
49. Ceravolo R, Volterrani D, Tognoni G, et al. Cerebral perfusional effects of cholinesterase inhibitors in Alzheimer disease. *Clin Neuropharmacol 2004;27:166–170.*
50. Vennerica A, Shanks MF, Staff RT, et al. Cerebral blood flow and cognitive responses to rivastigmine treatment in Alzheimer's disease. *Neuroreport 2002;13:83–87.*
51. Arahata H, Ohyagi Y, Matsumoto S, et al. [A patient with probable dementia with Lewy bodies, who showed improvement of dementia and parkinsonism by the administration of donepezil]. *Rinsho Shinkeigaku 2001;41:402–406.*
52. Lojkowska W, Ryglewicz D, Jedrzejczak T, et al. The effect of cholinesterase inhibitors on the regional blood flow in patients with Alzheimer's disease and vascular dementia. *J Neurol Sci 2003;216:119–126.*
53. Mori S. Responses to donepezil in Alzheimer's disease and Parkinson's disease. *Ann N Y Acad Sci. 2002;977:493–500.*
54. Scremin OU, Rovere AA, Raynald AC, Giardini A. Cholinergic control of blood flow in the cerebral cortex of the rat. *Stroke 1973;4:233–239.*

55. Blin J, Piercey MF, Giuffra ME, Mouradian MM, Chase TN. Metabolic effects of scopolamine and physostigmine in human brain measured by positron emission tomography. *J Neurol Sci 1994;123:44–51.*
56. Blin J, Ray CA, Piercey MF, Bartko JJ, Mouradian MM, Chase TN. Comparison of cholinergic drug effects on regional brain glucose consumption in rats and humans by means of autoradiography and position emission tomography. *Brain Res 1994;635:196–202.*
57. Nordberg A, Amberla K, Shigeta M, et al. Long-term tacrine treatment in three mild Alzheimer patients: effects on nicotinic receptors, cerebral blood flow, glucose metabolism, EEG, and cognitive abilities. *Alzheimer Dis Assoc Disord 1998;12:228–237.*
58. Kishikawa K, Kamouchi M, Okada Y, Inoue T, Ibayashi S, Iida M. Effects of carotid endarterectomy on cerebral blood flow and neuropsychological test performance in patients with high-grade carotid stenosis. *J Neurol Sci 2003;213:19–24.*
59. Fearn SJ, Hutchinson S, Riding G, Hill-Wilson G, Wesnes K, McCollum CN. Carotid endarterectomy improves cognitive function in patients with exhausted cerebrovascular reserve. *Eur J Vasc Endovasc Surg 2003;26:529–536.*
60. Bossema ER, Brand N, Moll FL, Ackerstaff RG, van Doornen LJ. Does carotid endarterectomy improve cognitive functioning? *J Vasc Surg 2005;41:775–781.*
61. Koide H, Kobayashi S, Kitani M, Tsunematsu T, Nakazawa Y. Improvement of cerebral blood flow and cognitive function following pacemaker implantation in patients with bradycardia. *Gerontology 1994;40:279–285.*
62. Barbe C, Puisieux F, Jansen I, et al. Improvement of cognitive function after pacemaker implantation in very old persons with bradycardia. *J Am Geriatr Soc 2002;50:778–780.*
63. Ruitenberg A, den Heijer T, Bakker SL, et al. Cerebral hypoperfusion and clinical onset of dementia: The Rotterdam study. *Ann Neurol 2005;57:789–794.*
64. Shiraishi H, Chang CC, Kanno H, Yamamoto I. The relationship between cerebral blood flow and cognitive function in patients with brain insult of various etiology. *J Clin Neurosci 2004;11:138–141.*
65. Waller SB, Nyberg P, Dietz NJ. Temporal profile of neurochemical recovery following injury by transient cerebral ischemia. *J Gerontol Biol Sci 1995;50A:B307–B314.*
66. De La Torre JC. Critically attained threshold of cerebral hypoperfusion: the CATCH hypothesis of Alzheimer's pathogenesis. *Neurobiol Aging 2000;21:331–342.*
67. Adunsky A, Chesnin V, Davidson M, Gerber Y, Alexander K, Haratz D. A cross-sectional study of lipids and ApoC levels in Alzheimer's patients with and without cardiovascular disease. *J Gerontol Med Sci 2002;57A:M757–M761.*

68. Rosenblum WI. Endothelial dependent relaxation demonstrated in vivo in cerebral arterioles. *Stroke 1986;17:494–497.*
69. Rosenblum WI. Selective impairment of response to acetylcholine after ischemia/reperfusion in mice. *Stroke 1997;28:448–451.*
70. Paris D, Humphrey J, Quadros A, et al. Vasoactive effects of A beta in isolated human cerebrovessels and in a transgenic mouse model of Alzheimer's disease:role of inflammation. *Neurol Res 2003;25:642–651.*
71. Iadecola C, Zhang F, Niwa K, et al. SOD1 rescues cerebral endothelial dysfunction in mice overexpressing amyloid precursor protein. *Nat Neurosci 1999;2:157–161.*
72. Zhang F, Eckman C, Younkin S, Hsiao KK, Iadecola C. Increased susceptibility to ischemic brain damage in transgenic mice overexpressing the amyloid precursor protein. *J Neurosci 1997;17:7655–7661.*
73. Yokota M, Saido TC, Tani E, Yamaura I, Minami N. Cytotoxic fragment of amyloid precursor protein accumulates in hippocampus after global forebrain ischemia. *J Cereb Blood Flow Metab 1996;16:1219–1223.*
74. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci 2004;5:347–360.*
75. Petersen RC, Thomas RG, Grundman M, et al. Vitamin E and donepezil for the treatment of mild cognitive impairment. *N Engl J Med 2005;352:2379–2388.*
76. Sarter M, Bruno JP. Developmental origins of the age-related decline in cortical cholinergic function and associated cognitive abilities. *Neurobiol Aging 2004;25:1127–1139.*
77. Sarter M, Parikh V. Choline transporters, cholinergic transmission and cognition. *Nat Rev Neurosci 2005;6:48–56.*
78. Sarter M, Hasselmo ME, Bruno JP, Givens B. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. *Brain Res Brain Res Rev 2005;48:98–111.*
79. Ances BM. Coupling of changes in cerebral blood flow with neural activity: what must initially dip must come back up. *J Cereb Blood Flow Metab 2004;24:1–6.*
80. Carusone LM, Srinivasan J, Gitelman DR, Mesulam MM, Parrish TB. Hemodynamic response changes in cerebrovascular disease: implications for functional MR imaging. *AJNR Am J Neuroradiol 2002;23:1222–1228.*
81. Detre JA, Wang J. Technical aspects and utility of fMRI using BOLD and ASL. *Clin Neurophysiol 2002;113:621–634.*
82. Le Bihan D, Mangin JF, Poupon C, et al. Diffusion tensor imaging: concepts and applications. *J Magn Reson Imaging 2001;13:534–546.*

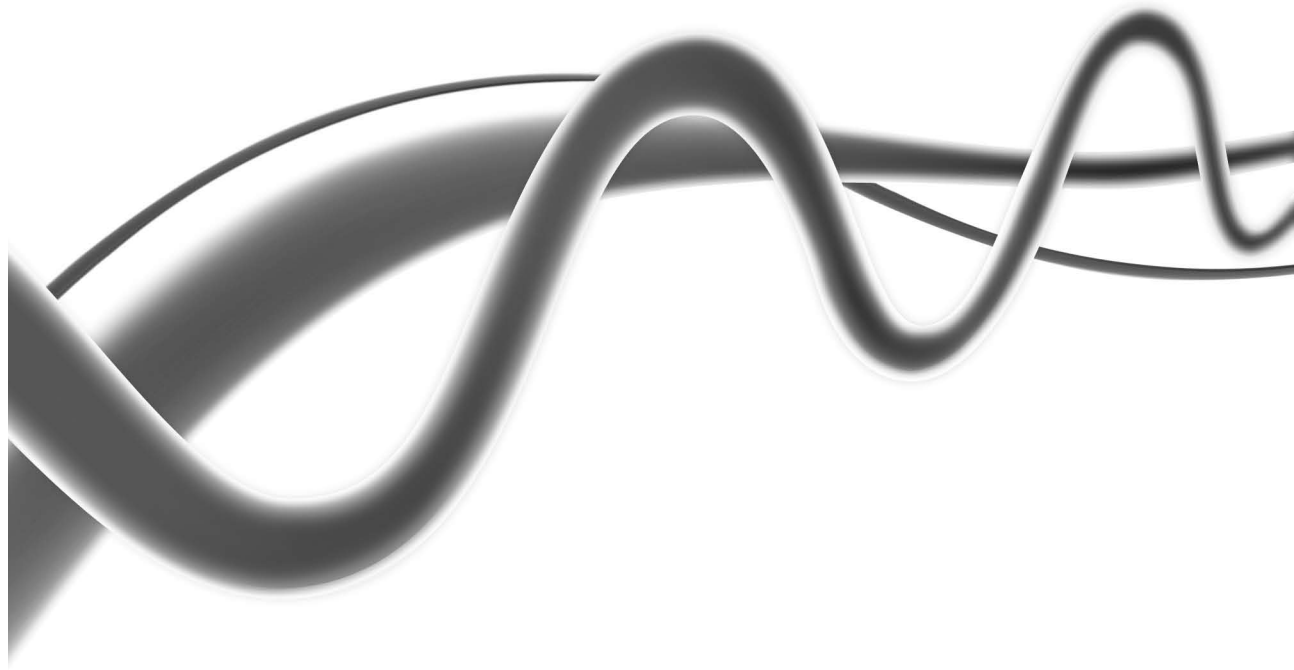
The emperor's pointer *(BMJ 2002;324:418)*

At first I thought it was just me. I was attending my first scientific meeting, a young doctor eager to acquire new knowledge. It was clear that the first speaker had firmly embraced the concept of the PowerPoint presentation, and he was treating his audience to beautifully coloured slides. Like so many of us, he tended to put just a little too much information on each slide. One of his slides showed a comparison between groups, and we were promised that the third group would differ dramatically from the other two. I saw two lines cross the slide, one close to the other, but no third line. I looked at the key at the bottom of the graph and again saw no more than two groups. I was still waiting for a sudden and victorious appearance of the third group on the graph when the speaker turned to the next slide as if nothing had happened. At another point, he showed us a pictogram of a human cell that was engaging in complex metabolic activity, with multiple schematics of receptors, proton pumps, and mitochondrial pathways. A small detail of this cell's activity was apparently of great interest to the speaker, for he was enthusiastically aiming his laser pointer at it. I waited for the dot or arrow to appear on the slide, but nothing happened. "Turn it on," I thought, but instead he went on to the next slide, leaving me in the dark about what had been so interesting in that cell. A similar thing occurred a few slides later, but no one in the audience seemed to bother to tell the speaker to turn on his magical pointing device. It had to be me, then. Apparently, his laser pointer was invisible to me, as were some of his wonderfully coloured lines and bars and legends. I couldn't see the emperor's clothes. And then it dawned on me: I was a man. And what are men, at least some men? Yes, they are colour blind. Presentation after presentation, I have failed to see the highlights in so many slides. And even the trusted journal BMJ joins in the conspiracy. For no particular reason, some issues appear without a date on the cover, such as the first issue in November 2001. Or is it that sometimes, some parts of this journal are invisible to me? But I can't be alone. Suppose I'm at a large international meeting with an audience of 1500 people, of whom 1000 are male. About 70 of them will be colour blind and therefore not able to see the little red dot or arrow being pointed at that interesting red line. There may be even more, because it seems that a lot of men who once had dreams of becoming a pilot but were turned down because of colour blindness have become doctors.

What can we do about this? Using a big flashing yellow arrow might help; or, as in Wheel of Fortune, using a female assistant to point out the area of interest on a slide with a cane (she herself can be guided by the speaker using his pointer); or, perhaps, just putting less data on each slide.

Part 2

Tools and methods to investigate cerebral hemodynamics



Chapter 4

Reproducibility of cerebral blood volume measurements by near infrared spectroscopy in 16 healthy elderly subjects

JAHR Claassen
WNJM Colier
RWMM Jansen

Physiol Meas 2006; 27: 255–264.

Abstract

Near infrared spectroscopy (NIRS) is a non-invasive method to monitor cerebral haemodynamics. Used either alone or in combination with other non-invasive methods such as transcranial Doppler sonography, this technique is well suited for use in cerebrovascular research in ageing. Reproducibility of NIRS, however, has only been determined in neonates and adults. We applied controlled desaturation (the O_2-method) to measure the cerebral blood volume (CBV) with NIRS in 16 healthy subjects aged 65 to 88. This method uses deoxygenated haemoglobin (the concentration of which is manipulated by desaturation) as an intravascular tracer for NIRS. We determined repeatability (between tests interval: 2 min), short-term reproducibility (intervals of 20 and 40 min) and long-term reproducibility (interval > 2 weeks). We found a coefficient of variation (CV) of 12.5% for repeatability and a CV of 11.7% for short-term reproducibility. The CV for long-term reproducibility was 15%. We conclude that NIRS can reproducibly measure CBV in subjects aged 65 and older, using the O_2-method. In this group of healthy subjects, this method was well tolerated.

Introduction

With advancing age, global cerebral blood flow declines.[1] Moreover, there is an increasing prevalence of factors such as orthostatic hypotension and postprandial hypotension that could further impair perfusion of the brain.[2-4] In clinical practice, these findings can lead to dizziness, loss of consciousness or syncope and cerebral ischaemia.[3] Research in this field requires continuous haemodynamic monitoring of both systemic blood pressure and cerebral perfusion. Magnetic resonance imaging (MRI) and positron emission tomography (PET) are two important techniques to investigate cerebral haemodynamics, but these methods are limited to monitoring in the supine position, and may have limited availability. Near infrared spectroscopy (NIRS) offers non-invasive monitoring of cerebral haemodynamics with high temporal resolution at low cost.[5] It can easily be adapted to allow continuous monitoring during a 90-min postprandial test, or during head-up tilt or active standing. NIRS can measure beat-to-beat variations in the concentrations of oxygenated (O_2Hb) and deoxygenated (HHb) haemoglobin. We have previously shown that these measurements are reproducible in healthy elderly subjects.[6] In addition, we have demonstrated that NIRS-recorded changes in O_2Hb and HHb, brought about by finger tapping, correlated well with changes in BOLD-signal recorded by fMRI.[7]

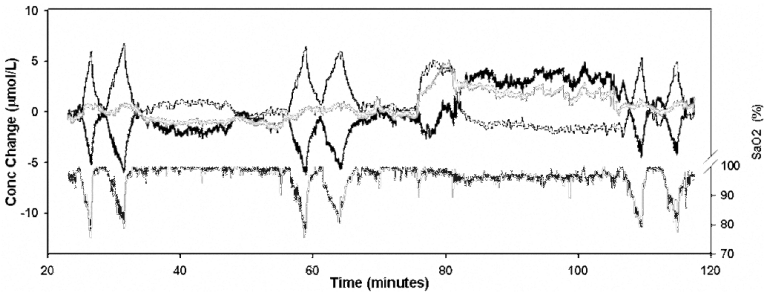
Techniques have been developed in neonatal research that enable NIRS to measure the more intuitive haemodynamic parameters, cerebral blood flow (CBF) and cerebral blood volume (CBV).[8-12] In contrast with the situation in neonates, in adults only CBV can be measured reproducibly.[9,10] CBV is affected by changes in cerebrovascular dilatation, arterial-to-venous-transit time and CBF.[13] To measure CBV, O_2Hb and HHb are used as an intravascular tracer, or 'dye'. A gradual decrease in the fraction of inspired oxygen results in a slow desaturation of haemoglobin, hence its name 'the O_2-method'.[11] The reproducibility of this method has been examined in neonates and adults, and its internal validity has been tested in an animal model, but no data are available for subjects above the age of 65.[10,11,14] Because ageing is associated with reduced cerebral blood flow, brain atrophy and changes in respiratory physiology, this method may be less reliable in older subjects. We designed this study to test the reproducibility of CBV measurements in subjects between 65 and 90 years old.

Methods

Study design

CBV was measured in two cortical regions: the frontal and frontoparietal cortex. It has previously been demonstrated that the frontal cortex is susceptible to deoxygenation in orthostatic hypotension.[3] The repeatability, short-term and long-term reproducibility were tested. Repeatability was assessed in three sets of repeated CBV measurements (see figure 1). Each set consisted of two identical desaturation periods with a normoxic interval of 1 min. Short-term reproducibility was assessed by inserting a 20 min interval between the first and the second set. During this interval, the subjects remained in a supine position. Between the second and the third set, we used a longer interval of 40 min. After 20 min, we performed an intervention that is likely to be used in future studies assessing CBV with NIRS. This intervention consisted of a 60° head-up tilt test for a period of 5 min. The purpose was to test the possible effect of a tilt-test procedure on optode position and fixation. Movement of the optodes due to tilting could have a negative effect on the reproducibility of the CBV measurements following this tilt test. We

Figure 1 Graphical overview of the experiment.



The bottom half shows the SaO_2-curve, where the desaturation episodes can easily be identified. The upper half of the graph shows the parameters measured by NIRS. Oxygenated haemoglobin decreases during desaturation (peaks facing downwards); deoxygenated haemoglobin rises during desaturation (upward peaks); and total haemoglobin (which represents the sum of the previous parameters) should remain constant during desaturation (a straight line).

waited 20 min before measuring CBV, to cancel out the effect that tilting itself has on CBV. Finally, long-term reproducibility was tested by repeating a CBV test after an interval of two or more weeks. All measurements were performed and analysed by the same researcher (JC).

This protocol was approved by the Ethics Committee of our institution.

Subjects

We recruited 16 healthy volunteers responding to a newspaper announcement, 12 men, 4 women, mean age 73 years, range 68–87. All subjects were examined by a geriatrician. They were leading an active and independent life, had no history of cardiovascular or cerebrovascular disease, had a normal cognitive screening (mini mental state examination score >24 and normal clock drawing test), and used no medication. They had normal ECGs, and normal duplex examination of the carotid and vertebral arteries. All subjects gave their written informed consent.

CBV measurements

We used a continuous wave NIRS instrument (Oxymon, Artinis Medical Systems, The Netherlands). A detailed description of the materials and methods used to measure CBV has been published previously.[10] The distance between the transmitter and the receivers was 5.5 cm to assure deep enough penetration of the infrared light into the brain to exclude substantial contamination from the extra-cerebral circulation. The probes were held in place by a custom-made elastic headband and were applied to the skin with light pressure. We applied a fixed differential path length factor of 6.6 (corresponding to age 50) in all subjects.[15] This factor corrects for the distance that the light travels through the cerebral tissue. No data are available on the actual variation of the differential path length factor with age above the age of 50. For the purpose of this reproducibility study, however, this is of little significance.

All experiments were performed in the morning. Beat-to-beat arterial pressure was recorded using a finger plethysmograph (Finapres, Ohmeda 2300, USA). Arterial saturation (SaO_2) was recorded with a pulse oximeter (N200 Nellcor Puritan Benett,

USA) with a reflectance sensor attached to the forehead, and end tidal CO_2 ($etCO_2$) was monitored using a capnograph (N1000, Nellcor Puritan Benett, USA). All analogue signals were synchronized and stored together with the NIRS data on a PC for off-line analysis.

During desaturation, the subjects were asked to breathe through a face mask, and hypoxia was induced using a stepwise computer controlled reduction of the inspired oxygen fraction (FiO_2) by changing an O_2/N_2 gas mixture (Bronckhorst Hitec, Ruurlo, The Netherlands). We aimed for a gradual decrease in SaO_2 of 10% below baseline within 3 to 5 min, after which FiO_2 was immediately increased to 33%. As a result of this, SaO_2 returned to normal values within 10 to 30 s.

CBV analysis

CBV analysis was performed off-line using a software application written inMatlab (Oxysoft, Artinis Medical Systems, The Netherlands). The start and end of each desaturation episode were visually identified from the graphic representation of the changes in [O_2Hb], [HHb] and SaO_2 (see figure 2). The theoretical explanation of CBV calculation from these data has been described in detail before.[10-12] In short, the concentrations of [O_2Hb] and [HHb] measured by NIRS vary with the concentration of cerebral haemoglobin [cHb] and its absorptive property, which depends on its oxygenation state (SaO_2). Because total cerebral haemoglobin remains constant during desaturation, [O_2Hb] and [HHb] will change equally but in opposite direction, and their average is used in the equation as the differential haemoglobin signal ($Hb_{diff} = ([O_2Hb] - [HHb])/2$). [cHb] is then derived from the relationship of the change in Hbdiff and SaO_2. This relationship can be calculated using the slope method, which applies linear regression (Hb_{diff} = slope $*$ SaO_2 + constant) to determine the slope, which equals [cHb]. CBV is derived from [cHb] by multiplying with a constant k that incorporates brain density, cerebral-to-large-vessel haematocrit ratio and unit conversion, and this result is divided by the subjects haemoglobin [Hb] concentration:

$CBV(mL\ 100\ g^{-1}) = k * [cHb]/[Hb]$

Statistical analysis

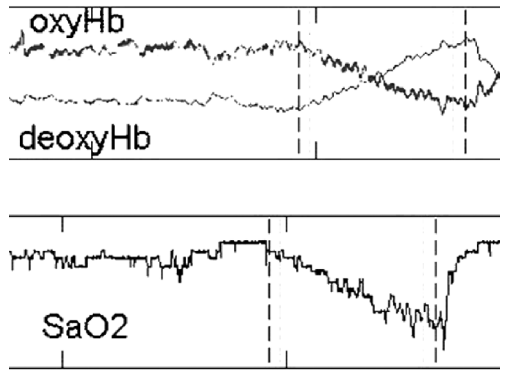
All CBV tests were processed regardless of their quality, except for those instances where CBV could not be calculated due to a disturbance in either the NIRS signal or the pulse oximetry signal, mostly due to motion artefacts or disconnection of the probes leading to signal loss. These instances are reported as missing values or uninterpretable tests.

For repeatability, we compared the two measurements in each of the three sets. For reproducibility, we compared the average values for the three sets. We calculated coefficients of variation (CV) and two-tailed Pearson correlation coefficients. Differences were tested with a paired sample *t*-test.

The overall CBV for each subject was calculated as the mean of the three CBV sets. Because of the small size of the group and the unevenly spread ages, we did not investigate the correlation between age or gender and CBV.

We determined mean values for blood pressure and $etCO_2$ measurements during 3 min of baseline recording and during 1 min at the end of the first desaturation episode, and compared these values using a paired-sample *t*-test.

Figure 2 Example of a CBV measurement.



The lower part of the graph shows the saturation curve, identifying the period of desaturation. The upper part shows the effect of desaturation on the parameters measured by NIRS, oxygenated and deoxygenated haemoglobin. The dotted lines mark the manually chosen points for the calculation of CBV using the slope method (see the text for details).

Results

Group values (mean ± SD) for cHb were 28.3 ± 9 μmol L^{-1}. The results of the CBV measurements are summarized in table 1. The CBV for the whole group was 0.98 ± 0.38 mL 100 g^{-1}. There was a small and non-significant difference between the CBV in the first and last set (–0.1 mL 100 g^{-1}, p = 0.2). Mean $etCO_2$ was 34.7 ± 3.6 mmHg during baseline and 34.8 ± 4.2 mmHg during desaturation. The average mean arterial pressure (MAP) was 75.7 ± 9.5 mmHg during baseline and 78.9 ± 9.9 mmHg during desaturation (p = 0.034).

Table 1

	Age		CBV (mL 100 g^{-1})			
Subject	**Gender**	**(years)**	**Baseline**	**20 min**	**40 min**	**≥ 1 week**
1	Female	70	0.52	0.56	0.51	0.52
2	Female	68	1.28	0.96	1.11	0.47
3	Female	70	0.64	0.86	0.81	0.61
4	Female	73	0.77	0.80	0.77	0.89
5	Male	69	1.20	1.06	1.05	1.17
6	Male	70	Missing	0.63	0.54	1.04
7	Male	68	1.06	1.25	1.39	1.23
8	Male	75	0.84	0.77	0.80	1.00
9	Male	69	1.17	1.29	1.11	1.00
10	Male	82	1.21	1.08	0.91	1.03
11	Male	74	0.87	0.64	0.50	Missing
12	Male	70	1.30	1.32	1.10	1.47
13	Male	73	1.76	1.27	1.56	1.33
14	Male	75	1.16	1.46	1.42	1.35
15	Male	75	0.65	0.68	0.58	1.17
16	Male	87	0.84	0.68	0.59	0.88
	Mean	73	1.02	0.96	0.92	1.01
	SD	5.2	0.33	0.29	0.34	0.30
	Min	68	0.52	0.56	0.50	0.47
	Max	87	1.76	1.46	1.56	1.47

Summary of individual data for age, gender and CBV values. This table provides age and CBV values for all subjects, as well as mean values for the whole group. Three sets of two CBV measurements were performed with intervals of 20 min, 40 min and more than 1 week. Each CBV value represents the average of the two CBV measurements in that particular set. Missing values are uninterpretable CBV measurements.

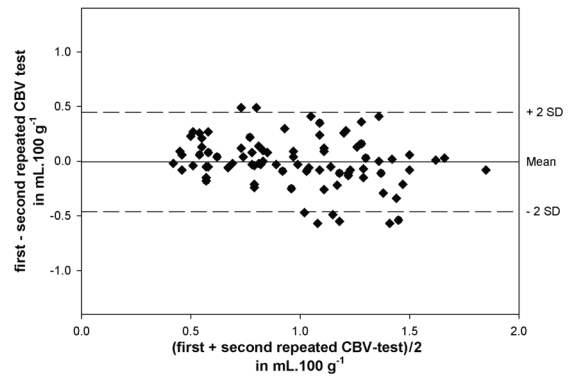
The results for repeatability are demonstrated in table 2. We found no difference in repeatability between the three sets, or between the two measurement sites, and therefore all measurements were pooled. The CV for these pooled data was 12.5% (95% CI: 9.3–15.8%). A Bland–Altman plot is presented in figure 3.

Table 2

Optode position	Missing values	CBV-set 1 CV (%) (95% CI)	CBV-set 2 CV (%) (95% CI)	CBV-set 3 CV (%) (95% CI)
Frontal	8	11.8 (5.9–17.6)	11.7 (7.4–16.1)	11.0 (5.2–16.8)
Parietal	15	10.9 (6.3–15.5)	14.3 (5–23.6)	17.3 (8.7–25.9)

Coefficients of variation (CV) for repeated CBV tests, expressed as mean values with 95% confidence interval. The first pair of CBV tests (CBV-set 1) was recorded at the baseline, the second (CBV-set 2) was performed after 20 min of supine rest, and the third (CBV-set 3) after 40 min of supine rest, interrupted midway by a tilt test for 5 min. Missing values are reported as the percentage of the total number of CBV-sets (48) that were collected.

Figure 3 Repeatability of CBV tests.



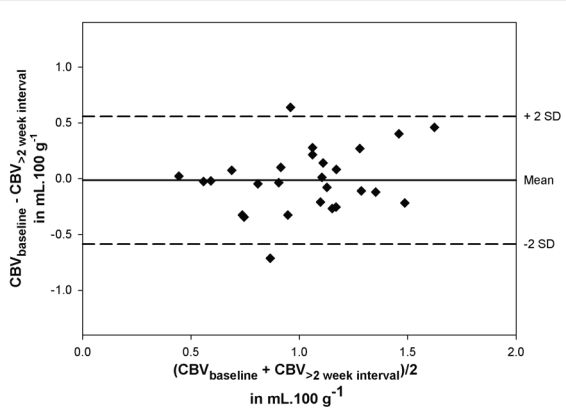
Analysis as proposed by Bland and Altman of repeated CBV tests calculated using the slope method (see the text for details). In 16 subjects, six sets of two repeated (interval: 1 min) CBV measurements were performed. The average of these two measurements is plotted against their difference

The mean CV for the third set of repeated CBV tests, which was obtained 20 min after a tilt table intervention, was not significantly different from that of previous CBV sets. Pearson's correlation coefficient for the repeated tests was $R = 0.81$, $p < 0.001$. 11 out of 96 sets (11%) could not be analysed because either or both CBV tests were uninterpretable.

For short-term reproducibility, we found a good correlation between the three CBV values ($R > 0.8$, $p = 0.01$), and the CV was 11.7% (95% CI: 8.3–15%). For the CBV values obtained on different days (with an interval of two or more weeks), correlation was acceptable (R=0.5, p=0.05) and the CV was 15% (95% CI 5–25%). A Bland–Altman plot for long-term reproducibility is presented in figure 4.

The quality of a measurement using the slope method can be expressed by the linear correlation coefficient (r^2) of the relationship between the changes in Hb_{diff} and SaO_2. Mean r^2 for our measurements was 0.92 ± 0.05. We found a correlation between the goodness of fit (a high value for r^2) and the CV ($R = 0.3$, $p = 0.05$). All CBV values with $r^2 > 0.95$ had a CV of 10% or less.

Figure 4 Long-term reproducibility of CBV measurements.



Analysis as proposed by Bland and Altman. In 16 subjects, CBV was determined twice with an interval of more than 2 weeks. The average of the two CBV values is plotted against their difference.

Discussion

The main finding of our study is that CBV measurements with NIRS can be performed with the same reliability in old subjects as they can in other age groups. Repeatability in our study resulted in a CV of 12.5%, which is almost equivalent to the CV of 11.7% that was found for this method in adults and the CV of 11.5% that was found in neonates.[10,11] Of note, the study of Van de Ven *et al* was performed in our institution using identical equipment and methodology. These investigators have discussed the differences with reproducibility results from studies using other methodology.[10]

An important new outcome of our study is that, even with a prolonged interval of several weeks, measurements of CBV with NIRS in this age group of healthy elderly subjects were reproducible with a CV of 15%, and showed an acceptable correlation. Over such a period, individual CBV is subject to considerable physiological change. The option to perform repeated measurements of CBV over time with a non-invasive method like NIRS offers an interesting perspective for cerebrovascular research.

The reproducibility of CBV measurements with NIRS in our study compares favourably to other methods. For example, PET measures CBV with a CV of 20% [16], and MRI measures CBV with a CV of 14% [17].

The fact that we did not validate our NIRS measurements with a second method to determine CBV is a limitation in the design of this study. However, even though there are several methods available to measure CBV, none of these can actually serve as a gold standard. Haemodynamic measurements with NIRS in animals, neonates and adults have been compared with MRI, immunolabelling, jugular venous occlusion plethysmography and PET.[18-21] The correlation between the two methods studied varied from very poor to excellent. Only one of these studies, comparing NIRS with MRI, actually used the desaturation method to determine CBV. In this study, the group mean value for CBV obtained with NIRS was similar to that found with MRI, but intra-individual correlation between the two methods was poor.[21] We judged that validation with a second neuroimaging tool that cannot serve as a gold standard would yield little extra information for the purpose of our reproducibility study.

A potential problem with the desaturation method is that it could influence CBV. Prolonged hypoxia, especially around or below a SaO_2 of 80%, can induce vasodilatation and increase CBF and CBV. On the other hand, desaturation could produce compensatory hyperventilation, which causes vasoconstriction and reduces CBF. In our study, we found no evidence for hyperventilation during desaturation, as $etCO_2$ remained stable. Blood pressure rose slightly during desaturation, which is consistent with previous investigations. Because we performed paired CBV measurements with a short interval, any substantial effect of the desaturation procedure itself on CBV would result in the second CBV measurement being systematically higher or lower than the first. We did not find such an effect in our study. In addition, we constantly monitored the total haemoglobin concentration measured by NIRS, which did not change during our desaturation procedures.

We did not investigate the possible effects of altered brain geometry with ageing on the outcome of CBV measurements. For instance, brain atrophy with ageing would increase the depth of the cerebral tissue relative to the probes. This could unfavourably affect the relative contribution of extracerebral tissue to the NIRS signal. In order to investigate this, a method comparing different source-detector distances would be needed. Kohri *et al*.[22] for example demonstrated that with source-detector distances of 3 cm, the cerebral tissue contributed to 55% of the signal, versus 69% when using a 4 cm distance. We did not investigate these aspects in this study for several reasons. First, we wished to compare results directly with the previous study by Van de Ven *et al* in younger subjects, using an identical technique. Second, the source-detector distance used with the Oxymon-device is 5.5 cm, which is larger than is generally available in other NIRS devices, and represents the optimal distance for this device to minimize noise from extra-cerebral tissues. Third, a previous study in our laboratory has measured the depth between NIRS optodes and cerebral cortical tissue using MRI in a small group of young (35 ± 9 years) and ageing (73 ± 3 years) subjects, and found no evidence for an increase with ageing.[7]

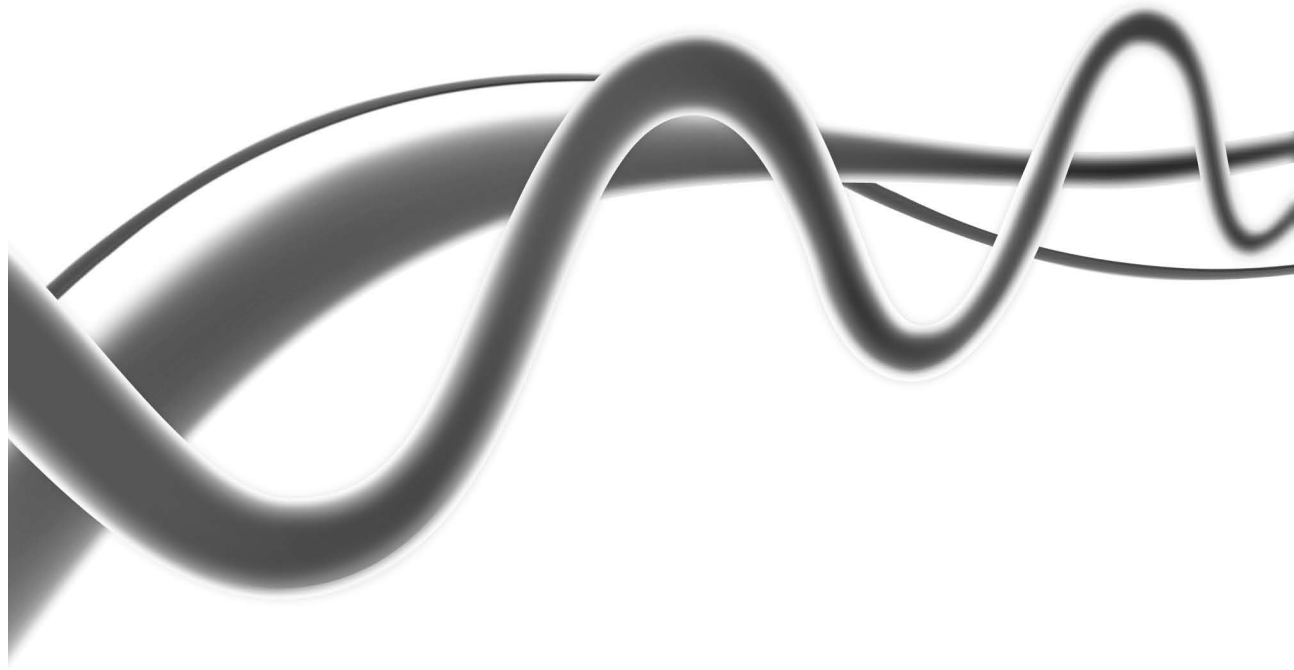
Possibly related to this matter is the finding of low CBV values in our group. Even though estimation of individual values for CBV was not the primary focus of this study, this finding merits further discussion. For comparison, mean CBV determined by PET was 3.8 ± 0.7 mL 100 g^{-1} in a group with a mean age of 51.8 (±15.1) years.[16] The study of Van de Ven *et al*, using NIRS, found a mean CBV value of 3.66 ± 0.82 mL 100 g^{-1} in a group of adults with a mean age of 31 ± 10 years. Future studies are needed to further investigate the low CBV values we found in our age group. Despite similar brain geometry in young and ageing subjects, a reduction with ageing in the deoxyhaemoglobin response to cortical activation was apparent both with NIRS and fMRI in a previous study.[7] One of many possible explanations for this finding is a reduced cortical haemoglobin content with ageing. A future study using different source-detector distances and measuring brain geometrics with MRI may serve to confirm in a larger group that there is no evidence for reduced brain tissue sample due to brain atrophy. In addition, a study investigating a larger, intermediate age group may demonstrate if a true reduction of CBV measured by NIRS age occurs with advancing age.

Conclusion

The O_2-method to measure changes in CBV with NIRS can be applied in subjects aged 65 years and older. Despite many possible sources of variation in older subjects, including reduced global cerebral perfusion, brain atrophy and altered respiratory physiology, we found that reproducibility in our study was comparable to investigations in younger subjects. We conclude that NIRS is a safe and reliable tool to monitor changes in CBV in cerebrovascular research in the ageing population.

References

1. Kamper AM, Spilt A, de Craen AJ, van Buchem MA, Westendorp RG, Blauw GJ. Basal cerebral blood flow is dependent on the nitric oxide pathway in elderly but not in young healthy men. *Exp Gerontol 2004;39:1245–8.*
2. Jansen RW, Lipsitz LA. Postprandial hypotension: epidemiology, pathophysiology, and clinical management. *Ann Intern Med 1995;122:286–95.*
3. Mehagnoul-Schipper DJ, Vloet LC, Colier WN, Hoefnagels WH, Jansen RW. Cerebral oxygenation declines in healthy elderly subjects in response to assuming the upright position. *Stroke 2000;31:1615–20.*
4. Vloet LC, Mehagnoul-Schipper DJ, Hoefnagels WH, Jansen RW. The influence of low-, normal-, and high-carbohydrate meals on blood pressure in elderly patients with postprandial hypotension. *J Gerontol A Biol Sci Med Sci 2001;56:M744–8.*
5. Obrig H, Villringer A. Beyond the visible–imaging the human brain with light. *J Cereb Blood Flow Metab 2003;23:1–18.*
6. Mehagnoul-Schipper DJ, Colier WN, Jansen RW. Reproducibility of orthostatic changes in cerebral oxygenation in healthy subjects aged 70 years or older. *Clin. Physiol 2001;21:77–84.*
7. Mehagnoul-Schipper DJ, van der Kallen BF, Colier WN, van der Sluijs MC, van Erning LJ, Thijssen HO, Oeseburg B, Hoefnagels WH, Jansen RW. Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. *Hum Brain Mapp 2002;16:14–23.*
8. Fallon P, Roberts I, Kirkham FJ, Elliott MJ, Lloyd-Thomas A, Maynard R, Edwards AD. Cerebral hemodynamics during cardiopulmonary bypass in children using near-infrared spectroscopy. *Ann Thorac Surg 1993;56:1473–7.*
9. van de Ven MJ, Colier WN, Walraven D, Oeseburg B, Folgering H. Cerebral blood flow in humans measured with near infrared spectroscopy is not reproducible. *Adv Exp Med Biol 1999;471:749–58.*
10. van de Ven MJ, Colier WN, van der Sluijs MC, Walraven D, Oeseburg B, Folgering H. Can cerebral blood volume be measured reproducibly with an improved near infrared spectroscopy system? *J Cereb Blood Flow Metab 2001;21:110–3.*
11. Wolf M, Bucher H U, Dietz V, Keel M, von Siebenthal K, Duc G. How to evaluate slow oxygenation changes to estimate absolute cerebral haemoglobin concentration by near infrared spectrophotometry in neonates. *Adv Exp Med Biol 1997;411:495–501.*
12. Wyatt JS, Cope M, Delpy DT, Richardson CE, Edwards AD, Wray S, Reynolds EO. Quantitation of cerebral blood volume in human infants by near-infrared spectroscopy. *J Appl Physiol 1990;68:1086–91.*
13. Rostrup E, Knudsen GM, Law I, Holm S, Larsson HB, Paulson OB. The relationship between cerebral blood flow and volume in humans. *Neuroimage 2005;24:1–11.*
14. Firbank M, Elwell CE, Cooper CE, Delpy DT. Experimental and theoretical comparison of NIR spectroscopy measurements of cerebral hemoglobin changes. *J Appl Physiol 1998;85:1915–21.*
15. Duncan A, Meek JH, Clemence M, Elwell CE, Fallon P, Tyszczuk L, Cope M, Delpy DT. Measurement of cranial optical path length as a function of age using phase resolved near infrared spectroscopy. *Pediatr Res 1996;39:889–94.*
16. Ito H, Kanno I, Kato C, Sasaki T, Ishii K, Ouchi Y, Iida A, Okazawa H, Hayashida K, Tsuyuguchi N, Ishii K, Kuwabara Y, Senda M. Database of normal human cerebral blood flow, cerebral blood volume, cerebral oxygen extraction fraction and cerebral metabolic rate of oxygen measured by positron emission tomography with 15O-labelled carbon dioxide or water, carbon monoxide and oxygen: a multicentre study in Japan. *Eur J Nucl Med Mol Imaging 2004;31:635–43.*
17. Henry ME, Kaufman MJ, Lange N, Schmidt ME, Purcell S, Cote J, Perron-Henry DM, Stoddard E, Cohen BM, Renshaw PF. Test-retest reliability of DSC MRI CBV mapping in healthy volunteers. *Neuro Report 2001;12:1567–9.*
18. Barfield CP, Yu VY, Noma O, Kukita J, Cussen LJ, Oates A, Walker AM. Cerebral blood volume measured using near-infrared spectroscopy and radiolabels in the immature lamb brain. *Pediatr Res 1999;46:50–6.*
19. Rostrup E, Law I, Pott F, Ide K, Knudsen GM. Cerebral hemodynamics measured with simultaneous PET and near-infrared spectroscopy in humans. *Brain Res 2002;954:183–93.*
20. Wickramasinghe Y A, Livera LN, Spencer SA, Rolfe P, Thorniley MS. Plethysmographic validation of near infrared spectroscopic monitoring of cerebral blood volume. *Arch Dis Child 1992;67:407–11.*
21. Wolf M, Weber O, Keel M, Golay X, Scheidegger M, Bucher HU, Kollias S, Boesiger P, Banziger O. Comparison of cerebral blood volume measured by near infrared spectroscopy and contrast enhanced magnetic resonance imaging. *Adv Exp Med Biol 1999;471:767–73.*
22. Kohri S, Hoshi Y, Tamura M, Kato C, Kuge Y, Tamaki N. Quantitative evaluation of the relative contribution ratio of cerebral tissue to near-infrared signals in the adult human head: a preliminary study. *Physiol Meas 2002;23:301–12.*



Chapter 5

Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing

Jurgen A.H.R. Claassen
Rong Zhang
Qi Fu
Sarah Witkowski
Benjamin D. Levine

J Appl Physiol 2007; 102: 870–877.

Abstract

Clinical transcranial Doppler assessment of cerebral vasomotor reactivity (CVMR) uses linear regression of cerebral blood flow velocity (CBFV) vs. end-tidal CO_2 (PET_{CO2}) under steady-state conditions. However, the cerebral blood flow (CBF)-PET_{CO2} relationship is nonlinear, even for moderate changes in CO_2. Moreover, CBF is increased by increases in arterial blood pressure (ABP) during hypercapnia. We used a modified rebreathing protocol to estimate CVMR during transient breath-by-breath changes in CBFV and PET_{CO2}. Ten healthy subjects (6 men) performed 15 s of hyperventilation followed by 5 min of rebreathing, with supplemental O_2 to maintain arterial oxygen saturation constant. To minimize effects of changes in ABP on CVMR estimation, cerebrovascular conductance index (CVCi) was calculated. CBFV-PET_{CO2} and CVCi-PET_{CO2} relationships were quantified by both linear and nonlinear logistic regression. In three subjects, muscle sympathetic nerve activity was recorded. From hyperventilation to rebreathing, robust changes occurred in PET_{CO2} (20–61 Torr), CBFV (-44 to +104% of baseline), CVCi (-39 to +64%), and ABP (-19 to +23%) (all $P < 0.01$). Muscle sympathetic nerve activity increased by 446% during hypercapnia. The linear regression slope of CVCi vs. PET_{CO2} was less steep than that of CBFV (3 vs. 5%/Torr; $P = 0.01$). Logistic regression of CBF-PET_{CO2} ($r^2 = 0.97$) and CVCi-PET_{CO2} ($r^2 = 0.93$) was superior to linear regression ($r^2 = 0.91$, $r^2 = 0.85$; $P = 0.01$). CVMR was maximal (6–8%/Torr) for PET_{CO2} of 40–50 Torr. In conclusion, CBFV and CVCi responses to transient changes in PET_{CO2} can be described by a nonlinear logistic function, indicating that CVMR estimation varies within the range from hypocapnia to hypercapnia. Furthermore, quantification of the CVCi-PET_{CO2} relationship may minimize the effects of changes in ABP on the estimation of CVMR. The method developed provides insight into CVMR under transient breath-by-breath changes in CO_2.

Introduction

The prominent cerebral blood flow (CBF) responses to changes in arterial CO_2 are a unique characteristic of the cerebral vasculature.[1,2] These responses have been quantified to reflect cerebral vasomotor reactivity (CVMR)[3], even though the underlying mechanisms are still not completely understood.[4] With the advent of transcranial Doppler (TCD) for measurement of CBF velocity (CBFV) with high temporal resolution, the Doppler-CO_2 test of CVMR has become a widely adopted method in research and clinical practice practice. Research exploring the physiological characteristics of CVMR has demonstrated that the relationship between arterial CO_2 and CBF is nonlinear and that this relationship is affected by CO_2-induced changes in arterial blood pressure (ABP).[3,5-12] In addition, the control mechanisms that govern the responses of CBF and ABP to CO_2 have dynamic properties.[5,9,11] Despite these complexities associated with the CBF-CO_2 relationship, measurement of CVMR has been widely applied in clinical practice to evaluate cerebral vascular function, e.g., in patients with carotid artery stenosis or hypertension.[3,13-15] These clinical studies have quantified CVMR using linear regression of steady-state responses of CBF to changes in CO_2, without incorporation of the effects of ABP. The likely reason for this simplicity is that well-controlled experiments and complex modeling methods may not be practical for clinical use.[9,16] In this regard, development of a new method that is clinically applicable and may assess CVMR reliably under conditions of breath-by-breath changes in end-tidal CO_2 (PET_{CO2}) is warranted.

We used a modified rebreathing protocol, consisting of a period of voluntary hyperventilation, followed by rebreathing, to obtain a wide range of changes in PET_{CO2} to assess CVMR under breath-by-breath conditions.[17] We hypothesized that the CBF-PET_{CO2} relationship is nonlinear during transient changes in PET_{CO2}. In addition, we speculated that measurement of cerebrovascular conductance index (CVCi) during this process may reveal direct effects of changes in ABP on CBF, leading to a more precise estimation of CVMR based on the CVCi-PET_{CO2} relationship. Finally, to explore the underlying mechanisms of increases in ABP during hypercapnia, we recorded muscle sympathetic nerve activity (MSNA) in three subjects in this study.

Methods

Ten healthy subjects (4 women) with a mean age of 37 yr (SD 8), height 177 cm (SD 10), and weight 84 kg (SD 18) voluntarily participated in the study. Participants were nonsmokers, were not on any medication, and were normotensive. Participants were free of known cardiovascular, cerebrovascular, or respiratory disease. Each subject was informed of the experimental procedures and signed a consent form, approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas.

Protocol. Each participant visited the laboratory twice, 3–4 wk apart (in three participants, the time interval was 3–4 mo). All experiments took place in a quiet laboratory with a room temperature of 22°C. The subjects refrained from heavy exercise and caffeinated or alcoholic beverages at least 12 h before the tests. The study was conducted with the subjects in the supine position. Therefore, assessment of CVMR would be comparable with those obtained with other imaging technology.[16] Participants wore a nose clip and breathed through a mouthpiece with a Y-valve, with one end connected to a 5-liter rubber bag and the other end open to room air. This system was held in place by an adjustable cable, rendering it practically weightless to avoid any discomfort for the subject. After at least 20 min of stabilization, 2 min of baseline data during spontaneous breathing were recorded on room air. A modified Read rebreathing protocol was used.[17,18,19] The procedure contains a period of voluntary hyperventilation preceding rebreathing to obtain a wider range of CO_2 changes. Specifically, a short period of 15 s of hyperventilation with a duty cycle of 1 s and maximal voluntary deep breaths were used. This short period of hyperventilation reduced PET_{CO2} to ~20 Torr but did not cause respiratory muscle fatigue or central hypoxia possibly associated with a prolonged hyperventilation as employed in the other rebreathing methods.[18,19] At the end of a deep inspiration, the respiratory valve was switched to the empty bag and then the bag was filled with the subjects' own breathing gas during expiration (with a PET_{CO2} of ~20 Torr, comparable to a gas mixture with 2.6% CO_2). Rebreathing was continued for 5 min, before returning to room air for recovery (4 min). During rebreathing, a small amount of oxygen was bled into the rebreathing bag at the subject's basal metabolic rate (estimated using the Harris-Benedict formula) to maintain arterial oxygen saturation (SaO_2) constant.[20]

Instrumentation and data acquisition. The middle cerebral artery (MCA) was insonated with a 2-MHz pulsed Doppler ultrasound system (Multi-Dope X2; DWL). The probe was securely attached to the cranium by a mold that was cast individually to fit the facial bone structure.[21] In this way, the exact position and angle of the probe were preserved during the repeat tests.

Throughout the test, ABP was monitored noninvasively using a Finapres (Ohmeda 2300) and was corroborated by concurrent electrosphygmomanometry (SunTech Medical) with measurements at intervals of 1 min. PET_{CO2} was recorded using a capnograph (POET TE; Criticare Systems), SaO_2 was measured using a pulse oximeter (Biox 3700; Ohmeda), and heart rate (HR) was monitored with a three-lead ECG connected to a cardiotachometer (Quinton Instruments).

MSNA was recorded in three participants. MSNA signals were obtained by the microneurographic technique.[22] Sympathetic bursts were identified by a computer program and then were confirmed by an experienced microneurographer. The number of bursts per minute (burst frequency) and the sum of the integrated burst area per minute (total activity) were used as quantitative indexes. Specifically, total activity was normalized to the baseline value (set at 100%) to reflect changes in sympathetic activity during CO_2 stimuli.

Data analysis. Off-line data analysis was performed with commercially available software (Acknowledge; BIOPAC Systems). For hyperventilation, the time delay between the onset of hyperventilation and the onset of changes in CBFV and ABP was measured. Similarly, at the end of rebreathing, the time delays for recovery between PET_{CO2}, CBFV, and ABP were measured. Values for CBFV, ABP, and PET_{CO2} at the last minute of recovery were compared with their pretest baseline. Breathing cycles were identified from the respiratory CO_2 signal. For each cycle, the corresponding value for PET_{CO2} and mean values for CBFV, ABP, and HR were measured. Averaging the number of cardiac cycles (usually 6) has been applied previously in an attempt to reduce respiratory hemodynamic variability.[23] We preferred to identify each respiratory cycle[24] to derive the breath-to-breath CBFV-PET_{CO2} relationship. During the short breathing cycles in hyperventilation, an average of data from two cycles was used. Because CBFV is affected directly by changes in ABP[2,5,6,10], CVCi was calculated by dividing mean CBFV by mean ABP

within each breath cycle to reveal intrinsic vascular responses to changes in CO_2. Furthermore, percent changes from baseline CBFV and CVCi values were calculated for data analysis. Data ranging from the nadir of PET_{CO2} during hyperventilation to the end of rebreathing were utilized for curve fitting to quantify the CBFV-PET_{CO2} and CVCi-PET_{CO2} relationship.

A four-parameter logistic function was used for curve fitting[25]:

$$f(x) = y0 - \frac{a}{1+e^{[bx\ (x-x0)]}} \qquad (1)$$

where $f(x)$ represents percent changes in CBFV or CVCi (x representing PET_{CO2}). The model parameter a represents the range of change in CBFV or CVCi, $y0$ is the maximum value of CBFV or CVCi during hypercapnia, $x0$ is the level of PET_{CO2} where the first-order derivative of the logistic function (the slope of the curve) is maximal, and b is related to the overall curvilinear properties of the sigmoidal curve (Fig. 1). Nonlinear curve fitting was performed with a Marquardt-Levenberg algorithm for model parameter identification (SigmaPlot 8.02; SPSS). The selection of this model was based on the feature of curvilinear distribution of breath-by-breath changes in CBFV or CVCi vs. PET_{CO2} (Fig. 2) and the fact that model parameters of the selected logistic function have clear physiological implications, as shown in Fig. 1.[25]

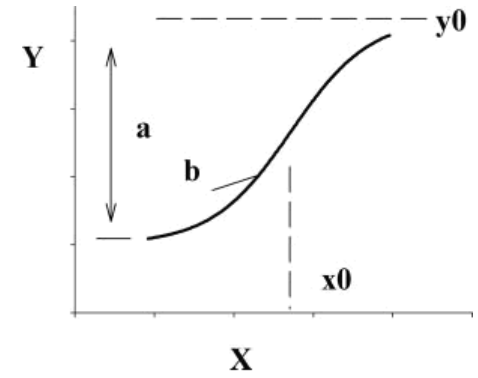
The first-order derivative of the logistic function was calculated with the following equation:

$$f'(x) = \frac{a \times b \times e^{[bx\ (x-x0)]}}{\{1+e^{[bx\ (x-x0)]}\}^2} \qquad (2)$$

This derivative function yields the specific CO_2 sensitivity (or CVMR) for each PET_{CO2}. At $x = x0$, CVMR becomes maximal (CVMRmax), and *Eq.* 2 becomes $(a \times b)/4$. Of note, CVMRmax derived from the CBFV-PET_{CO2} relationship was denoted as CBFVCVMRmax and CVMRmax derived from the CVCi-PET_{CO2} relationship was denoted as CVCi-CVMRmax.

For comparison, linear regressions of changes in CBFV and CVCi vs. PET_{CO2} were conducted for the entire range of changes in PET_{CO2}, from the nadir of hyperventilation to the end of rebreathing. The slopes of these linear regressions were defined as

Figure 1 Schematic representation of a logistic function with 4 parameters to be identified.



a Total range of changes in cerebrovascular conductance index (CVCi) or cerebral blood flow velocity (CBFV); $y0$, maximum value; $x0$, level of end-tidal CO_2 (PET_{CO2}) that exhibits highest CO_2 sensitivity; **b** curvilinear properties of the sigmoidal curve.

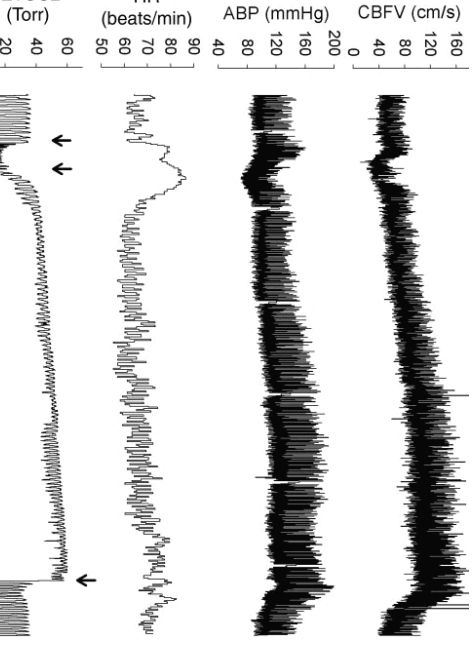
CBFV-CVMR0 and CVCi-CVMR0, respectively. In addition, to complement the assessment of CVMRmax with the derivative method, CVMR was estimated by the linear regressions in the steep part of the sigmoidal curves, and these results were defined as CBFV-CVMR1 and CVCi-CVMR1, respectively.

Statistics. The results of curve fitting were examined by the correlation coefficients (r^2) and by model residual analysis of individual data (SigmaPlot 8.02; SPSS). Thus individual data for r^2 as well as mean sum of squares were available for both linear and logistic curve fits, allowing comparisons of both methods using paired t-tests (SigmaStat 3.11; Systat Software). Comparisons of cerebral hemodynamics at baseline, during hyperventilation, and during rebreathing were made with one-way repeated-measures ANOVA. Comparisons between the estimates of CVMR by different methods were made with paired t-tests. Test-retest reproducibilities for curvefitting parameters and for CVMR estimation were examined by the analysis of typical error (the SD of the differences between the two tests divided by square root of 2), which was expressed as a coefficient of variation.[26] Data are presented as means and SD, and a $P < 0.05$ was considered to be statistically significant.

Results

Changes in cerebral and systemic hemodynamics. All 10 participants completed the experimental protocol, so that 20 data sets were collected. Data from one test could not be used because of poor signal quality; hence, reproducibility results were available only for nine subjects. Representative changes in CBFV, ABP, $PETC_{O2}$, HR, and MSNA are shown in Fig. 2. Individual values for Sa_{O2} varied within the range of 96–100% during baseline breathing of room air and remained unchanged during rebreathing, owing to the supplementation of oxygen. There was a delayed response of CBFV (mean: 7 s, range 6 –10) and ABP (mean: 14 s, range

Figure 2 A typical recording of baseline, hyperventilation, rebreathing, and recovery in 1 subject.



ABP, arterial blood pressure; HR, heart rate; MSNA, muscle sympathetic nerve activity. Note the significant increases in ABP and MSNA, concurrent with the increase in CBFV during rebreathing. Arrows indicate start and end of hyperventilation and rebreathing periods.

Table 2 Changes in Cerebral and Systemic Hemodynamics during the Test.

	Baseline				Hyperventilation				Rebreathing			
Subject	PET_{CO2}	CBFV	MABP	HR	PET_{CO2}	CBFV	MABP	HR	PET_{CO2}	CBFV	MABP	HR
1	46	60	89	59	23	31	65	76	67	101	101	64
2	42	82	77	72	22	42	57	87	61	142	92	77
3	41	89	90	62	24	45	63	70	58	155	142	80
4	42	45	89	56	16	23	73	68	62	98	101	59
5	43	71	86	45	17	36	65	79	61	151	103	57
6	40	56	96	70	13	29	75	94	61	127	108	78
7	37	60	103	59	20	31	95	73	55	122	123	70
8	41	41	94	69	21	21	77	81	65	104	108	72
9	37	55	103	65	23	38	86	85	61	128	139	75
10	42	62	99	76	22	32	89	97	62	128	119	86
Mean	41	62	93	63	20*	33*	75*	81*	61*	126*	114*	72*
SD	3	15	8	9	4	8	12	10	3	20	17	9

Values are means from all available tests in n=10 subjects.PET_{CO2}: end-tidal CO_2 (mmHg). CBFV: cerebral blood flow velocity (cm/s). MABP: mean arterial blood pressure (mmHg). HR: heart rate (bpm). Baseline: average of 2-minute recordings before hyperventilation. Hyperventilation: lowest values following 15 seconds of hyperventilation. Rebreathing: average of the last minute of data.
*: $p < 0.01$ for comparison of hyperventilation and rebreathing with baseline.

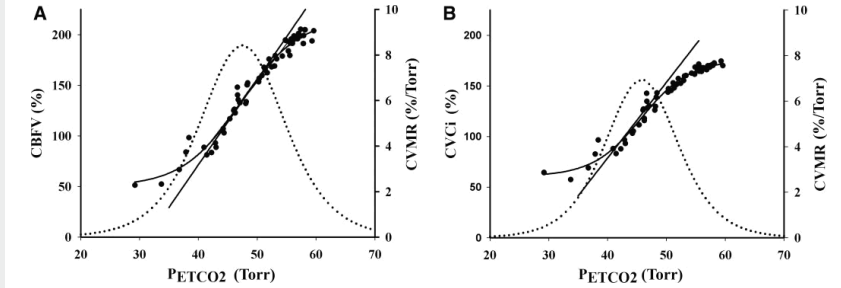
12–16) to changes in PET_{CO2} during hyperventilation. Correction for the time delay did not significantly alter the result of data analysis. Because the physiological validity of applying a time correction for CVMR estimation was uncertain, analysis of the raw (uncorrected) data is presented. After the rapid return of PET_{CO2} to the pretest baseline level at the onset of recovery, a considerable time delay was observed for the ensuing recovery of CBFV (mean: 21 s, range 14–35) and of ABP (mean: 22 s, range 12–30). However, this delay did not influence the data analysis because the recovery period was not included in the models used to assess CVMR in this study. Whereas ABP gradually returned to the pretest baseline after rebreathing, CBFV displayed an undershoot and then gradually returned to a sustained level, which was below the pretest baseline level (mean: 81% of baseline, range 65–90), even at the end of the 4 min of recovery. On average, PET_{CO2} was reduced by 22 Torr during hyperventilation and increased by 20 Torr during rebreathing from the pretest baseline ($P < 0.001$).

These changes in PET_{CO2} resulted in a respective reduction of CBFV by 46% and an increase by 104% relative to the baseline ($P<0.001$). CVCi fell by 39% during hyperventilation and increased by 64% during rebreathing ($P < 0.001$). Of note, ABP was reduced by 19% during hyperventilation and augmented by 23% during rebreathing ($P<0.01$). During rebreathing, increases in ABP appeared to be proportional to increases in PET_{CO2} but leveled off at high levels of PET_{CO2} (Fig. 2). HR increased by 29% during hyperventilation and by 14% during rebreathing ($P<0.01$) (Fig. 2, Table 1).

In the three subjects that underwent recording of MSNA, averaged burst frequency increased from 24 at baseline to 30 bursts/min during hyperventilation, and total nerve activity increased by 166% ($P=0.01$). MSNA returned rapidly to baseline after cessation of hyperventilation and subsequently increased to 41 bursts/min and 446% for total nerve activity during the last minute of rebreathing ($P=0.01$; Fig. 2). Changes in MSNA were similar in all three subjects.

Curve-fitting and model parameters. Figure 3 depicts the representative results of sigmoidal curve fitting of the CBFV/CVCi-PET_{CO2} relationship in one subject. The specific CVMR derived from the first-order derivative of the logistic function is also plotted.

Figure 3 Typical data of logistic regression of %changes in CBFV and CVCi to changes in end-tidal CO_2 (PET_{CO2}) in 1 subject.

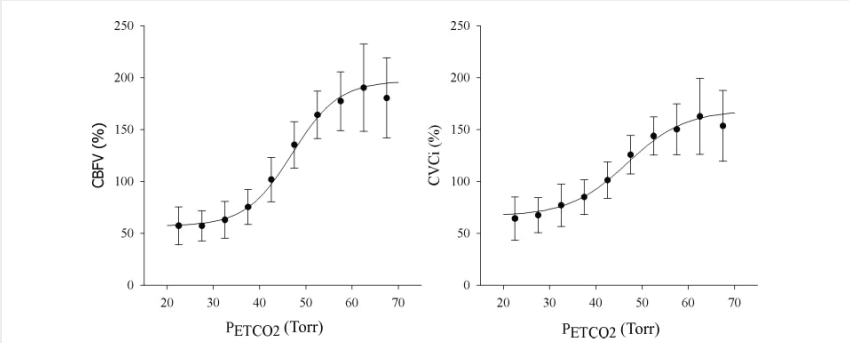


Sigmoidal curve represents the result of logistic regression; the dotted bell-shaped curve is the first-order derivative of the identified logistic function. Linear regression was performed in the steep portion of the sigmoidal curve to estimate cerebral vasomotor reactivity (CVMR), using CBFV and CVCi as the dependent variables, respectively.

The group-averaged data of changes in CBFV and CVCi as well as logistic regression of pooled data for all subjects are shown in Fig. 4. Because there were no significant differences between results from the first and second tests, all data were used in the pooled data analysis. Logistic regression of individual data demonstrated an excellent curve-fitting result (mean $r^2 = 0.95$, SD 0.04; average of mean sum of squares = 37, SD 19), superior to the use of linear regression (mean $r^2 = 0.88$, SD 0.06; average of mean sum of squares = 85, SD 51; $P<0.01$) for both the CBFV-PET_{CO2} and the CVCi-PET_{CO2} relationships. Specifically, logistic regression was performed well in all 18 tests for changes in CBFV ($r^2 = 0.97$, SD 0.02) and in 16 of 18 tests for CVCi ($r^2 = 0.93$, SD 0.06). In those two cases of CVCi for which logistic regressions could not be performed reliably, linear regressions yielded an r^2 of 0.71 and 0.76 for CBFV and CVCi, respectively.

Table 2 shows the group-averaged model parameters and the calculated CVMR indexes derived, respectively, from the logistic and linear regressions. Over the entire range of changes in PET_{CO2}, the linear regression slope of changes in CVCi (CVCi-CVMR0) was significantly smaller than that of CBFV (CBFV-CVMR0). The

Figure 4 Group (n = 10) averaged results for logistic regression of percent changes in CBFV and CVCi to changes in PET_{CO2}.



Sigmoidal curve represents the result of logistic regression of pooled group data from all subjects (parameters for CBFV: a = 140%, $y0$ = 198%, $x0$ = 47 Torr, b = 0.20 $Torr^{-1}$; parameters for CVCi: a = 102%, $y0$ = 169%, $x0$ = 47 Torr, b = 0.16 $Torr^{-1}$; note that these values are similar to the parameters obtained by averaging the individual curve fitting results as listed in Table 2). Dots and error bars, Group averages and SDs, respectively, for CBFV (%) and CVCi (%) within 5-Torr ranges of PET_{CO2} (e.g., 20–25 25–30, etc.).

steep ranges of the sigmoidal curves were found to lie between 40 and 50 Torr PET_{CO2} for most subjects (Figs. 3 and 4). As expected, in the steep ranges of the sigmoidal curves, linear regression slopes of changes in CBFV (CBFV-CVMR1) and CVCi (CVCi-CVMR1) were similar to those obtained from the first-order derivatives of the identified logistic function (CBFV-CVMRmax and CVCi-CVMRmax, respectively). Of note, both CVMRmax and CVMR1 were higher than the estimates of CVMR from the linear regressions over the entire range of changes in PET_{CO2} (CVMR0). Finally, for test-retest reproducibility, coefficients of variation for curve-fitting parameters and for estimates of CVMR are presented in Table 2.

Discussion

We have developed a clinically applicable method to assess CVMR that addresses the nonlinear relationship and interactions between transient changes in CO_2, CBFV, and ABP. Based on a modified rebreathing method, we have observed a

Table 2 Estimation of Logistic Function Parameters and CVMR.

	CBFV-PET_{CO2}	CV,%	CVCi-PET_{CO2}	CV,%
a, %	149 ± 34	17	99 ± 29*	26
y0, %	202 ± 35	10	166 ± 30*	11
x0, Torr	47 ± 2	6	46 ± 2	4
b, Torr-1	0.22 ± 0.06	36	0.28 ± 0.09	58
CVMRmax, %/Torr	8 ± 2†	23	6 ± 2†	31
CVMR0 , %/Torr	5 ± 1	18	3 ± 1*	18
CVMR1, %/Torr	7 ± 1†	9	6 ± 1*†	26

Values are mean ± SD, from 10 subjects. CV: coefficient of variation. CBFV: cerebral blood flow velocity; a, y0, x0, and b, identified logistic function parameters (Fig 1). CVMRmax: maximal cerebral vasomotor reactivity (CVMR), peak value of the first-order derivative of the identified logistic function. CVMR0: linear regression slope of percentage changes in CBFV over the entire range of changes in PET_{CO2}. CVMR1: linear regression slope of CBFV vs. PET_{CO2} in the steep portion of the sigmoidal curve.
* $p < 0.01$ for difference in parameters between CBFV-PET_{CO2} and CVCi-PET_{CO2}.
† $p < 0.01$ for CVMR0 vs. CVMRmax and CVMR1.

wide range of breath-by-breath changes in PET_{CO2} (~20–61 Torr) and corresponding changes in CBFV, CVCi, and ABP.

The main findings of this study are twofold. First, consistent with our hypothesis, transient, breath-by-breath responses of both CBFV and CVCi to changes in PET_{CO2} demonstrate nonlinear properties that can be quantified by a logistic function. Second, the magnitude of increases in CBFV during hypercapnic rebreathing exceeded CVCi (104% vs. 64%) because of the presence of significant increases in ABP by 23%. Consistently, during hypocapnic hyperventilation, the reduction in CBFV (46%) surpassed CVCi (39%), whereas ABP fell by 19%. These observations suggest that changes in ABP during CO_2 stimuli have direct effects on CBF.

Study limitations. The reproducibility of model parameters and estimates of CVMR are comparable with other Doppler-CO_2 tests.[3,27,28] Nonetheless, the coefficient of variation of the model parameter b was relatively large compared with the other parameters (Table 2). Parameter b reflects the overall curvilinear properties of the sigmoidal curve. Therefore, estimation of parameter b may have been affected by the lack of precise control of levels of PET_{CO2} reached during voluntary hyperventilation between the tests. The technique of dynamic end-tidal forcing may

provide much better control of PET_{CO2} to improve the reproducibility of model parameter's estimation.[29] An additional limitation, inherent to the use of TCD, is that CBFV was measured to reflect changes in CBF. The TCD method has been well validated against other modalities used to measure CBF.[30-32] However, changes in CBFV reflect changes in CBF only if the diameter of the insonated MCA remains constant. Direct and indirect measurements of the MCA diameters and comparisons of changes in CBFV with changes in CBF measured by different modalities during either hypo- or hypercapnia suggest the validity of using TCD.[33,34] We cannot exclude the possibility that a vasodilatation in the MCA may occur at high levels of CO_2.[35] If this were the case, the magnitude of maximal changes in CBF may have been underestimated by the measurement of CBFV during hypercapnia. However, the maximum changes in CBFV at a PET_{CO2} level of 60 Torr (~100% above baseline) are consistent with results of direct measurements of CBF in other studies with a similar level of changes in CO_2.[2,7]

Magnitude of changes in CBFV and ABP. We found marked changes in CBFV with a range of nearly 150% from hyperventilation to rebreathing, with rebreathing accounting for an estimated doubling of baseline CBF. This range of changes in CBF is comparable with those found in other studies using TCD or other techniques to measure CBF.[2,7,19,29,36,37] Prominent CBF responses to arterial CO_2 are well established.[2-4,12] These changes in CBF have been attributed mainly to cerebral vasodilatation during hypercapnia and vasoconstriction during hypocapnia.[38,39]

However, changes in arterial CO2 elicit complicated autonomic reflexes and exert vascular effects not only in the cerebral but also in the systemic circulation.[40,41] ABP increases substantially even during moderate increases in arterial CO_2, due largely to sympathetic activation[5,10,42,43], which clearly occurred in our study as well. This chemoreflexmediated sympathetic activation may have confounding and opposing effects for the cerebral circulation, especially in the setting of CO_2-induced cerebral vasodilatation. For example, systemic hypertension may increase CBF prominently when normal autoregulatory mechanisms are impaired by CO_2.[6,8,44] Conversely, sympathetic activation in the brain may restrain CO_2-induced vasodilatation[39,45-47], although it is less certain whether such effects occur consistently in humans.[48] Both of these opposing effects will influence the measurement of CVMR during rebreathing.

In the present study, we found that estimates of CVMR based on CBFV (during either hyper- or hypocapnia) exceeded those based on CVCi. For example, model parameters a and y0 (which reflect the range and maximal changes in CBFV and CVCi, respectively) derived from the CBFV-PET_{CO2} relationship were significantly higher than those obtained from the CVCi-PET_{CO2} relationship. Likewise, linear regressions resulted in higher values for CVMR when derived from CBFV instead of CVCi.

According to Ohm's law, the much higher increases in CBFV (104%) during hypercapnia relative to the increases in CVCi (64%) could be attributed to the 23% increase in ABP. Consequently, increases in ABP led to an overestimation of CVMR based on the linear regression of changes in CBFV vs. PET_{CO2} (CBFV-CVMR0) (Table 2).[6] Together, these findings suggest that the net CBF responses to acute hypercapnia in humans are dominated by an overwhelming vasodilatory effect of CO_2, which impairs cerebral autoregulation and thereby enhances the effects of ABP on CBFV.

Estimation of CVCi. This study applies changes in cerebrovascular conductance to assess CVMR during transient changes in PET_{CO2}. The use of CVCi, in contrast to changes in CBFV, may better reflect the physiological process of cerebral vasodilatation during hypercapnia and cerebral vasoconstriction during hypocapnia. When an index of cerebrovascular resistance (the inverse of conductance) is used rather than CBFV to assess CVMR, the outcome was less influenced by changes in ABP.[6] However, the use of resistance, rather then its inverse conductance, to assess CVMR may lead to difficulties in data interpretation, especially under conditions with increased CBF.[49] Because of their reciprocal relationship, in a condition where resistance is low and blood flow is high, a large increase in conductance (vasodilatation) leads to only a small (further) decrease in resistance. Therefore, if vasomotor responses are estimated from changes in resistance, such a small decrease in resistance could be falsely interpreted to represent a small increase in vasodilatation. Because CVMR testing generates high blood flow, conductance rather than resistance was used in this study.

Alternatively, studies have analyzed the ABP-CBFV relationship within each cardiac cycle and used linear extrapolations to calculate the zero-flow pressure (also known as critical closing pressure) or resistance-area product indexes.[24,50,51] Hypocapnia

increases and hypercapnia reduces zero-flow pressure; therefore, changes in these indexes may serve as alternative measures to assess cerebrovascular responses to CO_2.[23,24] Whereas the clinical relevance of assessment of CVMR has been demonstrated in follow-up studies in patients at risk for cerebrovascular events[14,15], similar findings have not been reported for zero-flow pressure or resistance-area product parameters.

Temporal characteristics of changes in CBFV and ABP. The CBF response to transient changes in CO_2 has a time delay of ~5–7 s.[9,52] It is therefore of interest to consider whether this factor could affect the estimation of CVMR using the proposed modeling. Identification of the time delay between transient changes in CBF and PET_{CO2}, as performed in these studies, requires complex and stringent control of respiration and CO_2 levels. However, in the present study, we are able to approximate the time delay between changes in PET_{CO2}, CBFV, and ABP based on the individual data from each subject. The average time delay of the reduction in CBFV in response to the reduction in PET_{CO2} during hyperventilation was ~7 s, consistent with the reported time delay after a negative step change in PET_{CO2} (6.8 s) [52] and with a time delay of ~5 s found when spontaneous CO_2 oscillations were analyzed.[11] This time delay is possibly explained by CO_2 transportation time.[53] The identification of a similar time delay during hypercapnic rebreathing is less evident because, as can be seen in Fig. 2, rebreathing caused a ramplike increase in PET_{CO2}. Despite the time delay between transient changes in CBFV and CO_2, it had little effect on the estimation of CVMR. With a total duration of hyperventilation of 15 s, a (short) plateau phase with minimum and stable CO_2 and CBFV values was reached in all individuals. Values at the end of hyperventilation (i.e., in this plateau phase) were used as a starting point for calculation of CVMR. Therefore, "correction" for the time delay did not affect the modeling and data outcome. Similarly, the observed reduction in ABP during hyperventilation also showed a time delay, lagging behind the changes in CBFV by an additional 7 s. This delayed response in ABP may partly explain why there was a smaller difference between changes in CVCi and CBFV during hyperventilation when compared with hypercapnic rebreathing.

During rebreathing, CBFV increased steadily, associated with a continuous increase in PET_{CO2}, and then appeared to level off at high levels of PET_{CO2}. Two mechanisms may have contributed to the saturation of CBFV. First, cerebral vasodilatation may

have reached its maximal levels at or greater than a PET_{CO2} of 60 Torr.[3,12] Second, substantial sympathetic activation may constrain the magnitude of the CBFV responses.[39,45,48]

With the onset of recovery, PET_{CO2} returned rapidly to the pretest baseline level within ~1–2 breaths of room air (<10 s). However, there was a substantial time delay of ~20 s for CBFV to reach a new sustained level, which was lower than the pretest baseline value. Of note, ABP returned to the pretest baseline level without a similar undershoot. These data suggest a sustained cerebral vasoconstriction during recovery from prolonged acute hypercapnia. In addition, these data confirm that adaptive processes modify the response in both ABP and CBF to CO_2, leading to limitations in the use of steady-state data to estimate CVMR.[7,52] However, the time delay in the recovery of CBFV appears to be much longer than that reported with a step change in CO_2 (6 s).[29] These discrepancies might be related to the differences in the duration as well as the intensities of CO_2 stimuli used.

Assessment of CVMR. A sigmoidal distribution of data was present in most subjects when breath-by-breath changes in CBFV were plotted against PET_{CO2}. Furthermore, similar distributions were observed for changes in CVCi, indicating the presence of threshold and saturation properties of cerebrovascular changes in response to transient changes in PET_{CO2}, providing further evidence for the nonlinear properties of CBF responses to changes in CO_2.[3,12,29,52,53] However, the specific physiological mechanisms underlying the nonlinear CBF-CO_2 relationship cannot be determined in the present study.

Data with a sigmoidal distribution could be fitted equally well with several nonlinear functions based on the norm of least-square curve-fitting procedures.[12] A modified logistic function was employed for curve fitting.[25] Compared with other curve-fitting methods, one advantage of the logistic function used in this study is that the model parameters identified have clear physiological meanings (Fig. 1). In addition, the first-order derivative of the identified logistic function readily provides an estimation of CVMR for each level of PET_{CO2}.

The linear regression slope of the CBFV-PET_{CO2} relationship (CVMR0, 5%/Torr) over the entire range of changes in PET_{CO2} is consistent with previous findings. [2,7,29,54]

However, this method of analysis, although practical, evidently underestimates the maximal cerebral vasodilatory effect of CO_2, as indicated by the estimates of CVMRmax using either the first-order derivatives of the identified logistic function (8%/Torr) or the specific linear regressions in the steep portion of sigmoidal curves (7%/Torr) (Table 2).

The inflection point of the logistic function (parameter *x*0), which corresponds to the maximal rate of changes in CBFV or CVCi to CO_2 (CVMRmax), was almost identical for both the CBFV-PET_{CO2} and CVCi-PET_{CO2} relationships (Table 2). This demonstrates that CVMRmax occurs during moderate hypercapnia, slightly above the baseline level of PET_{CO2}, consistent with previous studies suggesting that estimation of CVMR is lower during hypocapnia than during hypercapnia.[6,19,29,36]

Previous studies have used similar rebreathing methods to estimate CVMR.[19,37,54,55] However, these studies investigated only the hypercapnic stimuli and used linear regressions to derive a CVMR from 2.8 to 5.2%/Torr.[37,54,55] Only one study included the hypocapnic range using hyperventilation before rebreathing and applied a bilinear model, resulting in a smaller CVMR in the hypocapnic range than in the hypercapnic range (1.6 vs. 2.8 $cm \cdot s^{-1} \cdot Torr^{-1}$).[19] Direct comparisons to these previous findings are difficult because hypoxic and/or hyperoxic conditions were also employed in these studies.

In conclusion, a clinically applicable rebreathing method was developed to assess CVMR during transient changes in PET_{CO2}. We found that both CBFV and CVCi responses to transient changes in PET_{CO2} are nonlinear and that these responses can be quantified well by a modified logistic function. Furthermore, we found that the magnitude of change in CBFV during CO_2 rebreathing (and hyperventilation) is higher than that of CVCi, suggesting direct effects of increases (and decreases) in ABP on changes in CBFV. For assessment of CVMR, the range of changes in CO_2 to be studied and the confounding influence of changes in ABP on CBFV must be considered for application of an appropriate modeling method to quantify CBFV or CVCi responses.

References

1. Ainslie PN, Ashmead JC, Ide K, Morgan BJ, Poulin MJ. Differential responses to CO_2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol 2005;566:613–624.*
2. Kety S, Schmidt C. The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young man. *J Clin Invest 1948;27:484–492.*
3. Ringelstein EB, Sievers C, Ecker S, Schneider PA, Otis SM. Noninvasive assessment of CO2-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions. *Stroke 1988;19:963–969.*
4. Hurn P, Traystman RJ. Changes in arterial gas tension. In: Edvinsson L, Krause D (eds). Cerebral Blood Flow and Metabolism. *Philadelphia, PA: Lippincott Williams & Wilkins, 2002: 384–394.*
5. Dumville J, Panerai RB, Lennard NS, Naylor AR, Evans DH. Can cerebrovascular reactivity be assessed without measuring blood pressure in patients with carotid artery disease? *Stroke 1998;29: 968–974.*
6. Edwards MR, Topor ZL, Hughson RL. A new two-breath technique for extracting the cerebrovascular response to arterial carbon dioxide. *Am J Physiol Regul Integr Comp Physiol 2003;284: R853–R859.*
7. Ellingsen I, Hauge A, Nicolaysen G, Thoresen M, Walloe L. Changes in human cerebral blood flow due to step changes in PaO_2 and $PaCO_2$. *Acta Physiol Scand 1987;129:157–163.*
8. Harper AM, Glass HI. Effect of alterations in the arterial carbon dioxide tension on the blood flow through the cerebral cortex at normal and low arterial blood pressures. *J Neurol Neurosurg Psychiatry 1965;28:449–452.*
9. Mitsis GD, Ainslie PN, Poulin MJ, Robbins PA, Marmarelis VZ. Nonlinear modeling of the dynamic effects of arterial pressure and blood gas variations on cerebral blood flow in healthy humans. *Adv Exp Med Biol 2004;551:259–265.*
10. Panerai RB, Evans DH, Naylor AR. Influence of arterial blood pressure on cerebrovascular reactivity. *Stroke 1999;30:1293–1295.*
11. Panerai RB, Simpson DM, Deverson ST, Mahony P, Hayes P, Evans DH. Multivariate dynamic analysis of cerebral blood flow regulation in humans. *IEEE Trans Biomed Eng 2000;47:419-423.*
12. Reivich M. Arterial PCO2 and cerebral hemodynamics. *Am J Physiol 1964;206:25–35.*

13. Blaser T, Hofmann K, Buerger T, Effenberger O, Wallesch CW, Goertler M. Risk of stroke, transient ischemic attack, and vessel occlusion before endarterectomy in patients with symptomatic severe carotid stenosis. *Stroke 2002;33:1057–1062.*
14. Kleiser B, Widder B. Course of carotid artery occlusions with impaired cerebrovascular reactivity. *Stroke 1992;23:171–174.*
15. Widder B, Kleiser B, Krapf H. Course of cerebrovascular reactivity in patients with carotid artery occlusions. *Stroke 1994;25:1963–1967.*
16. Mitsis GD, Zhang R, Levine BD, Marmarelis VZ. Cerebral hemodynamics during orthostatic stress assessed by nonlinear modeling. *J Appl Physiol 2006;101:354–366.*
17. Duffin J, McAvoy GV. The peripheral-chemoreceptor threshold to carbon dioxide in man. *J Physiol 1988;406:15–26.*
18. Mohan R, Duffin J. The effect of hypoxia on the ventilatory response to carbon dioxide in man. *Respir Physiol 1997;108:101–115.*
19. Vovk A, Cunningham DA, Kowalchuk JM, Paterson DH, Duffin J. Cerebral blood flow responses to changes in oxygen and carbon dioxide in humans. *Can J Physiol Pharmacol 2002;80:819–827.*
20. Harris J, Benedict F. A biometric study of human basal metabolism. *Proc Natl Acad Sci USA 1918;4: 370–373.*
21. Giller CA, Giller AM. A new method for fixation of probes for transcranial Doppler ultrasound. *J Neuroimaging 1997;7:103–105.*
22. Vallbo AB, Hagbarth KE, Torebjork HE, Wallin BG. Somatosensory, proprioceptive, and sympathetic activity in human peripheral nerves. *Physiol Rev 1979;59:919–957.*
23. Hancock SM, Mahajan RP, Athanassiou L. Noninvasive estimation of cerebral perfusion pressure and zero flow pressure in healthy volunteers: the effects of changes in end-tidal carbon dioxide. *Anesth Analg 2003;96:847–851.*
24. Hsu HY, Chern CM, Kuo JS, Kuo TB, Chen YT, Hu HH. Correlations among critical closing pressure, pulsatility index and cerebrovascular resistance. *Ultrasound Med Biol 2004;30:1329–1335.*
25. Kent BB, Drane JW, Blumenstein B, Manning JW. A mathematical model to assess changes in the baroreceptor reflex. *Cardiology 1972;57:295–310.*
26. Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med 2000;30:1–15.*
27. Rohrberg M, Brodhun R. Measurement of vasomotor reserve in the transcranial Doppler-CO_2 test using an ultrasound contrast agent (Levovist). *Stroke 2001;32:1298–1303.*

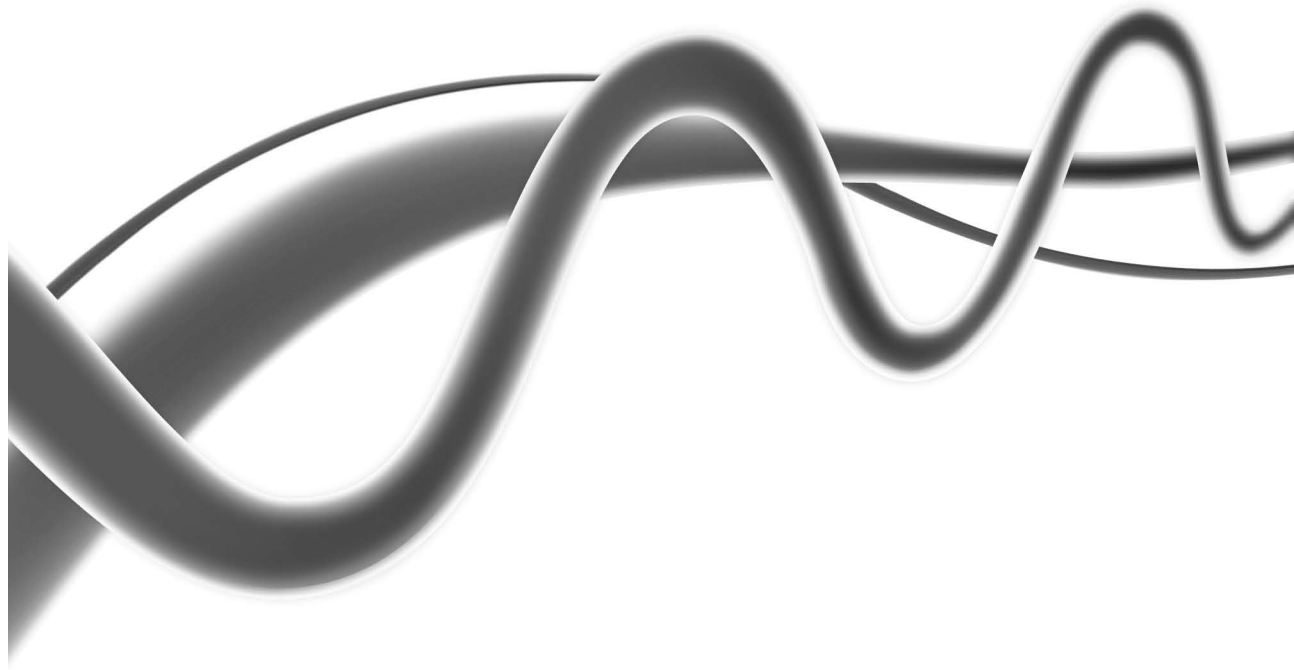
28. Totaro R, Marini C, Baldassarre M, Carolei A. Cerebrovascular reactivity evaluated by transcranial Doppler: reproducibility of different methods. *Cerebrovasc Dis 1999;9:142–145.*
29. Poulin MJ, Liang PJ, Robbins PA. Dynamics of the cerebral blood flow response to step changes in end-tidal PCO2 and PO2 in humans. *J Appl Physiol 1996;81:1084–1095.*
30. Bishop CC, Powell S, Rutt D, Browse NL. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke 1986;17:913–915.*
31. Dahl A, Lindegaard KF, Russell D, Nyberg-Hansen R, Rootwelt K, Sorteberg W, Nornes H. A comparison of transcranial Doppler and cerebral blood flow studies to assess cerebral vasoreactivity. *Stroke 1992;23:15–19.*
32. Lindegaard KF, Lundar T, Wiberg J, Sjoberg D, Aaslid R, Nornes H. Variations in middle cerebral artery blood flow investigated with noninvasive transcranial blood velocity measurements. *Stroke 1987;18:1025–1030.*
33. Giller CA, Bowman G, Dyer H, Mootz L, Krippner W. Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery 1993;32:737–741.*
34. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke 2000;31:1672–1678.*
35. Valdueza JM, Draganski B, Hoffmann O, Dirnagl U, Einhaupl KM. Analysis of CO_2 vasomotor reactivity and vessel diameter changes by simultaneous venous and arterial Doppler recordings. *Stroke 1999;30:81–86.*
36. Ide K, Eliasziw M, Poulin MJ. The relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol 2003;95:129–137.*
37. Pandit JJ, Mohan RM, Paterson ND, Poulin MJ. Cerebral blood flow sensitivity to CO_2 measured with steady-state and Read's rebreathing methods. *Respir Physiol Neurobiol 2003;137:1–10.*
38. Atkinson JL, Anderson RE, Sundt TM Jr. The effect of carbon dioxide on the diameter of brain capillaries. *Brain Res 1990;517:333–340.*
39. Wei EP, Kontos HA, Patterson JL Jr. Dependence of pial arteriolar response to hypercapnia on vessel size. *Am J Physiol Heart Circ Physiol 1980;238:H697–H703.*
40. Kontos HA, Richardson DW, Patterson JL Jr. Effects of hypercapnia on human forearm blood vessels. *Am J Physiol 1967;212:1070–1080.*
41. Richardson DW, Wasserman AJ, Patterson JL Jr. General and regional circulatory responses to change in blood pH and carbon dioxide tension. *J Clin Invest 1961;40:31–43.*

42. Braune S, Hetzel A, Prasse A, Dohms K, Guschlbauer B, Lucking CH. Stimulation of sympathetic activity by carbon dioxide in patients with autonomic failure compared with normal subjects. *Clin Auton Res 1997;7:327–332.*
43. Shoemaker JK, Vovk A, Cunningham DA. Peripheral chemoreceptor contributions to sympathetic and cardiovascular responses during hypercapnia. *Can J Physiol Pharmacol 2002;80:1136–1144.*
44. Panerai RB, Deverson ST, Mahony P, Hayes P, Evans DH. Effects of CO2 on dynamic cerebral autoregulation measurement. *Physiol Meas 1999;20:265–275.*
45. Busija DW, Heistad DD. Effects of activation of sympathetic nerves on cerebral blood flow during hypercapnia in cats and rabbits. *J Physiol 1984;347:35–45.*
46. Edvinsson L, Hamel E. Perivascular nerves in brain vessels. In: Edvinsson L, Krause D (eds). Cerebral Blood Flow and Metabolism. *Philadelphia, PA: Lippincott Williams & Wilkins, 2002: 43–67.*
47. Zhang R, Zuckerman JH, Iwasaki K, Wilson TE, Crandall CG, Levine BD. Autonomic neural control of dynamic cerebral autoregulation in humans. *Circulation 2002;106:1814–1820.*
48. Jordan J, Shannon JR, Diedrich A, Black B, Costa F, Robertson D, Biaggioni I. Interaction of carbon dioxide and sympathetic nervous system activity in the regulation of cerebral perfusion in humans. *Hypertension 2000;36:383–388.*
49. O'Leary DS. Regional vascular resistance vs. conductance: which index for baroreflex responses? *Am J Physiol Heart Circ Physiol 1991;260:H632–H637.*
50. Aaslid R, Lash SR, Bardy GH, Gild WH, Newell DW. Dynamic pressure-flow velocity relationships in the human cerebral circulation. *Stroke 2003;34:1645–1649.*
51. Panerai RB. The critical closing pressure of the cerebral circulation. *Med Eng Phys 2003;25:621–632.*
52. Poulin MJ, Liang PJ, Robbins PA. Fast and slow components of cerebral blood flow response to step decreases in end-tidal PCO2 in humans. *J Appl Physiol 1998;85:388–397.*
53. Wilson DA, Traystman RJ, Rapela CE. Transient analysis of the canine cerebrovascular response to carbon dioxide. *Circ Res 1985;56:596–605.*
54. Clivati A, Ciofetti M, Cavestri R, Longhini E. Cerebral vascular responsiveness in chronic hypercapnia. *Chest 1992;102:135–138.*
55. Hida W, Kikuchi Y, Okabe S, Miki H, Kurosawa H, Shirato K. CO2 response for the brain stem artery blood flow velocity in man. *Respir Physiol 1996;104:71–75.*

Epidemiology: friend or foe? *(BMJ 2004;329:467)*

Geriatric research is rapidly growing. Twenty years ago it was nearly impossible to find a trial that included old people. The major subject heading (MeSH) term "aged, 80 and over" wasn't introduced until 1986. But in the past 10 years more than 10,000 randomised controlled trials were published that included this age group. Alzheimer's disease is a good example of a geriatric disease that has long been ignored. It took us a while to realise that this devastating and incurable disease is about to become a pandemic. In the past decade we started a frantic search for clues that might lead to its cure. Since no one really knew where to start, we asked our friend epidemiology for help. Large cohorts of elderly people were studied. Epidemiologists looked for correlations, hoping to identify factors associated with a reduced risk of dementia. And with success. They gave us, among others, hormone replacement therapy in women and two groups of drugs, statins and non-steroidal anti-inflammatory drugs (NSAIDs). People using either of these types of drug were less likely to have Alzheimer's disease, they said. These hopeful findings were welcomed like a drop of water in the desert, and they were published in high ranking journals. All this inspired more research. The gold standard of evidence based medicine, the randomised controlled trial, was applied to confirm the data in a prospective setting. And in laboratories worldwide sophisticated in vitro and in vivo studies were designed to discover the pathophysiological mechanisms by which oestrogen, statins, and NSAIDs protect against Alzheimer's disease. To our surprise, the randomised controlled trials gave us bad news. The drugs had no effect at all on Alzheimer's disease. How can we explain this? It seems that the original observational studies suffered from the epidemiologist's biggest enemy: bias. Even worse, with hindsight this bias could and should have been easily identified. Doctors do not readily prescribe cholesterol lowering agents to patients with dementia. Likewise, patients with cognitive impairment are not likely to see their doctor to have their cholesterol checked. This explains why patients without dementia are more likely to use a statin than patients with dementia. The same holds for the use of hormone replacement therapy in women. In the case of the NSAIDs it was chance that played a trick on us. Chance findings are a recognised risk of doing multiple regression analysis on large volumes of variables in population studies: you can end up finding that patients born on a Monday are more likely to develop dementia than patients born on other days of the week. When we think about these events it seems that epidemiology—or rather its careless use—provided us with false clues. This led to a waste of valuable time and money. When scientists are so eager to find results, epidemiology can present as a friend but behave as our foe. What came next is puzzling. The laboratories started to publish their data. They had discovered that inflammatory processes are involved in the pathogenesis of Alzheimer's and that NSAIDs can modify these. They also identified several mechanisms of action for statins. For example, statins reduce the formation of

amyloid, one of the hallmarks of Alzheimer's. These data were enough to indicate that both drugs do have the potential to reduce the risk of the disease in certain patients. So, where does this leave us? Were the epidemiological findings true associations after all? This would mean that the randomised controlled trials have led us astray. This is troubling news for a gold standard. It is, however, not inconceivable that the design of these trials was inadequate. The process resulting in Alzheimer's disease probably starts many years before clinical symptoms present. If the therapeutic effect of statins and NSAIDs lies at a point 10 to 20 years before the onset of symptoms, any trial that starts at a much later stage will never find an effect. The alternative explanation is that the problem lies in the interpretation of the data. Maybe the inflammatory response seen in Alzheimer's is a non-specific reactive process and modifying it with NSAIDs has no true benefit. And maybe the amyloid that is reduced by statins is merely an innocent bystander and not a factor contributing to the disease. I think we're simply not clever enough at this point to have the broad view needed to solve the puzzle of Alzheimer's. It is like trying to describe a painting when your view is limited to a corner of it. We started out looking for clues to a cure for Alzheimer's disease. With the help of epidemiology we found several. Unfortunately, not one of them has turned out to be useful, despite lots of research effort. What we are left with is a number of new hypotheses. So how should we judge epidemiology in this case: friend or foe? I still vote for friend. Friends aren't always right, but they do make life much more interesting.



Chapter 6

Dynamic cerebral autoregulation during repeated squat-stand maneuvers

Jurgen A.H.R. Claassen
Benjamin D. Levine
Rong Zhang

J Appl Physiol. 2008; in press.

Abstract

Transfer function analysis of spontaneous oscillations in blood pressure (BP) and cerebral blood flow (CBF) can quantify the dynamic relationship between BP and CBF. However, such oscillation amplitudes are often small and of questionable clinical significance, vary substantially, and cannot be controlled. At the very low frequencies (< 0.07 Hz) coherence between BP and CBF often is low (< 0.50) and their causal relationship is debated. How these factors affect transfer function analysis is not clear. Eight healthy subjects performed repeated squat-stand maneuvers to induce large oscillations in BP at frequencies of 0.025 and 0.05 (very low frequency) and 0.1 Hz (low frequency) respectively. BP (Finapres), CBF velocity (CBFV, TCD), and end-tidal CO_2 (capnography) were monitored. Spectral analysis was used to quantify oscillations in BP and CBFV and to estimate transfer function phase, gain and coherence. Compared with spontaneous oscillations, induced oscillations had higher coherence (mean 0.8 SD 0.11, > 0.5 in all subjects at all frequencies) and lower variability in phase estimates. However, gain estimates remained unchanged. Under both conditions, the "high-pass filter" characteristics of dynamic autoregulation were observed. In conclusion, using repeated squat-stand maneuvers, we were able to study dynamic cerebral autoregulation in the low frequencies under conditions of hemodynamically strong and causally related oscillations in BP and CBFV. This enhances not only the confidence of transfer function analysis as indicated by high coherence and improved phase estimation, but strengthens also the clinical relevance of this method as induced oscillations in BP and CBFV mimic those associated with postural changes in daily life.

Introduction

Continuous measurements of cerebral blood flow velocity (CBFV) using transcranial Doppler (TCD) have shown that CBFV is affected by dynamic perturbations in blood pressure (BP) considered to lie within the "autoregulatory range".[1,2] Transfer function analysis of spontaneous oscillations in BP and CBFV quantifies this dynamic pressure-flow relationship of the cerebral circulation, and has suggested that cerebral autoregulation is more effective at low compared to high frequencies (the "high pass filter" effect).[1,3-5] However, this method may be limited by the relatively small magnitude of spontaneous oscillations. Also, assessment of dynamic cerebral autoregulation based on spontaneous changes in blood pressure and CBFV exhibits substantial individual variability under most experimental conditions.[4,6] In addition, coherence between these oscillations at low frequencies (<0. 07Hz) generally is low (<0.5), and thus it is uncertain whether the quantification of the transfer function is statistically reliable[5] and whether oscillations in CBFV simply may not be causally related to changes in BP at low frequencies.[7] In practice, most studies exclude data with coherence <0.5 for analysis, which can amount to a third of all collected measurements.[8,9]

Squatting followed by standing produces dramatic changes in BP.[10] In daily life comparable maneuvers occur frequently, e.g. when standing up after tying shoelaces or picking something from the floor, and these maneuvers are often accompanied by symptoms of light-headedness suggestive of cerebral hypoperfusion. Previous studies demonstrated that when such squat-stand maneuvers are performed repeatedly, large and periodic changes in BP and CBFV are created.[11] Thus, the magnitude of these changes is representative of daily life challenges for cerebral autoregulation.

We conducted this study to address the following hypotheses:

1) Oscillatory changes in BP and CBFV at specific low frequencies (0.025, 0.05 and 0.1 Hz) can be induced with repeated squat-stand maneuvers, thus creating relatively narrow band, but high amplitude signals.
2) With increased correlation between BP and CBFV, and reduced signal to noise, transfer function assessment of dynamic cerebral autoregulation at the low and very low frequencies would be improved, associated with reduced variability in phase and gain estimates.

Methods

Subjects. We studied eight healthy young subjects, four males, age 30 (SD 4) years, weight 72 (SD 18) kg, systolic/diastolic BP 114 (SD 5.4)/70 (SD 8.7) mmHg. These subjects were free of known disease, used no medication, and did not smoke.

Ethical approval. The study complies with the standards set by the latest revision of the Declaration of Helsinki and was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. All participants provided informed consent in writing.

Instrumentation. BP was measured in the finger by photoplethysmography (Finapres, Ohmeda). We and others have shown previously that this method reliably assesses dynamic changes in beat-to-beat BP that correlate well with intra-arterial recordings and can be used to quantify to the dynamic pressure-flow relationship of the cerebral circulation.[5,12,13] The servo-reset mechanism was turned off during data collection for a maximum of 5 min to allow uninterrupted data recording.[14] Intermittent BP was also measured in the arm by electrosphygmomanometry (Suntech), with a microphone placed over the brachial artery and the Korotkoff sounds gated to the electrocardiogram (ECG). The arm pressure was used to verify the accuracy of the finger pressure recordings throughout the experiment.

CBFV was obtained in the middle cerebral artery (MCA) by transcranial Doppler ultrasonography. This technique allows non-invasive and repeatable estimates of changes in CBF on a beat-to-beat basis. A 2-MHz Doppler probe (DWL Elektronische Systeme, Germany) was placed over the temporal window and fixed at a constant angle and position with a custom made individually shaped mould. End-tidal CO_2 ($ETCO_2$) was monitored with a nasal cannula using capnography (Criticare Systems). In addition, peripheral arterial saturation (pulse-oximetry) and 3-lead ECG were registered.

Experimental Procedures. All experiments were performed in the morning, at least 2 h after a light breakfast and 12 h after the last caffeinated beverage or alcohol, in a quiet, environmentally controlled laboratory with an ambient temperature of 22°C. After at least 10 min rest in sitting position, 5-min segments of BP and CBFV data were recorded during spontaneous respiration. These data were used for spectral analysis of spontaneous oscillations in BP and CBFV. Next, repeated squat-stand maneuvers were performed. After careful instruction and practice, participants were coached into performing these maneuvers at a frequency of 0.025 Hz (20 s squat followed by 20 s standing up), 0.05 Hz (10 s squat, 10 s stand) and 0.1 Hz (5 s squat, 5 s stand) for 5 min each, separated by 10 min of recovery between the maneuvers. During these maneuvers, subjects were instructed to keep normal breathing and to avoid performing a Valsalva maneuver.

Data processing. The analog finger BP and Doppler CBFV signals were sampled simultaneously at 100 Hz (Multi-Dop X2, DWL). Real time beat-to-beat mean values of pressure and velocity were calculated as waveform integration of the sampled pressure and velocity signal within each cardiac cycle, divided by the corresponding pulse interval and stored for off-line analysis. Beat-to-beat changes in mean BP and CBFV were aligned with the time of R wave peaks of the ECG and linearly interpolated, then resampled at 2 Hz to convert the unequally spaced beat-to-beat time series to a uniformly spaced time series for spectral and transfer function analysis. The time series were detrended with third-order polynomial fitting and then subdivided into 128-point segments (64 s) with 50% overlap for spectral estimation. This process resulted in eight segments of data for the segment periodogram average, with a spectral resolution of ~0.0156 Hz. In this way, each segment contains at least one full period of oscillations at the lowest frequency (0.025 Hz) and up to 6 full periods of oscillations at the frequency of 0.1 Hz. Fast Fourier transforms were implemented with each Hanning-windowed segment and averaged to calculate the autospectrum of BP and CBFV. This analysis was repeated after adjustment of the window length to the cycle length of the squat-stand maneuvers, to the effect that each window contained 3 cycles. Because this did not alter transfer function estimates, the described standard window-lengths were used for both spontaneous and induced oscillations. Breath-to-breath values for ETCO2 were linearly interpolated and resampled at 2Hz and spectral analysis was performed to estimate the variability in ETCO2. Respiratory frequency was obtained form spectral analysis of continuous recording of CO2 waveforms.

Transfer function estimation. Estimation of transfer function gain, phase and coherence function was based on the cross-spectral method which has been described in detail

previously.[5] Transfer gain quantifies how changes in BP are transmitted into CBFV; a lower gain suggests that oscillations in CBFV in response to changes in BP are either buffered by active changes in cerebrovascular resistance out of phase with changes in BP (dynamic autoregulation)[8,15,16] and/or by increases in steady-state cerebrovascular resistance.[17] The phase spectrum describes the temporal relationship between changes in BP and CBFV at different frequencies.[3,8,18] Finally, estimates of coherence function quantify to what extent changes in CBFV are linearly correlated with changes in BP.

Based on our previous studies[5], transfer function analysis for spontaneous oscillations was carried out in the three frequency domains: very low frequency (0.02–0.07 Hz), low frequency (0.07–0.2 Hz) and high frequency (0.2–0.35 Hz). Because we wished to make comparisons, the oscillations induced by repeated squat-stand maneuvers were analyzed in the same frequency domains as used for spontaneous oscillations: squat-stand maneuvers at 0.025 Hz and 0.05 Hz were analyzed in the very low frequency domain (0.02–0.07 Hz); 0.1 Hz maneuvers were analyzed in the low frequency domain (0.07–0.14 Hz).

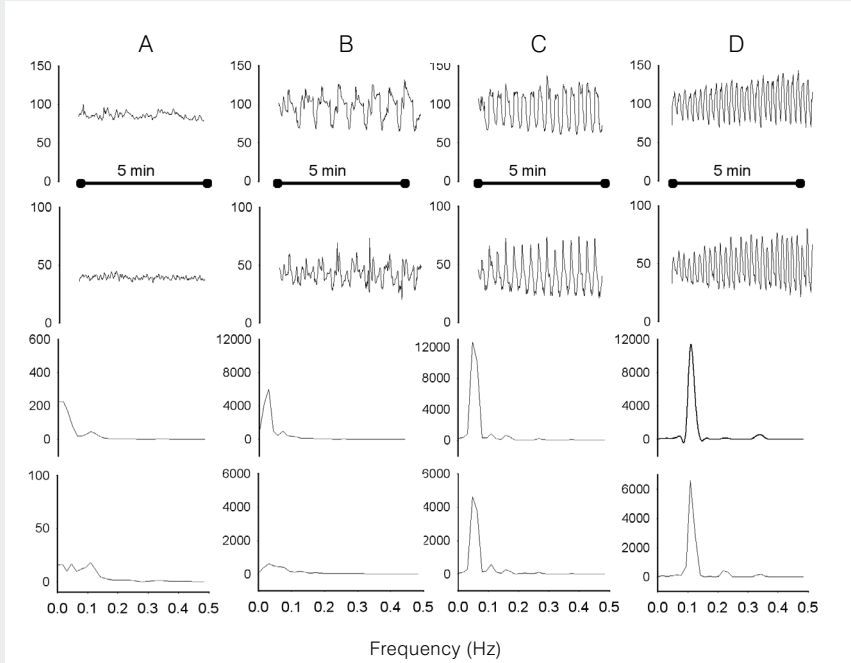
For induced oscillations, we also performed the analysis around each of the three evoked frequencies. Thus, 0.025 Hz maneuvers were analyzed in the range 0.02–0.06 Hz and 0.05 Hz maneuvers were analyzed in the range 0.03–0.08 Hz in all subjects. 0.1 Hz maneuvers were analyzed in the range 0.08–0.14 Hz in 7 subjects and 0.11-0.17 Hz in 1 subject with the peak at 0.14 Hz (see results). Transfer function gain, phase and coherence did not differ significantly between the choices of the frequency bands. Therefore, we will only report the results based on the same frequency ranges as used for spontaneous oscillations.

Statistical analysis. Comparisons between baseline and squat-stand maneuvers and between different frequencies were performed using analysis of variance for repeated measures. The data for BP, CBFV, HR, and CO_2 had a normal distribution (Kolmogorov-Smirnov test), and repeated measures ANOVA with Bonferroni post-test for multiple comparisons if $p < 0.05$ was applied. Normal distribution could not be assumed for the data for spectral power, transfer function gain, phase and coherence. Therefore, the nonparametric Friedman test with Dunn post-test was applied. Statistical significance was set at $p < 0.05$. Data are presented as mean and standard deviation (SD).

Results

Hemodynamics effects of squat-stand maneuvers. Large oscillations in BP and CBFV were observed at all three frequencies of repeated squat-stand maneuvers (figures 1 and 2). There was a low inter-subject variability in the frequency induced by the repeated squat-stand maneuvers, determined from the peak of the BP and CBFV power spectra: maneuvers at 0.1 Hz induced a peak at 0.11 Hz in 7 and 0.14 Hz in 1 subject; maneuvers at 0.05 Hz and 0.025 Hz induced peaks respectively at 0.047 and 0.031 Hz in all 8 subjects.

Figure 1 Spectral analysis of hemodynamic changes in rest and during squatting maneuvers.



Data from one subject, showing beat-to-beat variability in blood pressure (BP) and cerebral blood flow-velocity (CVBFV) (top panel), and results of spectral analysis of these data (bottom panel). A: resting condition (spontaneous oscillations), B: oscillations induced by squat-stand maneuvers at 0.025 Hz, C: oscillations induced at 0.05 Hz, D: oscillations induced at 0.1 Hz.

Table 1 shows that the mean values of the main hemodynamics variables BP, HR, $ETCO_2$ and respiratory frequency did not change significantly due to the repeated squat-stand maneuvers, despite the increased oscillation amplitude. Fluctuations in $ETCO_2$ were slightly enhanced during these maneuvers (Table 2).

Magnitude of induced oscillations. The repeated squat-stand maneuvers at 0.025 and 0.05 Hz resulted in respectively 15-fold and 40-fold increases in BP spectral power (compared to spontaneous VLF oscillations), while at 0.1Hz, a 100-fold increase occurred relative to spontaneous LF oscillations (figure 2 and table 2).

Table 1 Main hemodynamic parameters during the different tests.

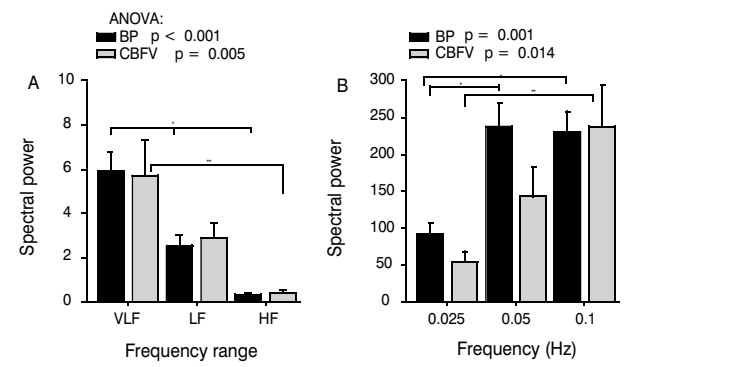
	Rest	Periodic squatting			P value
		0.025 Hz	0.05 Hz	0.1 Hz	
BP (mmHg)	84 (8)	90 (9)	86 (9)	90 (3)	0.18
CBFV (cm/s)	55 (13)	53 (8)	52 (9)	55 (11)	0.24
HR (bpm)	69 (15)	84 (17)	88 (17)	90 (17)	0.1
$ETCO_2$ (mmHg)	38 (3)	38 (3)	38 (4)	40 (3)	0.08
Resp.freq. (Hz)	0.29 (0.07)	0.31 (0.07)	0.34 (0.05)	0.28 (0.07)	0.34

BP: blood pressure (Finapres). CBFV: cerebral blood flow-velocity (TCD). ETCO2: end-tidal CO_2 (capnograph). Resp. freq: respiratory frequency (spectral analysis of the CO_2 waveform). Values represent mean and standard deviation (SD) during each test. P values: repeated measures analysis of variance.

These augmented oscillations in BP led to 10, 20 and 100-fold increases in CBFV spectral power at 0.025, 0.05 Hz and 0.1 Hz, respectively. Thus, increases in CBFV oscillations were relatively smaller than increases in BP oscillations at 0.025 and 0.05 Hz but not at 0.1 Hz, indicating more effective damping at the lower frequencies (Fig 2B). As expected this was corroborated by lower gain in these frequencies (see below and Fig 3).

Transfer Function Gain, Phase and Coherence. Coherence between BP and CBFV was much higher for repeated squat-stand maneuvers than for spontaneous oscillations, with values > 0. 6 for all subjects (range: 0.61-0.96).

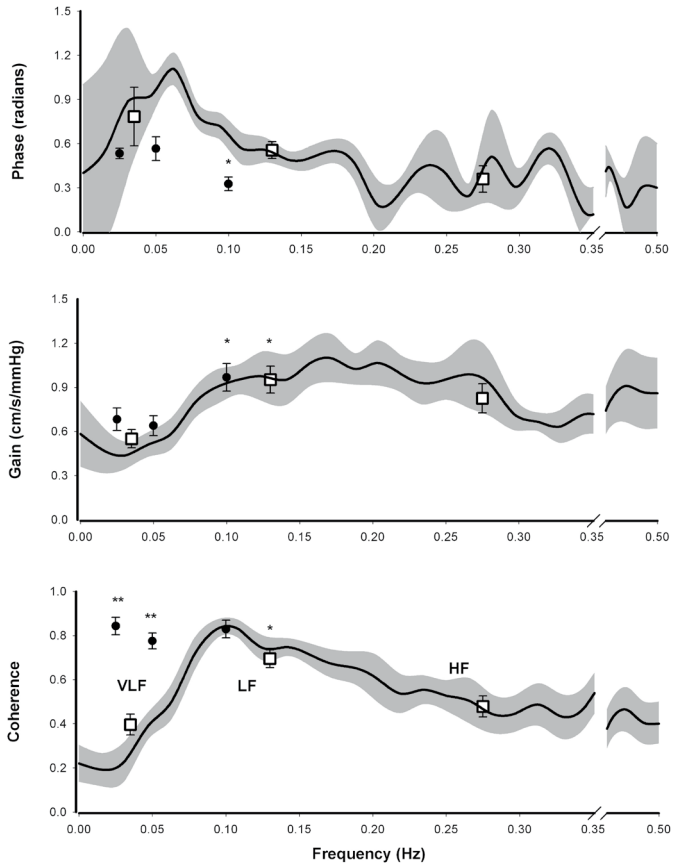
Figure 2 Spectral power of BP and CBFV for spontaneous and induced oscillations.



A. Spectral power of spontaneous oscillations in blood pressure (BP, in mmHg2) and cerebral blood flow-velocity (CBFV, in (cm/s)2). Bars represent group mean and standard error. VLF: very low frequency band (0.02-0.07 Hz), LF: low frequency (0.07-0.2) HF: high frequency (0.2-0.35 Hz). P-values: results of Friedman Test. * significant post-hoc (Dunn) test for BP; ** for CBFV. B. Spectral power of BP and CBFV during oscillations induced by periodic squatting at three frequencies: 0.025 and 0.05 Hz (VLF range) and 0.1 Hz (LF range). Note the different Y-axis scale (factor 30) for spectral power compared with 2A. P-values and *,** as in 2A. Differences in spectral power between spontaneous and induced oscillations were highly significant ($p < 0.0001$).

Figure 3 summarizes transfer function gain, phase and coherence in the frequency domains of VLF, LF and HF for spontaneous oscillations, and for oscillations induced at 0.025, 0.05 and 0.1Hz by the repeated squat-stand maneuvers. For comparison, these parameters are superimposed on the frequency plots of transfer function gain, phase and coherence for spontaneous oscillations. Despite the poor coherence at the very low frequencies for spontaneous oscillations there was a remarkable consistency between estimates of gain from spontaneous and induced oscillations: no significant differences were observed at the VLF and LF ranges between spontaneous and induced oscillations, and the variability of the estimates was comparable (see size of the standard errors in Fig 3). However, findings for phase were different. As seen in Fig 3, phase estimates from induced oscillations were lower. Moreover, estimation of VLF phase from the induced oscillations (0.025 and 0.05 Hz) showed much less inter-individual variability (mean 0.5 radians, range 0.3-0.7, associated with a coherence of 0.8) than VLF phase estimated from spontaneous oscillations (mean 0.8 radians, range 0.1–1.9, associated with a coherence of 0.4).

Figure 3 Results of transfer function analysis of spontaneous and induced oscillations.



Group results of transfer function analysis of spontaneous oscillations (resting conditions) and oscillations induced by repeated squat-stand maneuvers at 3 different frequencies. The background plots are frequency distribution graphs, obtained from spontaneous oscillations, of mean (black line) and standard error (SE, gray area) phase, gain and coherence plotted against oscillation frequency. Squared boxes represent mean (SE) phase, gain and coherence within the three frequency bands VLF, LF and HF (see Fig 2). For comparison, results obtained from repeated squat-stand maneuvers are plotted as black dots (mean and SE) at their respective frequencies (0.025, 0.05 and 0.1 Hz). Statistical comparisons were made using Friedman Test with Dunn post-test if $p < 0.05$; *: significant difference within group and **: between groups

Both for spontaneous and induced oscillations, it was clear that with increasing frequency, gain increased and phase decreased (Fig 3), in line with high-pass filter properties of the dynamic pressure-flow relationship of the cerebral circulation.

Discussion

There are several new findings in this study. During repeated squat-stand maneuvers at three specific frequencies of 0.25 Hz, 0.05 Hz and 0.1 Hz, large oscillatory changes in BP and CBFV were generated around each of these frequencies. Increases in oscillations in BP and CBFV led to a significant increase in coherence in the very low frequency range and improved estimations of transfer function phase. Interestingly, estimation of transfer function gain yielded identical results with spontaneous and induced oscillations. The “high-pass filter” behavior of the dynamic pressure-flow relationship, deduced from these augmented and causally related oscillations in BP and CBFV, fits the model based on analysis of spontaneous oscillations. These findings show that the large oscillations in BP and CBFV induced during repeated squat-stand maneuvers not only provided strong and physiologically relevant hemodynamics perturbations, but also led to improved estimation of transfer function to assess dynamic cerebral autoregulation at the very low frequencies.

Previous methods to induce hemodynamics oscillations. Previous studies have induced oscillations in BP and CBFV in order to advance the understanding of dynamic pressure-flow relationship of the cerebral circulation.[8,11,13,18-21] In one of the first studies describing the clinical application of the phase relationship between BP and CBF, Diehl et al. used periodic breathing to induce sinusoidal oscillations at 0.1 Hz (6 breaths per min).[18] Forced breathing carries the risk of altering arterial CO_2, which affects CBF. For obvious reasons, this technique can not be used to induce oscillations at frequencies much lower than 0.1 Hz. Augmented oscillations have also been induced by repeated head-up tilt to 45° (< 2 s) with a period of 10 s.[8] The purpose of this study was to investigate the phase relationship between BP and cerebrovascular resistance, in order to explain the phase lead of CBFV to BP in studies of autoregulation. Compared with spontaneous oscillations, coherence between BP and CBFV was increased by these maneuvers. A disadvantage of this technique was the uncertain effect of the gravitational force on cerebral perfusion pressure when going from supine to tilt.[8] Recently, a method of cyclic thigh cuff compression and release was used to induce oscillatory changes in BP and CBF at a single frequency of 0.033 Hz.[19] On average, BP increased by ~10 mmHg during cuff compression, and decreased by ~6 mmHg during cuff release. However,

continuous recordings did not reveal clearly discernible sinusoidal changes in BP and CBFV in response to cyclic changes in cuff pressure.[19] Finally, oscillatory lower body negative pressure (OLBNP) has been applied to create oscillations in BP and CBFV.[13,20,21] This technique has important limitations in that changes in pressure of LBNP chambers may not transfer immediately and effectively into changes in BP with increases in oscillatory frequencies.[21] In addition, LBNP pressures that were high enough to be effective in generating large BP and CBFV oscillations are poorly tolerated by subjects.[20] Despite these technical difficulties, Hamner et al. have applied OLBNP to induce oscillations in BP and CBFV at the frequencies of 0.03, 0.05 and 0.1 Hz, with the purpose of increasing low frequency coherence.[21] Even at a maximum change of LBNP pressure from zero to -40 mmHg and at the most effective frequency of 0.05 Hz, spectral power of BP and CBFV increased only by a factor of 10 and 5 respectively.[21] Of note, 14 out of 36 LBNP trials in nine subjects still had coherence < 0.3 at the frequencies of 0.03 and 0.05 Hz, indicating the limitations of using oscillatory LBNP to generate large and coherent low frequency changes in BP and CBFV.[21]

Rationale for using repeated squat-stand maneuvers. More than half a century ago, Sharpey-Schafer described the strong hemodynamics changes provoked by a squat-stand maneuver.[10] Briefly, squatting induces a rapid but transient rise in BP with its peak after about 2 to 3 seconds. The increase in BP has been attributed mainly to a sudden increase in cardiac output from increased venous return from the lower limbs.[22] The reduction in BP during standing up has been attributed to a combination of reduction in peripheral vascular resistance and a reduction in cardiac output due to rapid translocation of central blood volume to the lower limbs.[23] Here, we observed large and coherent oscillations in BP and CBFV, induced by repeated squat-stand maneuvers at three distinct frequencies. These results clearly demonstrated that low frequency, transient changes in CBFV occur in response to dynamic changes in arterial pressure that should lie within the "autoregulatory range".

Different frequencies induced oscillations with different magnitude. Oscillations in BP at 0.05 and 0.1 Hz were much larger than at 0.025 Hz. A likely explanation is that at 0.025 Hz, baroreflex sympathetic control of BP would have sufficient response time to counteract dynamic changes in BP.[24] Conversely, large oscillations

in BP at 0.05 and 0.1 Hz may be compounded by cardiovascular resonance in response to external perturbations to BP.[25,26] In fact, the frequencies of 0.05 and 0.1 Hz of repeated squat-stand maneuvers in this study are close to the baroreflex resonance frequencies observed in humans.[25,26]

Low-frequency coherence. Low-frequency coherence (<0.07 Hz) was increased significantly in all subjects. Previous studies have suggested that low coherence may indicate that changes in CBFV are relatively independent of changes in BP, and thus may reflect an intact cerebral autoregulation. Conversely, increases in coherence have been interpreted to reflect impairment of autoregulation.[1] Could the higher coherence indicate that autoregulation was less efficient for the large hemodynamics changes during the repeated squat-stand maneuvers? Given the fact that transfer function gain was not different from spontaneous oscillations, indicating a similar degree of damping for the much stronger induced oscillations in BP, this is very unlikely.[15] Furthermore, phase shifts for induced oscillations were within the normal range and showed a pattern similar to spontaneous oscillations, indicating intact autoregulation. Therefore, we propose that the increased coherence reflects improved "signal to noise ratio" for induced oscillations. Finally, it can be speculated that the high coherence for induced oscillations may reflect nonlinear synchronization or entrainment of the two oscillatory systems (BP and CBFV) with periodic external stimuli, similar to what has been observed in other biological regulator systems.[27]

Transfer function phase and gain. Phase estimates at the VLF frequencies from spontaneous oscillations had large inter-individual variability (observe the high standard error in figure 3); this most likely results from the low coherence, since phase for augmented oscillations in BP and CBFV, associated with higher coherence, showed considerably less inter-individual variability (Fig 3). Decreases in phase with increases in frequency confirmed previous observations based on spontaneous oscillations under resting conditions that phase shift is larger at the VLF than the LF frequencies. Phase in induced oscillations (0.6 radians, SD 0.23) was slightly lower than, but consistent with, observations in previous studies using enhanced oscillations. These studies found a phase lead of CBFV of 0.6–0.8 radians in the very low frequency region < 0.07 Hz.[11,20,21] Since the magnitude of enhanced BP and CBFV oscillations in this study is significantly higher than in those

previous studies, these data suggest that estimation of phase also may be influenced by the magnitude of oscillations in BP and CBFV.

Transfer function gain was similar for spontaneous and induced oscillations even though coherence was low for spontaneous oscillations (0.4, SD 0.13) at frequencies below 0.07 Hz. These findings suggest that, compared with phase, estimation of gain is less affected by the magnitude of BP and CBFV oscillations and by low coherence.

The characteristics of transfer function gain and phase in the present study, under conditions of augmented oscillations in BP and CBFV, extend previous observations of the "high-pass filter" characteristics of dynamic cerebral autoregulation.[21] The consistency of transfer function gain and phase estimates between spontaneous and augmented changes in BP and CBFV suggests that for each specific frequency, CBFV changed in proportion with changes in BP, revealing linear dynamic system properties.[28] These observations are interesting given the fact that BP and CBFV spectral power during repeated squat-stand maneuvers was substantially higher than for spontaneous oscillations (10 to 100 fold), suggesting that linear transfer function analysis can be applied in a wide range of changes in BP and CBFV.

$ETCO_2$ during repeated maneuvers. Fluctuations in ETCO2 were enhanced slightly during repeated squat-stand maneuvers (Table 2). Considering that CBF changes 3% for each mmHg change in $ETCO_2$, the spectral power of these fluctuations translates into fluctuations in CBFV with a maximum of 2.5%, which is small relative to the > 20% of change induced by BP. Moreover, the repeated body movements may introduce artifacts in capnography registration of the CO_2 waveform, which do not affect mean $ETCO_2$ estimation, but could cause overestimation of fluctuations. We did not find a temporal relationship between fluctuations in $ETCO_2$ and oscillations in BP or CBFV.

Study limitations. Repeated squat-stand maneuvers can be considered as aerobic exercise. Thus, these maneuvers per se may modulate the dynamic pressure-flow relationship of the cerebral circulation. However, previous studies have shown that moderate to high intensity aerobic exercise did not alter dynamic cerebral autoregulation.[29] Moreover, the absence of changes in mean blood pressure, heart rate and $ETCO_2$ between resting conditions and repeated squat-stand maneuvers (Table 1) argues against significant hemodynamics exercise effects.

The number of subjects in this study is relatively small. However, the increases in spectral power and coherence were robust and statistically significant.

Finally, flow-velocity in the MCA is determined by CBF and by the diameter of the MCA. Therefore, changes in CBFV reflect changes in CBF only if the MCA diameter is constant. During acute, moderate changes in BP, MCA diameter showed less than 4% change.[30,31] This suggests that in our study, small changes in MCA diameter can not be excluded. In theory, this would have the following effects on our data. Vasoconstriction during an increase in BP overestimates the associated rise in CBF. Conversely, vasodilatation induced by a decrease in BP overestimates the reduction in CBF. Collectively, these effects, if they did occur, would overestimate transfer function gain, but would not affect phase, as this parameter is not affected by the amplitude but by the timing of the oscillations. In a similar vein, pressure-induced passive changes in diameter would underestimate changes in CBFV and thus transfer function gain, but not phase.

Clinical significance. In clinical practice, the consequences of sudden changes in BP are frequently encountered in patients complaining of feeling light headed or dizzy when standing up, and in patients evaluated for unexplained falls or syncope. Diagnostic evaluation generally focuses on BP measurements, with the inexplicit assumption that a reduction beyond a certain defined cut-off value (e.g. 20 mmHg systolic) induces cerebral hypoperfusion. However, dynamic cerebral autoregulation mediates the consequences of BP changes on CBF, and therefore plays an important role in determining the clinical consequence of hemodynamic instability. Quantification of dynamic cerebral autoregulation has provided insight in these syndromes[32] as well as in other important clinical conditions such as stroke[32,33], hypertension[9,34], and will be important to investigate in Alzheimer disease.[35,36]

Conclusions

We have demonstrated that large and coherent oscillations in BP and CBFV at the low frequencies of 0.025, 0.05 and 0.1 Hz can be induced by repeated squat-stand maneuvers. This study is the first to succeed in inducing high amplitude oscillations in the low frequencies, in combination with high coherence, thereby allowing confident comparisons of the behavior of dynamic cerebral autoregulation in low versus high frequencies. The magnitude of these hemodynamics changes as well as the maneuvers through which they are induced arguably are representative of the daily physiological challenges put to cerebral autoregulation, such as orthostatic hypotension. Phase estimates at the low frequencies below 0.07 Hz were substantially improved by the augmented BP and CBFV oscillations. Therefore, we conclude that addition of these maneuvers to an analysis based solely on spontaneous oscillations enhances the information obtained from transfer function analysis and may be an important step towards its translation and implementation in clinical medicine.

References

1. Giller CA. The frequency-dependent behavior of cerebral autoregulation. *Neurosurgery 1990;27:362-368.*
2. Zhang R, Zuckerman JH, Levine BD. Spontaneous fluctuations in cerebral blood flow: insights from extended-duration recordings in humans. *Am J Physiol Heart Circ Physiol 2000;278:H1848-H1855.*
3. Diehl RR, Linden D, Lucke D, Berlit P. Spontaneous blood pressure oscillations and cerebral autoregulation. *Clin Auton Res 1998;8:7-12.*
4. Panerai RB, White RP, Markus HS, Evans DH. Grading of cerebral dynamic autoregulation from spontaneous fluctuations in arterial blood pressure. *Stroke 1998;29:2341-2346.*
5. Zhang R, Zuckerman JH, Giller CA, Levine BD. Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol 1998;274:H233-H241.*
6. Taylor JA, Carr DL, Myers CW, Eckberg DL. Mechanisms underlying very-low-frequency RR-interval oscillations in humans. *Circulation 1998;98:547-555.*
7. Giller CA, Hatab MR, Giller AM. Oscillations in cerebral blood flow detected with a transcranial Doppler index. *J Cereb Blood Flow Metab 1999;19:452-459.*
8. Hughson RL, Edwards MR, O'Leary DD, Shoemaker JK. Critical analysis of cerebrovascular autoregulation during repeated head-up tilt. *Stroke 2001;32:2403-2408.*
9. Lipsitz LA, Mukai S, Hamner J, Gagnon M, Babikian V. Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke 2000;31:1897-1903.*
10. Sharpey-Schafer EP. Effects of squatting on the normal and failing circulation. *BMJ 1956;1:1072-1074.*
11. Birch AA, Dirnhuber MJ, Hartley-Davies R, Iannotti F, Neil-Dwyer G. Assessment of autoregulation by means of periodic changes in blood pressure. *Stroke 1995;26:834-837.*
12. Sammons EL, Samani NJ, Smith SM, Rathbone WE, Bentley S, Potter JF, Panerai RB. Influence of noninvasive peripheral arterial blood pressure measurements on assessment of dynamic cerebral autoregulation. *J Appl Physiol 2007;103:369-375.*
13. Zhang R, Zuckerman JH, Iwasaki K, Wilson TE, Crandall CG, Levine BD. Autonomic neural control of dynamic cerebral autoregulation in humans. *Circulation 2002;106:1814-1820.*
14. Omboni S, Parati G, Frattola A, Mutti E, Di RM, Castiglioni P, Mancia G. Spectral and sequence analysis of finger blood pressure variability. Comparison with analysis of intra-arterial recordings. *Hypertension 1993;22:26-33.*
15. Kolb B, Rotella DL, Stauss HM. Frequency response characteristics of cerebral blood flow autoregulation in rats. *Am J Physiol Heart Circ Physiol 2007;292:H432-H438.*

16. Kontos HA, Wei EP, Navari RM, Levasseur JE, Rosenblum WI, Patterson JL, Jr. Responses of cerebral arteries and arterioles to acute hypotension and hypertension. *Am J Physiol 1978;234:H371-H383.*
17. Zhang R, Witkowski S, Fu Q, Claassen JA, Levine BD. Cerebral hemodynamics after short- and long-term reduction in blood pressure in mild and moderate hypertension. *Hypertension 2007;49:1149-1155.*
18. Diehl RR, Linden D, Lucke D, Berlit P. Phase relationship between cerebral blood flow velocity and blood pressure. A clinical test of autoregulation. *Stroke 1995;26:1801-1804.*
19. Aaslid R, Blaha M, Sviri G, Douville CM, Newell DW. Asymmetric dynamic cerebral autoregulatory response to cyclic stimuli. *Stroke 2007;38:1465-1469.*
20. Birch AA, Neil-Dwyer G, Murrills AJ. The repeatability of cerebral autoregulation assessment using sinusoidal lower body negative pressure. *Physiol Meas 2002;23:73-83.*
21. Hamner JW, Cohen MA, Mukai S, Lipsitz LA, Taylor JA. Spectral indices of human cerebral blood flow control: responses to augmented blood pressure oscillations. *J Physiol 2004;559:965-973.*
22. Krediet CT, de B, I, Ganzeboom KS, Linzer M, van Lieshout JJ, Wieling W. Leg crossing, muscle tensing, squatting, and the crash position are effective against vasovagal reactions solely through increases in cardiac output. *J Appl Physiol 2005;99:1697-1703.*
23. Wieling W, Krediet CT, van DN, Linzer M, Tschakovsky ME. Initial orthostatic hypotension: review of a forgotten condition. *Clin Sci (Lond) 2007;112:157-165.*
24. Eckberg DL and Sleight P. Human Baroreflexes in Health and Disease. *Oxford: Clarendon Press, 1992.*
25. Hammer PE, Saul JP. Resonance in a mathematical model of baroreflex control: arterial blood pressure waves accompanying postural stress. *Am J Physiol Regul Integr Comp Physiol 2005;288: R1637-R1648.*
26. van de Vooren H, Gademan MG, Swenne CA, TenVoorde BJ, Schalij MJ, van der Wall EE. Baroreflex sensitivity, blood pressure buffering, and resonance: what are the links? Computer simulation of healthy subjects and heart failure patients. *J Appl Physiol 2007;102:1348-1356.*
27. Matsugu M, Duffin J, Poon CS. Entrainment, instability, quasi-periodicity, and chaos in a compound neural oscillator. *J Comput Neurosci 1998;5:35-51.*
28. Van de Vegte J. Feedback control systems. Englewood Cliffs, NJ: Prentice Hall, 1994.
29. Ogoh S, Dalsgaard MK, Secher NH, Raven PB. Dynamic blood pressure control and middle cerebral artery mean blood velocity variability at rest and during exercise in humans. *Acta Physiol (Oxf) 2007; 191:3-14.*

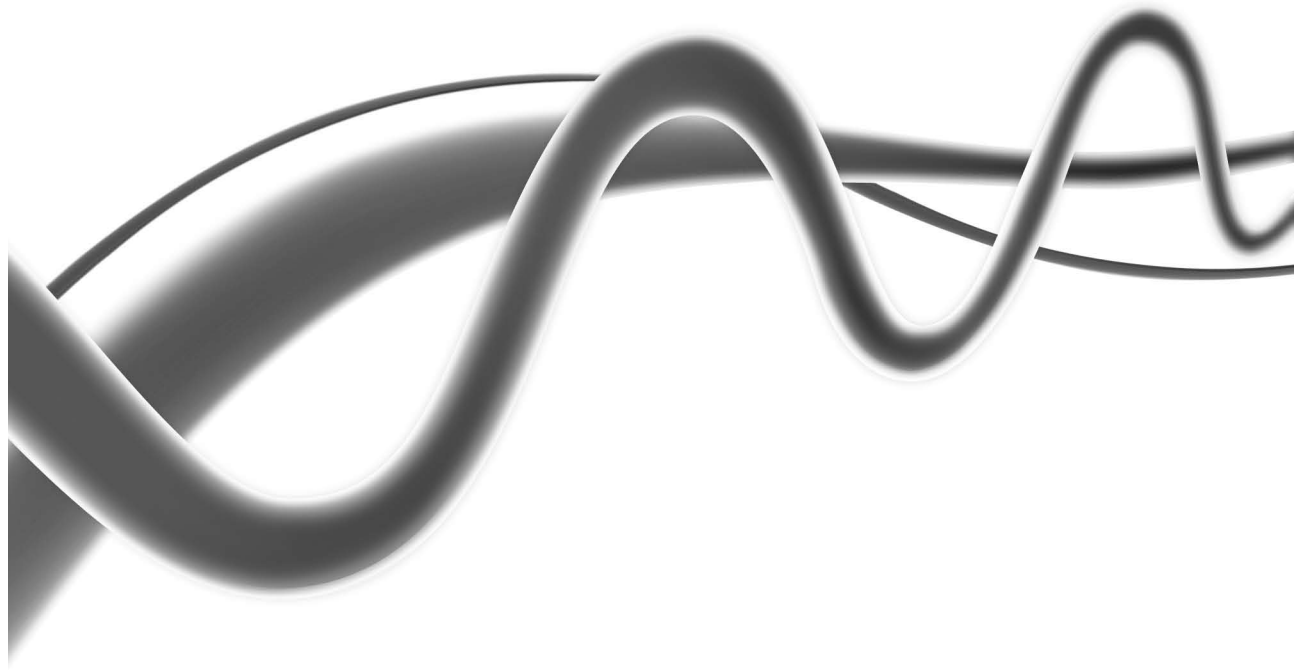
30. Giller CA, Bowman G, Dyer H, Mootz L, Krippner W. Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery 1993;32:737-741.*
31. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke 2000;31:1672-1678.*
32. van Beek AH, Claassen JA, Rikkert MG, Jansen RW. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab 2008;28: 1071-1085.*
33. Immink RV, van Montfrans GA, Stam J, Karemaker JM, Diamant M, van Lieshout JJ. Dynamic cerebral autoregulation in acute lacunar and middle cerebral artery territory ischemic stroke. *Stroke 2005;36: 2595-2600.*
34. Immink RV, van den Born BJ, van Montfrans GA, Koopmans RP, Karemaker JM, van Lieshout JJ. Impaired cerebral autoregulation in patients with malignant hypertension. *Circulation 2004;110:2241-2245.*
35. Claassen JA, Jansen RW. Cholinergically mediated augmentation of cerebral perfusion in Alzheimer's disease and related cognitive disorders: the cholinergic-vascular hypothesis. *J Gerontol A Biol Sci Med Sci 2006;61:267-271.*
36. Suter OC, Sunthorn T, Kraftsik R, Straubel J, Darekar P, Khalili K, Miklossy J. Cerebral hypoperfusion generates cortical watershed microinfarcts in Alzheimer disease. *Stroke 2002;33:1986-1992.*

Tellymedicine *(BMJ 2005;331:1185)*

In the past, doctors had to see a patient before they could make a diagnosis. Doctors were also restricted in whom they could tell about this diagnosis -the patient and his or her trusted next of kin, the nurse, and perhaps the odd interested colleague. Recent advances in technology first brought us telemedicine, where doctors can assist other doctors in making a diagnosis, guide surgical procedures, or even perform surgery themselves, without being physically near the patient. More recently, we have seen tellymedicine. This enables us to make a diagnosis in patients for whom we have no responsibility. We don't need to examine them, nor do we need a formal invitation to become involved in their medical care. In fact, we may only know of these patients from watching them on television or from reading about them in a newspaper. One of the most accomplished tellydoctors is Dr Gupta, a neurosurgeon who is also a senior medical correspondent for the US Cable News Network (CNN) (www.cnn.com/2005/HEALTH/03/30/otsc.gupta/). Dr Gupta diagnosed coronary artery disease in former US president Bill Clinton almost before his cardiologist did. Another recent triumph of tellymedicine was recorded during the death of the late pope. The reluctance of the pontiff's doctors to release information threatened to turn things into a private Vatican matter. Fortunately, tellydoctors in several countries used their skills in remote diagnostics to provide the international media with detailed information on the pope's medical condition during his last days. Forensic medicine is one particular field that can benefit from tellymedicine. For instance, Volkert van der Graaf, the assassin of Dutch politician Pim Fortuyn, was admitted to a clinic for forensic psychiatry so that a single expert witness could report to the court. Because Volkert refused cooperation, this psychiatric evaluation was extremely lengthy. Fortunately, several "Tele" psychiatrists, after studying the media, were able to conclude that Volkert suffered from Asperger's syndrome, and informed the court and public of this through the media long before his trial. In the UK, Professor David Southall sought a child protection investigation after watching a television programme. He claimed to see what others had not but for his pains was found guilty of serious professional misconduct by the General Medical Council. Tellymedicine implies that an insightful diagnosis can be made through careful observation alone, and that modern physicians have lost this gift and have come to rely too much on their tradition of diagnostic tests and procedures, requiring the physical presence of the patient. Tellymedicine obviates all these costly and time consuming tests. Patients are spared pointless visits to our clinics. As an additional benefit, they can read their diagnosis in the newspapers or watch it on television in the comfort of their home.

Part 3

Application in clinical research



Chapter 7

Preserved cerebral vasomotor reactivity before and after blood pressure reduction in hypertensive patients

Jurgen A.H.R. Claassen
Benjamin D. Levine
Rong Zhang

Abstract

In hypertension, cerebral vasomotor reactivity to CO_2 may be reduced as a consequence of cerebrovascular remodelling and endothelial dysfunction. In untreated patients, BP (blood pressure) reduction may further impair CO_2 reactivity as cerebral pressure autoregulation will cause compensatory cerebral vasodilatation. Treatment with an AT (angiotensin) II receptor blocking agent may modulate CO_2 reactivity under these conditions as AT II mediates vascular remodelling and endothelial function. 11 patients with newly diagnosed, untreated mild to moderate hypertension, 9 men, 2 women, age (mean (SD)) 52 (9) years, and 8 controls, 7 men, 1 woman, age 46 (10) years were studied. Patients received losartan/ hydrochlorothiazide (50/12.5 or 100/25 mg) to reduce BP to $<$ 140/$<$90 mmHg within 1-2 weeks. BP, heart rate, CBFV (cerebral blood flow-velocity), cerebrovascular resistance, and CO_2 reactivity were measured at baseline, after the rapid BP reduction, and after long- term treatment (3-4 months). At baseline, hypertension was not associated with reduced CO_2 reactivity. Treatment effectively lowered BP from 148(12)/ 89(7) to 130(15)/80(9) after 1-2 weeks and 125(10)/ 77(7) mmHg after 3-4 months ($p=0.003$). Cerebrovascular resistance was reduced (indicating vasodilatation) and CBFV remained stable, however, CO_2 reactivity was not affected. In conclusion, in this small sample, neither elevated BP nor rapid BP lowering affected CO_2 reactivity. In addition, no effect was observed of prolonged inhibition of AT II with losartan.

Introduction

Hypertension is the major risk factor for stroke[1], and large epidemiological studies have demonstrated that high blood pressure (BP) in midlife is also a major risk factor for Alzheimer's disease and vascular dementia.[2] These important clinical associations are supported by the profound disruptive effects of hypertension on the brain's circulation. Prolonged hypertension leads to remodelling of cerebral arteries with an increase in wall thickness/lumen ratio.[3] In addition, hypertension impairs endothelium-mediated cerebral vasodilatation.[4]

Substantial evidence suggests that angiotensin II is implicated in cerebral vascular remodelling and endothelial dysfunction in hypertension.[4] Specifically, angiotensin II, via AT1 receptor binding, attenuates endothelium-dependent responses of the cerebral circulation.[4] Thus, antihypertensive treatment with ACE inhibitors or AT1 receptor blockers may reverse cerebrovascular changes not only through the lowering of BP, but also through direct vascular effects of these agents acting on the vascular renin-angiotensin system.[2,5]

Two major regulatory mechanisms for the cerebrovascular effects of hypertension are implicated that can both be evaluated clinically: 1) pressure autoregulation (cerebral autoregulation), describing the cerebral vasomotor responses to changes in perfusion pressure[6], and 2) CO_2 reactivity, describing cerebral vasomotor responses to changes in arterial CO_2 (CVMR).[7] These two mechanisms differ importantly in that most evidence suggests that CVMR is, and cerebral autoregulation is not, affected by endothelial dysfunction.[6,8,9] Moreover, angiotensin II may exert differential effects on CVMR and cerebral autoregulation.[4]

Recently, we have reported that dynamic cerebral autoregulation and cerebral vasodilatatory response to BP reduction were preserved in hypertensive patients receiving treatment with the AT1 receptor blocker losartan combined with hydrochlorothiazide (Hyzaar).[6] These findings support the notion that angiotensin receptor blockade causes a leftward shift of the lower limit of static cerebral autoregulation (to lower pressures), thus protecting the brain against hypoperfusion when BP is lowered.[10]

However, it is not clear whether hypertension-induced cerebrovascular changes may impair vascular reactivity to CO_2. Moreover, pressure autoregulation may interact in the way that the vasodilatation required to buffer BP reduction may attenuate or exhaust any further vasodilatatory response to CO_2. Furthermore, whereas blockade of the effects of angiotensin II may improve pressure autoregulation, its effects on CVMR are insufficiently known. The present study was performed to test the hypothesis that CVMR is reduced in untreated mild to moderate hypertension, and that these changes are exacerbated after acute reduction in BP associated with autoregulatory vasodilatation. In addition, we hypothesized that prolonged treatment with losartan, through a beneficial effect on vascular remodelling or endothelial function may restore CVMR.

Materials and Methods

Subjects

This study was conducted in a subgroup of subjects who participated in research to determine the effects of short and long-term reduction in BP on dynamic cerebral autoregulation in patients with mild and moderate hypertension.[6] 11 patients with newly diagnosed mild to moderate hypertension (9 men, 2 women) with a mean age of 52 (SD 9) years (range, 39–66), and 8 healthy subjects (7 men, 1 woman) with a mean age of 46 (SD 10) years (range, 38-66) participated in this study (Table 1). Based on the variance of he method used to measure CVRM, obtained in a previous study[7], this study had 90 % power to detect a 10 % change in total CVMR, or a difference in CVMR equal to or greater than 1.5 %/Torr.

None of the patients had received prior antihypertensive treatment. Classification of hypertension (JNC 7 and ESC/ESH 2007; mild to moderate, grade 1-2) was based on the average of awake recordings from 24-h ambulatory BP.[1] All participants underwent a thorough medical history and physical examination, as well as blood chemistry evaluation and echocardiography to exclude angina pectoris, myocardial infarction, heart failure, diabetes, renal disease, lung disease, or history of stroke. None were current smokers. During the screening process, patients with severe hypertension (Grade 3; systolic pressure > 180 or diastolic pressure > 110 mmHg) were referred to emergency medical care and were not included in this study.

The study was conducted in accordance with the guidelines set by the Declaration of Helsinki, and the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas approved the study. All participants provided informed consent in writing.

Instrumentation

Finger photoplethysmography (Finapres, Ohmeda) was used to measure beat-to-beat changes in BP. Intermittent cuff BP was measured at the upper arm using electrosphygmomanometry (SunTech). CBF-velocity (CBFV) was recorded in the middle cerebral artery (MCA) using transcranial Doppler (TCD, Multiflow, DWL). The Doppler probe was placed over the subject's temporal window and fixed at a constant angle with a probe holder that was custom made to fit each subject's facial bone structure. This technique allowed CBFV to be measured precisely at the same acoustic window and at the same angle for repeated studies.[7] Heart rate was monitored using ECG. End-tidal CO_2 ($ETCO_2$) was monitored via a nasal cannula using a capnograph (Criticare Systems Inc.).

Protocol

All experiments were performed in the morning at least two hours after a light breakfast. The subjects refrained from heavy exercise and caffeinated or alcoholic beverages at least 24 hours before the tests. Baseline measurements, consisting of six min of beat-to-beat BP and CBFV during spontaneous breathing, were recorded after at least 30 min of supine rest.

We used a previously described protocol to estimate CVMR using breath-by-breath changes in $ETCO_2$, CBFV and BP.[7] The study was conducted with the subjects in the supine position to allow comparison with studies using other imaging technology such as MRI or PET.[11,12] To obtain a wide range of CO_2 changes (from ~20 to 60 mmHg $ETCO_2$), a modified rebreathing protocol was used with a period of voluntary hyperventilation preceding rebreathing. Rebreathing was continued for 5 min, before returning to room air for recovery (4 min). During rebreathing, a small amount of oxygen was bled into the rebreathing bag at the subject's basal metabolic rate (estimated using the Harris-Benedict formula) to maintain arterial oxygen saturation (SaO_2) constant.[7]

Antihypertensive treatment

After the baseline test, patients received low dose losartan/hydrochlorothiazide (50/12.5 mg once daily) for one week. If the reduction in BP was not below a prior determined level of systolic pressure < 140 mmHg and diastolic pressure < 90 mmHg the dose was increased to 100/25 mg. The short-term test (one to two weeks after start of treatment) was performed when this goal of BP control was reached. The long-term test was scheduled after three to four months of continued treatment. In patients, 24-h ambulatory BP (Accutracker II, SunTech) was measured before treatment and repeated on the days before the short and long-term tests. In healthy subjects, 24-h BP was measured once at baseline and once before the long-term test. The patients took their losartan/hydrochlorothiazide in the morning 2-3 h before the study to ensure its optimal BP lowering effects.

Data analysis

Off-line data analysis was performed using commercially available software (Acknowledge, BIOPAC systems). Baseline BP, HR and CBFV were obtained from the average of beat-to-beat data; $ETCO_2$ was derived from the breath-to-breath data. Cerebrovascular conductance index (CVCI) was calculated as mean CBFV divided by mean BP for all beat-to-beat data.[7] Since CBFV may be affected directly by changes in BP[13,14], estimations of CVMR based on CVCI may reveal intrinsic vascular responses to changes in CO_2. During hyperventilation and rebreathing, breath-to-breath values of mean CBFV, BP, CVCI, HR and $ETCO_2$ were obtained. Towards the end of rebreathing, CBFV reached a plateau-phase; a similar plateau was observed for hypoventilation, indicating that a steady-state had been reached. To allow comparison with previous studies that have only measured steady-state conditions, the first part of data analysis was performed using these plateau values. Thus, maximum hypocapnic (~20 mmHg below baseline $ETCO_2$) reduction in CBFV and CVCI (in %), maximum hypercapnic (~20 mmHg above baseline) increase in CBFV and CVCI (in %), and total range of change in CBFV and CVCI (hypocapnic + hypercapnic, in %) were derived. Next, CVMR was expressed as the ratio of % change in CBFV or CVCI over the whole range of changes in $ETCO_2$ (hypocapnic + hypercapnic).

The second part of data-analysis focused on the dynamic responses of CBFV to the transient changes in $ETCO_2$. Here, during hyperventilation and rebreathing, CVMR was estimated nonlinearly for breath-to-breath changes in CBFV as well as CVCI, as described in detail previously.[7] In short, a 4 parameter logistic function was used for sigmoidal curve fitting, where model parameter a represents the total range of change in CBFV or CVCi; *y*0 is the maximum value of CBFV or CVCI during hypercapnia; *x*0 is the level of $ETCO_2$ where vascular reactivity to changes in CO_2 is maximal; and *b* is a constant that determines the sigmoidal shape of the curve (low values for b result in an almost flat curve) (Fig. 1). Finally, linear regression of changes in CBFV and CVCI in the range of $ETCO_2$ between 40-50 Torr was performed. We have shown previously that the slope of this regression is a robust method to estimate maximum vascular reactivity for each individual subject.[7] This maximum vascular reactivity is expected to be very sensitive to altered myogenic and structural cerebrovascular properties, associated with hypertension.[3,15,16]

Statistics

T-tests and analysis of variance were used to compare the differences and interactions between the subject groups and the effects of antihypertensive treatment on baseline hemodynamics and CVMR (SigmaStat, version 3.11 Systat Software Inc.). Data are presented as mean (SD), with their associated P values.

Results

Reduction in BP and changes in CBFV

24 h ambulatory BP was reduced significantly within 1-2 weeks of treatment. Reductions in BP in patients with hypertension were well maintained over the period of 3-4 months with the same dose of drugs used to reduce BP acutely (Table 1). For brevity, nocturnal BP data are not shown, however, all participants were “normal dippers”, and treatment affected nocturnal BP similarly as awake BP.

Before treatment, no differences in CBFV were observed between control subjects and patients with hypertension. CBFV did not change after short and long-term reductions in BP.

Estimation of CVMR

Before treatment, maximum (plateau) changes in CBFV and CVCI with hyperventilation and rebreathing were similar in control subjects and patients with hypertension. In patients, neither short-term nor long-term treatment affected these values (Table 2).

Table 1 Characteristics of hypertensive patients and controls at baseline and follow-up.

	Baseline		1-2 weeks		3-4 months	
Variables	**Control**	**Hypertension**	**Control**	**Hypertension**	**Control**	**Hypertension**
Age, y	46 (10)	52 (9)				
Height, cm	180 (9)	178 (10)				
Weight, kg	87 (16)	88 (19)				
LDL, mg/dL	117 (35)	111 (32)			111 (25)	108 (26)
SBP, mmHg	120 (7)	148 (12)*	--	130 (15)†	124 (7)	125 (10)†
DBP, mmHg	74 (6)	89 (7)*	--	80 (9)†	76 (7)	77 (7)†
HR, bpm	72 (8)	77 (8)	--	78 (7)	72 (8)	77 (10)
CBFV, cm/s	49 (11)	49 (12)	--	51 (14)	51 (13)	51 (14)
CVCI, cm/s/mmHg	0.51 (0.11)	0.45 (0.11)	--	0.53 (0.13)	0.52 (0.10)	0.55 (0.15)
CO_2, mmHg	38 (5)	36 (4)	--	36 (5)	38 (3)	38 (5)

Data are mean (SD), n=8 for control, n=11 for hypertension. LDL, low-density-lipoprotein fraction of cholesterol. SBP, systolic blood pressure, DBP, diastolic blood pressure, HR, heart rate. These measurements were obtained from 24 h ambulatory recordings (awake). CBFV, cerebral blood flow velocity, CVCI, cerebrovascular conductance, $ETCO_2$, end-tidal CO2. These measurements were obtained under supine resting conditions. * P=0.0001 for comparison between control subjects and patients with hypertension. † P=0.003 for comparisons between baseline and treatment for patients with hypertension. The increase in mean CVCI from baseline following treatment was not significant (1-2 weeks: p=0.17; 3-4 months, p=0.09).

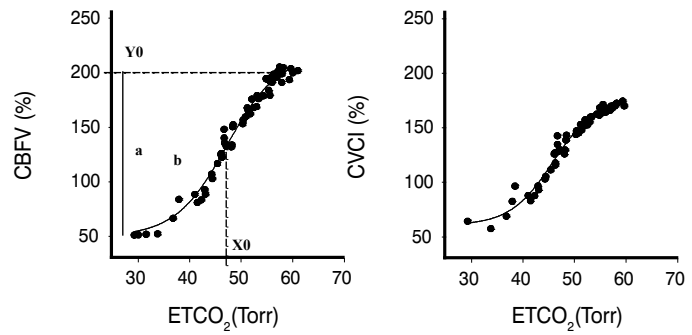
Table 2 Estimates of CVMR in controls and patients with hypertension before and during treatment.

Vasomotor reactivity		**Control**			**Hypertension**	
		Baseline	**3-4 months**	**Baseline**	**1-2 weeks**	**3-4 months**
Hypocapnia						
minimum flow	CBFV, %	60 (9)	65 (9)	66 (12)	66 (13)	66 (11)
minimum conductance	CVCI, %	65 (10)	67 (10)	73 (9)	77 (7)	75 (12)
Hypercapnia						
maximum flow	CBFV, %	216 (26)	218 (29)	220 (35)	220 (33)	214 (42)
maximum conductance	CVCI, %	172 (26)	176 (16)	180 (28)	180 (25)	172 (31)
Logistic model parameters						
	a-CBFV, %	178 (26)	160 (31)	197 (73)	187 (53)	169 (57)
	a-CVCI, %	114 (22)	106 (15)	121 (73)	104 (33)	107 (44)
	Y0-CBFV, %	227 (24)	213 (35)	241 (56)	242 (55)	221 (57)
	Y0-CVCI, %	180 (25)	177 (19)	200 (64)	183 (31)	182 (47)
	X0-CBFV, Torr	48 (3)	45 (6)	49 (6)	49 (7)	51 (8)
	X0-CVCI, Torr	47 (3)	46 (2)	47 (4)	45 (4)	47 (3)
	b-CBFV, constant	0.17 (0.0)	0.22 (0.0)	0.17 (0.1)	0.18 (0.1)	0.18 (0.1)
	b-CVCI, constant	0.25 (0.1)	0.30 (0.1)	0.28 (0.1)	0.33 (0.1)	0.31 (0.1)
Estimations of CVMR						
Logistic regression	CVMRmax-CBFV, %/Torr	7.5 (0.8)	8.6 (1.7)	8.8 (1.7)	9.1 (4.1)	8.9 (2.5)
	CVMRmax-CVCI, %/Torr	7.0 (1.5)	8.0 (1.8)	7.3 (1.7)	7.9 (2.5)	7.4 (1.4)
Linear regression 40-50 Torr	CVMRmax-CBFV, %/Torr	7.8 (0.9)	7.9 (1.6)	7.9 (1.5)	7.6 (1.5)	8.3 (1.4)
	CVMRmax-CVCI, %/Torr	5.9(1.6)	6.0 (1.9)	6.0 (1.1)	6.6 (1.3)	6.3 (1.4)
Ratio	CVMR-CBFV, %/Torr	3.8 (0.6)	4.0 (0.6)	4.2 (1.0)	3.9 (1.2)	4.0 (1.2)
	CVMR-CVCI, %/Torr	2.8 (0.5)	2.9 (0.4)	4.2 (1.0)	4.0 (1.3)	3.9 (1.4)

Data are mean (SD). N=7 for control, n=11 for hypertension. CBFV, cerebral blood flow velocity, CVCI, cerebrovascular conductance index. Minimum and maximum values during hypocapnia and hypercapnia represent observed values for CBFV and CVCI. Logistic model parameters represent values for CBFV and CVCI predicted from logistic regression. a-CBFV, total change in CBFV from maximum hypocapnia to maximum hypercapnia in % of baseline. Note that a-CBFV compares to the difference between maximum flow and minimum flow. a-CVCI, similar for CVCI. Y0-CBFV, maximum value for CBFV during hypercapnia, relative to baseline. Note that YO-CBFV compares to maximum flow. Y0-CVCI, similar for CVCI. X0, value for end-tidal CO_2 where CVMRmax, from logistic regression, occurs. CVMRmax, maximum vasomotor reactivity identified with either logistic regression or linear regression in the $ETCO_2$ range between 40-50 Torr (see text). CVMR, index of vasomotor reactivity representing the ratio of change in CBFV or CVCI over $ETCO_2$ in the total hypocapnic and hypercapnic range. There were no significant differences between controls and patients or between baseline and follow-up (P > 0.1).

Examples of sigmoidal curve fitting of changes in CBFV and CVCI vs. $ETCO_2$ are presented in Fig 1 and 2 respectively. The results of non-linear parameter identification are summarized in Table 2. Before treatment, no differences in the model parameters were observed between control subjects and patients with hypertension. Model parameters did not change after reductions in BP in patients. The steep portion of changes in CBFV or CVCI to $ETCO_2$ was identified in the range between 40-50 Torr (Fig 1, 2). Estimation of CVMRmax in this range of $ETCO_2$ also did not differ between patients and controls nor was it influenced by treatment (Table 2).

Figure 1 Example of the analysis of vasomotor reactivity in a healthy subjects.

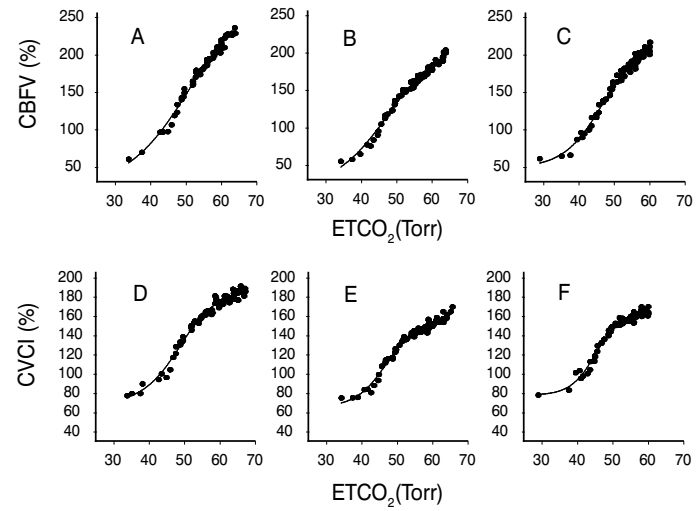


Results of the modified rebreathing test and sigmoidal curve-fitting, displaying the changes in cerebral blood flow velocity (CBFV, left) and cerebrovascular conductance index (CVCI, right) in response to end-tidal CO_2 ($ETCO_2$) in a healthy control subject. The black dots represent data averaged over the period of one breath, during hyperventilation (data points below 100 %) and CO_2-rebreathing (data points above 100%). The sigmoid line is the result of 4-parameter exponential curve fitting of these data points (see text and[7] for more details). The left graph offers a graphic representation of these 4 identified parameters. "a" is the total range of changes in CVCi or CBFV, "Y0" is the maximum value, "X0" is the level of $ETCO_2$ that exhibits highest CO_2 sensitivity, and "b" is a factor that determines the shape of the regression curve.

Effects of CO_2 on BP and HR

Patients and controls had similar changes in ETCO2 during hyperventilation and rebreathing (Fig 1, 2). In controls, hyperventilation decreased BP by 16 (SD 3) mmHg and increased HR by 17 (SD 11) bpm. Rebreathing increased BP by 27 (SD 14) mmHg without a change in HR. In patients, hyperventilation lowered BP by 21 (SD 12) mmHg and increased HR by 14 (SD 10) bpm. During rebreathing, BP

Figure 2 Example of vasomotor reactivity analysis in a hypertensive patient.



Results of the modified rebreathing test and sigmoidal curve-fitting in a hypertensive patient. Representation of black dots and sigmoid curve as in figure 1. Top: Changes in cerebral blood flow velocity (CBFV) in response to end-tidal CO2 (ETCO2) at baseline (A), and after 1-2 weeks (B) and 3 months (C) of treatment with losartan/hydrochlorothiazide. Bottom: Changes in cerebrovascular conductance index (CVCI) in response to ETCO2 at baseline (D), and after 1-2 weeks (E) and 3 months (F) of treatment.

increased by 25 (SD 8) mmHg, without a change in HR. Neither short-term nor long-term treatment altered the BP and HR response to CO_2.

Discussion

This study assessed cerebral vasomotor reactivity to CO_2 in patients with newly diagnosed mild to moderate hypertension, using a modified rebreathing method recently developed in our laboratory.[7] In these untreated patients, there was no evidence that the elevated BP caused impairment in CVMR. Acute lowering of BP within 1-2 weeks with losartan/hydrochlorothiazide led to compensatory cerebral vasodilatation (reduction in resistance) and therefore did not lower CBFV, consistent

with intact pressure autoregulation. These hemodynamic changes also did not impair CVMR. Furthermore, long-term antihypertensive treatment with the inhibition of angiotensin II, which in theory would cause beneficial cerebrovascular remodelling and/or improved endothelial function, did not further change cerebrovascular resistance nor did it augment CVMR. Finally, there was no difference in the BP and HR responses to CO_2 stimuli between patients and controls, and these responses were not influenced by antihypertensive treatment.

The findings that CVMR was not altered in untreated hypertension and was not reduced by acute reduction in BP were in contrast with our hypothesis. Below, we will further discuss these findings in relation with the existent literature.

CVMR in hypertension

It has been demonstrated repeatedly that CBF in patients with hypertension, measured directly or estimated from measurements of CBF-velocity, is similar to that observed in age and sex matched normotensive controls.[6,17,18] According to Ohm's law, this implies that cerebrovascular resistance is elevated in patients with hypertension, most likely due to cerebral vasoconstriction.[17] Therefore, cerebrovascular capacity for further vasoconstriction may be reduced.[19] Furthermore, cerebrovascular remodelling and/or endothelial dysfunction associated with prolonged hypertension may reduce the capacity for vasodilatation as demonstrated in animal studies.[3] In the present study, however, patients with mild to moderate hypertension had vasoconstrictive and vasodilatatory responses to changes in arterial pressure and CO_2 that were similar to normal controls. Specifically, the estimates of CVMR using traditional linear regression methods, as well as the recently developed and more sensitive transient estimates of CVMR did not differ between hypertensive patients and controls.

The findings in this study appear to be in contrast with previous reports of a reduced CVMR in patients with hypertension.[20-22] Troisi et al. estimated CVMR with TCD in young subjects, free from cerebrovascular disease or diabetes, using a breath hold index, obtained during 30 s voluntary breath hold.[22] This index was lower in subjects with hypertension (aged 34 (SD 7) y), and was partially restored following treatment with atenolol. Unfortunately, the breath hold index describes vascular reactivity only in a small hypercapnic range. Moreover, it does not provide information on changes

in $ETCO_2$ or BP, the parameters that determine the increase in CBFV and thus are essential to evaluate CVMR. Serrador et al. also reported reduced CVMR in subjects with both controlled and uncontrolled hypertension without co-morbidity.[23] Breath-by-breath changes in CVCI were obtained to estimate CVMR during hypercapnic rebreathing. Their CVCI data were derived after subtraction of a hypothetical component induced by changes in BP, assuming that cerebral autoregulation was unaltered in hypercapnic states. Interestingly, when CVMR was estimated from the unmodified CVCI data, there was no difference between patients and control subjects.[23] Maeda et al. found a reduced CVMR in hypertension using linear regression analysis of CBFV in the hypocapnic to hypercapnic range and estimated CVMR from the regression slope of these data. However, CVMR was reduced only in a subgroup with advanced cerebrovascular disease. Kario et al. used perfusion MRI, with acetazolamide infusion to assess CVMR[20]. Only patients with both hypertension and diabetes had reduced reactivity. Tominaga et al. found normal CVMR, using the Xenon clearance method, in hypertensive patients who did not manifest cerebrovascular disease.[24] Oku et al. found normal CVMR with PET in patients with mild to moderate hypertension with minimal or no cerebrovascular disease.[12] None of these studies accounted for changes in BP during assessment of CVMR. Our study adds to these earlier reports by exploring a wider range of changes in CO_2, and by investigating transient changes in CO_2, CBFV and CVCI, therefore accounting for alterations in BP during CO_2 stimuli.

In all these studies, it was unknown how long hypertension had already been present at the time of study. Also, presence or absence of cerebrovascular disease in subjects was mostly judged on clinical history. Only subjects with signs or symptoms of cerebrovascular disease, and/or diabetes had reduced CVMR.[20,25,26] Therefore, our observation of normal CVMR in hypertensive patients without evidence of cerebrovascular disease and without diabetes seems consistent with careful analysis of previous reports. Consistent with this interpretation is that in spontaneously hypertensive rats, CVMR remains normal, whereas CVMR is impaired in the stroke-prone genetic variant, at advanced age when cerebrovascular disease has developed.[27]

Normal CVMR after BP lowering

CVMR is reduced in patients with carotid artery stenosis, who have reduced perfusion pressure and compensatory cerebral vasodilatation.[28] Reduction in

CVMR also was observed after acute reduction in BP in animal experiments[29] and in human subjects.[19] In contrast with these observations, we found no effect of either short or long-term BP lowering on CVMR, despite clear indications that cerebral vasodilatation occurred. Four factors may explain these observations.

First, the reduction in BP was smaller than in the acute hypotension experiments, where BP was reduced below the normal autoregulation range.[19] The reduction in BP is also not likely to approximate the reduction in perfusion pressure caused by a severe carotid artery stenosis.

Second, the magnitude of vasodilatation induced by CO_2 exceeds the magnitude of vasodilatation induced by pressure autoregulation. For example, for a 20% reduction in perfusion pressure, CBF can be maintained constant by an increase in cerebral blood vessel diameter of ≈6%. However, maximum hypercapnia, associated with a 100% increase in flow, brings about a 20% increase in diameter, assuming that BP is unchanged.

Third, CVMR is largely determined by the properties of smaller cerebral arteries, arterioles and capillaries[30], whereas hypertension has major effects on large cerebral arteries.[31] Under normal conditions, large arteries (diameter >200 mm) account for ≈40% of total cerebral vascular resistance, but their relative contribution increases in chronic hypertension.[31] In hypertensive rats with a mean aortic BP ≈160 mmHg (controls: 98 mmHg), the nearly doubled large artery resistance resulted in a pial arteriolar pressure of only ≈80 mmHg (control 60 mmHg).[32] Thus, microvessels may retain normal vascular reactivity to CO_2 as they are protected by the resistance changes in larger arteries[31]; alternatively, the adaptations that take place in larger arteries as a result of either hypertension or BP lowering are not reflected in CVMR.

Fourth, losartan, an AT1 receptor antagonist that blocks the deleterious effects of angiotensin II, may have beneficial cerebrovascular effects that are independent of its BP lowering effects.[5] Specifically, effects of losartan on cerebral remodelling and endothelial function may have augmented the vasomotor response to CO_2, thus compensating for the effects of blood pressure reduction on CVMR. Indeed, Oku et al. similarly found no change in CVMR after follow-up of 8-22 weeks with losartan monotherapy.[12] However, we caution that there are several arguments against this

contention. First, Lipsitz et al. found a preserved CVMR following BP reduction with a wide range of antihypertensive drugs, which strongly argues against a specific drug class effect.[33] Second, we observed these effects already after 1-2 weeks of treatment, which would mean that effects of angiotensin II inhibition on remodelling and endothelial function should have taken place within this short time frame.

Careful analysis of the studies that have reported an improvement of CVMR with long-term antihypertensive treatment, demonstrates that this effect was observed only in patients with known cerebrovascular disease and with diabetes.[20]

BP response to CO_2

Hypercapnia, through central chemoreceptors, causes sympathetic activation which in turn results in elevated BP due to the combined effects of an increase in cardiac output and peripheral vascular resistance.[7,34,35] Hypertension is associated with increased sympathetic activity.[36,37] Thus far, the issue whether this increased baseline sympathetic activity leads to an enhanced effect of hypercapnia on BP in hypertensive patients had not been addressed specifically. There was no difference in BP and HR response between untreated patients with hypertension and controls. The few available studies on CVMR in hypertension that have measured the BP response to CO_2 support these findings.[12,23,24,33] One explanation is that despite the difference in baseline activity, sympathetic responses are similar in hypertension and controls, as has been observed for example in tilt-table testing.[36] A second explanation is that the magnitude of CO_2 induced sympathetic activation obscures the much smaller difference in baseline activity.[35-37]

Antihypertensive treatment did not alter the BP and HR responses to CO_2. Previous research regarding interactions between baroreflex activation by changes in BP and central chemoreflex activation by changes in CO_2 are inconsistent.[34,35] Our findings suggest that baroreflex resetting after BP lowering does not alter the BP response to hypercapnia.

The observed hypotensive response to hyperventilation, with an associated increase in HR, has been described previously.[7] This response, too, was not influenced by hypertension or antihypertensive treatment.

Limitations of this study

The limitations imposed by the measurement of CBF using TCD, as well as the limitations specific to the method used to estimate CVMR have been discussed previously.[7,38-40] The flow-velocity in the MCA measured by TCD is determined by CBF and by the diameter of the MCA. Therefore, changes in CBFV reflect changes in CBF only if the MCA diameter is constant. During hypotension, as well as during hypocapnia and hypercapnia, MCA diameter was unchanged or showed less than 4% change.[39,40] These studies were performed in healthy subjects and neurosurgical patients. Studies in hypertensive patients are lacking. Measurements of isolated MCA from spontaneously hypertensive rats indicate a 10 % change in diameter for a large range of changes in mean BP from 40 to 140 mmHg.[41] This suggests that in our study, small changes in MCA diameter cannot be excluded. In theory, this would have the following effects on our data. Under conditions of equivalent CBF, the relatively narrower MCA lumen in hypertensive patients[41] would increase CBFV and lead to overestimation of CBF, whereas a lumen increase in MCA following normalization of BP would lower CBFV and underestimate CBF. This then would have led to a reduction in measured CBFV following treatment. In summary, our finding of unaltered CBFV represent unaltered CBF is MCA diameter was constant, or increased CBF should MCA diameter have been affected by BP changes.

Similarly, should hypocapnia cause MCA vasoconstriction, this would augment CBFV and underestimate the hypocapnic reduction in CBF, and vice versa for a vasodilatatory effect of hypercapnia.

Nevertheless, our results are concordant with results obtained with other modalities (such as MRI and PET) to measure CBF during hypo- and hypercapnia [12,20,24], which suggests that possible confounding effects of changes in MCA diameter in our study had minimal effect on outcome or interpretation.

This is a study in a small number of subjects. We previously found good reproducibility of our method, in line with other methods to estimate CVMR.[7] It has been shown that a clinically significant reduction in CVMR can be identified even with this small number of subjects.[28] Indeed, this study was adequately powered to detect small differences in CVMR.

Finally, because we only investigated losartan-hydrochlorothiazide, we can not tell whether our observations are specific for this drug class, or whether they will also apply to other antihypertensive drugs, as is suggested by the study of Lipsitz et al.[33]

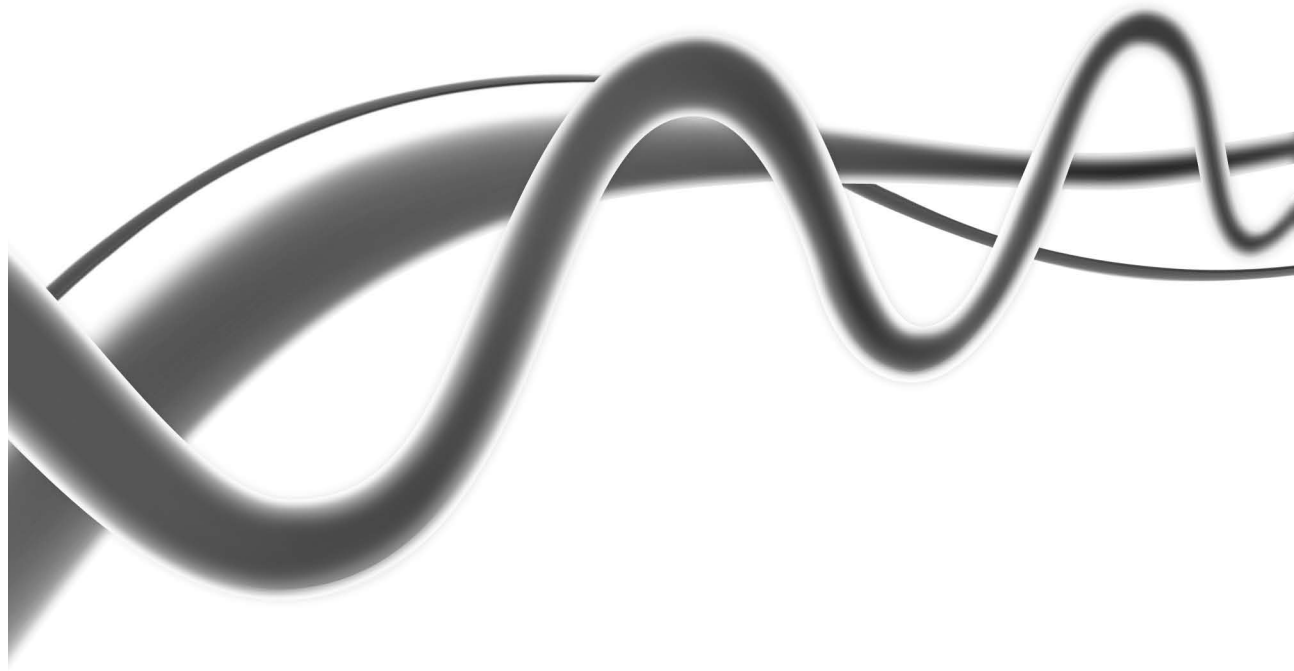
In summary, we have studied patients with mild to moderate hypertension of unknown duration, in the absence of diabetes or cerebrovascular disease, and have shown that CVMR is not affected. As discussed above, we conclude that previous reports of impaired CVMR with hypertension were based on inclusion of hypertensive subjects with co-morbid severe cerebrovascular disease and/or diabetes.

We have also shown that despite significant hemodynamic alterations induced by the initiation of antihypertensive treatment, CVMR remained unaffected. These findings add to our previous observation that cerebral autoregulation was not altered after short-term and long-term antihypertensive treatment.[6] Together these observations suggest that the cerebrovascular adaptations to a reduction in perfusion pressure are not associated with exhaustion of cerebrovascular reserve, with the caveat that these were patients with mild to moderate hypertension.

References

1. Mancia G, De Backer G, Dominiczak A, et al. 2007 ESH-ESC Practice Guidelines for the Management of Arterial Hypertension: ESH-ESC Task Force on the Management of Arterial Hypertension. *J Hypertens 2007;25:1105-1187.*
2. Staessen JA, Richart T, Birkenhager WH. Less atherosclerosis and lower blood pressure for a meaningful life perspective with more brain. Hypertension 2007;49:389-400.
3. Baumbach GL, Heistad DD. Remodeling of cerebral arterioles in chronic hypertension. *Hypertension 1989;13:968-972.*
4. Girouard H, Park L, Anrather J, Zhou P, Iadecola C. Angiotensin II attenuates endothelium-dependent responses in the cerebral microcirculation through nox-2-derived radicals. *Arterioscler Thromb Vasc Biol 2006;26:826-832.*
5. Dahlöf B, Devereux RB, Kjeldsen SE, et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet 2002;359:995-1003.*
6. Zhang R, Witkowski S, FuQ, Claassen JA, Levine BD. Cerebral hemodynamics after short and long-term reduction in blood pressure in mild and moderate hypertension. *Hypertension 2007;49:1149-1155.*
7. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol 2007;102:870-977.*
8. Lavi S, Gaitini D, Milloul V, Jacob G. Impaired cerebral CO2 vasoreactivity: association with endothelial dysfunction. *Am J Physiol Heart Circ Physiol 2006;291:H1856-H1861.*
9. Zhang R, Wilson TE, Witkowski S, Cui J, Crandall GG, Levine BD. Inhibition of nitric oxide synthase does not alter dynamic cerebral autoregulation in humans. *Am.J.Physiol Heart Circ.Physiol 2004;286: H863-H869.*
10. Takada J, Ibayashi S, Ooboshi H, et al. Valsartan improves the lower limit of cerebral autoregulation in rats. *Hypertens Res 2006;29:621-626.*
11. Mitsis GD, Zhang R, Levine BD, Marmarelis VZ. Cerebral hemodynamics during orthostatic stress assessed by nonlinear modeling. *J Appl Physiol 2006;101:354-366.*
12. Oku N, Kitagawa K, Imaizumi M, et al. Hemodynamic influences of losartan on the brain in hypertensive patients. *Hypertens Res 2005;28:43-49.*
13. Edwards MR, Topor ZL, Hughson RL. A new two-breath technique for extracting the cerebrovascular response to arterial carbon dioxide. *Am J Physiol Regul.Integr.Comp Physiol 2003;284:R853-R859.*
14. Kety S, Schmidt C. The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young man. *J Clin Invest 1948;27:484-492.*
15. Heagerty AM, Izzard AS. Small-artery changes in hypertension. *J.Hypertens 1995;13:1560-1565.*
16. Heistad DD, Mayhan WG, Coyle P, Baumbach GL. Impaired dilatation of cerebral arterioles in chronic hypertension. *Blood Vessels 1990;27:258-262.*
17. Kety S, Hafkenschiel J, Jeffers W, Leopold IH, Shenkin H. The blood flow, vascular resistance, and oxygen consumption of the brain in essential hypertension. *J.Clin.Invest 1948;27:511-514.*
18. Strandgaard S. Autoregulation of cerebral blood flow in hypertensive patients. The modifying influence of prolonged antihypertensive treatment on the tolerance to acute, drug-induced hypotension. *Circulation 1976;53:720-727.*
19. Schmidt JF, Waldemar G, Paulson OB. Arterial CO2 tension and cerebral vascular reactivity during the induction of acute hypertension and hypotension in the awake human. *J.Neurosurg Anesthesiol 1990;2:92-96.*
20. Kario K, Ishikawa J, Hoshide S, et al. Diabetic brain damage in hypertension: role of renin-angiotensin system. *Hypertension 2005;45:887-893.*
21. Maeda H, Matsumoto M, Handa N, et al. Reactivity of cerebral blood flow to carbon dioxide in hypertensive patients: evaluation by the transcranial Doppler method. *J Hypertens 1994;12:191-197.*
22. Troisi E, Attanasio A, Matteis M, et al. Cerebral hemodynamics in young hypertensive subjects and effects of atenolol treatment. *J.Neurol Sci 1998;159:115-119.*
23. Serrador JM, Sorond FA, Vyas M, Gagnon M, Iloputaife ID, Lipsitz LA. Cerebral pressure-flow relations in hypertensive elderly humans: transfer gain in different frequency domains. *J Appl Physiol 2005;98:151-159.*
24. Tominaga S, Strandgaard S, Uemura K, Ito K, Kutsuzawa T. Cerebrovascular CO2 reactivity in normotensive and hypertensive man. *Stroke 1976;7:507-510.*
25. Gregorio F, Ambrosi F, Carle F, et al. Microalbuminuria, brain vasomotor reactivity, carotid and kidney arterial flow in Type 2 diabetes mellitus. *Diabetes Nutr Metab 2004;17:323-330.*
26. Gur AY, Gucuyener D, Uzuner N, et al. Cerebral vasomotor reactivity of patients with acute ischemic stroke: Cortical versus subcortical infarcts: an Israeli-Turkish collaborative study. *J Neurol Sci 2007;257:121-125.*
27. Yamori Y, Horie R. Developmental course of hypertension and regional cerebral blood flow in stroke-prone spontaneously hypertensive rats. *Stroke 1977;8:456-461.*

28. Widder B, Kleiser B, Krapf H. Course of cerebrovascular reactivity in patients with carotid artery occlusions. *Stroke 1994;25:1963-1967.*
29. Artru AA, Colley PS. Cerebral blood flow responses to hypocapnia during hypotension. *Stroke 1984;15: 878-883.*
30. Hurn P, Traystman RJ. Changes in arterial gas tension. In: Edvinsson L, Krause D (eds). Cerebral blood flow and metabolism. *Philadelphia: Lippincott Williams & Wilkins, 2002: 384-394.*
31. Baumbach GL, Heistad DD. Cerebral circulation in chronic arterial hypertension. *Hypertension 1988;12:89-95.*
32. Werber AH, Heistad DD. Effects of chronic hypertension and sympathetic nerves on the cerebral microvasculature of stroke-prone spontaneously hypertensive rats. *Circ.Res 1984;55:286-294.*
33. Lipsitz LA, Gagnon M, Vyas M, et al. Antihypertensive therapy increases cerebral blood flow and carotid distensibility in hypertensive elderly subjects. *Hypertension 2005;45:216-221.*
34. Shoemaker JK, Vovk A, Cunningham DA. Peripheral chemoreceptor contributions to sympathetic and cardiovascular responses during hypercapnia. *Can J Physiol Pharmacol 2002;80:1136-1144.*
35. Somers VK, Mark AL, Abboud FM. Interaction of baroreceptor and chemoreceptor reflex control of sympathetic nerve activity in normal humans. *J.Clin.Invest 1991;87:1953-1957.*
36. Fu Q, Zhang R, Witkowski S, et al. Persistent sympathetic activation during chronic antihypertensive therapy: a potential mechanism for long term morbidity? *Hypertension 2005;45:513-521.*
37. Somers VK, Anderson EA, Mark AL. Sympathetic neural mechanisms in human hypertension. *Curr Opin Nephrol Hypertens 1993;2:96-105.*
38. Bishop CC, Powell S, Rutt D, Browse NL. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke 1986;17:913-915.*
39. Giller CA, Bowman G, Dyer H, Mootz L, Krippner W. Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery 1993;32:737-741.*
40. Serrador JM, Picot PA, Rutt BK, Shoemaker JK, Bondar RL. MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke 2000;31:1672-1678.*
41. Izzard AS, Horton S, Heerkens EH, Shaw L, Heagerty AM. Middle cerebral artery structure and distensibility during developing and established phases of hypertension in the spontaneously hypertensive rat. *J.Hypertens 2006;24:875-880.*



Chapter 8

Cerebral Hemodynamics in Early Alzheimer Disease

Jurgen A.H.R. Claassen
Ramon Diaz-Arrastia
Kristin Martin-Cook
Benjamin D. Levine
Rong Zhang

Abstract

Background and Purpose - Cerebrovascular disease may contribute to the development and progression of Alzheimer's disease (AD). This study determined whether impairments in cerebral hemodynamics can be observed in early-stage AD.

Methods - 9 patients with mild AD and 8 cognitively normal controls matched for age underwent brain MRI and neuropsychological evaluation, followed by assessment of steady-state cerebral blood flow velocity (CBFV, transcranial Doppler), blood pressure (BP, Finapres) and cerebrovascular resistance index (BP/CBFV). To detect changes in cerebral hemodynamics and to quantify the dynamic pressure-flow relation of the cerebral circulation, spectral and transfer function analysis of beat-to-beat variability in BP and CBFV under resting conditions and during repeated squat-stand maneuvers were measured.

Results - Alzheimer patients had lower CBFV and higher cerebrovascular resistance index, even when normalized for total brain volume. Low-frequency variability of BP was enhanced in AD patients, suggesting impaired arterial baroreflex function. CBFV variability was reduced despite enhanced BP variability in AD. Consistent with this finding, transfer function gain at frequencies of 0.1 and 0.05 Hz during squat-stand maneuvers was significantly reduced by 40% in patients with AD ($p < 0.01$), indicating enhanced cerebral vasoconstriction in damping of CBFV to BP fluctuations.

Conclusions - Patients had a distinct pattern of altered cerebral hemodynamics, including reduced CBFV and increased cerebrovascular resistance. Induced BP fluctuations were exaggerated, suggesting baroreflex impairment. Cerebral blood flow was severely compromised during dynamic standing, demonstrating impaired vasomotor reserve in patients with early AD.

Introduction

Vascular pathology is likely to play an important role in the development and progression of Alzheimer's disease (AD).[1-5] Epidemiological studies have shown that vascular risk factors increase the risk of developing AD.[6] In addition, brain autopsy studies have observed that both large and small-vessel disease are extensively present in AD.[1,3] Furthermore, in animal models of AD, cerebrovascular disease is found not only in the form of anatomical (e.g. luminal obstruction and microvascular deformation),[7] but also as functional (endothelium-related)[8] alterations, linked to early vascular deposition of amyloid-beta peptide, which may precede overt AD neuropathology.[7,8] Additionally, independent of the presence of amyloid-beta, brain vascular smooth muscle cells in AD patients display hypercontractile properties, and brain endothelial cells have reduced expression of genes regulating vascular differentiation, leading to enhanced cerebral vasoconstriction and possibly reduced capillary density.[9,10]

These vascular changes may contribute to the neuronal degeneration in AD[5] and are even considered responsible for the initiation and progression of AD neuropathology.[1] Indeed, in vivo measurements of CBF in patients with AD, using imaging modalities such as MRI and PET, have consistently identified reduced global and regional CBF associated with AD severity.[5] A point of discussion remains, however, that the reduced CBF may equally be secondary to the loss of neuronal function (reduced metabolic demand) and/or brain atrophy. Therefore, further studies of cerebral hemodynamics in patients presenting with early AD are warranted to elucidate the role of vascular disease in AD.

Transcranial Doppler ultrasonography (TCD) allows measurements of cerebral blood flow velocity (CBFV) in the major cerebral arteries with high temporal resolution. When registered together with blood pressure (BP), synchronized data of beat-to-beat variability in BP and CBFV are obtained, providing important information on the regulation of the cerebral circulation.[11,12] Transfer function analysis, a method based on spectral analysis of these beat-to-beat data, can then be used to quantify the dynamic pressure-flow relationship of the cerebral circulation.[11,12] We hypothesized that vascular abnormalities in AD at an early stage alter cerebral hemodynamics, which can be measured via beat-to-beat changes in

BP and CBFV using TCD. We proposed that changes in cerebral hemodynamics identified using this method potentially can be used for selection of patients at risk for vascular pathology in AD and as a biomarker for clinical trials of therapies aimed at reducing vascular risk in AD.

Materials and Methods

Subjects

We included 9 patients with a diagnosis of probable AD according to NINCDS-ADRDA criteria, with a clinical dementia rating scale of 0.5 (n=3) or 1.0 (n=6), and 8 controls, matched for age and level of education, recruited from the Alzheimer Disease Center at UT Southwestern Medical Center, Dallas. Participants were selected from 11 patients and 12 controls who were screened. Subjects with acute or chronic knee or hip conditions which would prevent them from performing squat-stand maneuvers (2 controls) and subjects without an adequate acoustic window, preventing high quality TCD signal detection (1 control, 1 AD) were excluded during the initial screening process. Subsequently, 2 subjects (1 control, 1 AD) were excluded because of poor finger BP and TCD signal quality during measurements. The study was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. All participants signed an informed consent form. In AD patients, additional informed consent was obtained by proxy. AD patients and their controls underwent a comprehensive clinical-neuropsychological evaluation and brain imaging using MRI. Characteristics of the study participants are listed in Table 1.

Hemodynamic Measurements

BP was measured in the finger by photoplethysmography (Finapres, Ohmeda). Finger arterial pressure waves measured with this technique were validated against central aortic root measurements and can be used reliably to quantify cerebral hemodynamics.[13] CBFV was obtained in the middle cerebral artery (MCA) on one side by transcranial Doppler ultrasonography (DWL Elektronische Systeme, Germany).[11] End-tidal CO_2 was monitored with a nasal cannula using capnography (Criticare Systems). In addition, peripheral arterial saturation (pulse-oximetry) and 3-lead ECG were recorded.

After at least 10 min rest in sitting position, 5-min segments of BP and CBFV data were recorded during spontaneous respiration. These data were used for spectral analysis of spontaneous oscillations in BP and CBFV.

To enhance the magnitude of alterations in BP and CBF – and to mimic such changes in daily life associated with changes in body posture- oscillations in BP and CBFV were induced by repeated squat-stand maneuvers. After careful instruction and practice, participants were coached into performing these maneuvers at a frequency of 0.025 Hz (20 s squat followed by 20 s standing up) for 5 min, 0.05 Hz (10 s squat, 10 s stand) for 4 min and at 0.1 Hz (5 s squat, 5 s stand) for 3 min, separated by 10 min of recovery. Physical assistance during standing up from squat was provided to aid the performance of these maneuvers.

Transfer Function Analysis of the Dynamic Pressure-Flow Relationship of the Cerebral Circulation

The method of transfer function analysis, which provides the parameters of gain, phase and coherence, has been described in detail previously.[11]

Transfer gain quantifies how the amplitudes of the changes (oscillations) in BP at different frequencies are transmitted to CBFV; a lower gain implies that these oscillations are reduced (damped) either by dynamic cerebral autoregulation or increases in the cerebrovascular impedance.[14] In this study, transfer function gain is expressed not only as changes in CBFV per mmHg changes in BP, but also as the percentage change in CBFV per mmHg change in BP to reduce the potential effects of individual differences in MCA diameter on the gain estimate. Transfer function phase reflects the time relationship between oscillations in BP and CBFV. Coherence can be compared to a linear correlation coefficient, and quantifies to what extent changes in CBFV are linearly associated with changes in BP.

MRI Measurements

T2-weighted and Fluid-Attenuated Inversion Recovery (FLAIR) images were obtained using a Philips Intera 1.5T scanner. Images were converted from DICOM to ANALYZE format to facilitate analysis. Segmentation of skull and connective tissue was conducted using the skull strip function in the DICOM image editor MRIcro. Leukoaraiosis volume (LV) and whole brain volume (WBV) were measured

Table 1 Overview of the various dynamic quantifications of cerebral autoregulation in the time domain.

	Alzheimer's Disease (N= 9)	Aging Controls (N= 8)	P Value
Age-yrs	67.9 (5.5)	64.5 (4.0)	0.17
Gender -male:female	3:6	4:4	
MMSE	25 (3.2)	29 (0.5)	0.03
Word list recall- no. of words	2 (1.9)	8 (1.3)	0.0001
CDR- sum of boxes	4 (1.2)	0.1 (0.2)	0.0001
Total brain volume-ml	1081 (65)	1108 (79)	0.53
White matter lesions-ml	14.3 (10.4)	7.5 (5.8)	0.25
Vascular risk factors	(no. of subjects)		
Hypertension	4	3	
Hypercholesterolemia	6	1	
Diabetes	0	0	
Smoking	0	0	
Medication			
Antihypertensive drugs	4	3	
lipid-lowering drugs	4	2	
cholinesterase-inhibitor	8	-	
NMDA-receptor antagonist	8	-	

MMSE: Mini mental state examination. CDR: Clinical dementia rating scale

using an image processing tool developed with MATLAB (The MathWorks, Inc., Natick, MA, USA).[15]

24 hr Ambulatory Blood Pressure Monitoring

In 5 aging controls and 6 AD patients, 24 hr ambulatory BP recordings (Accutracker II, Sun Tech) were measured. Mean values for systolic and diastolic BP and heart rate were collected during awake hours and during sleep.

Standing Up Following a Singe Squat

In these same 5 aging controls and 6 AD patients, we measured the hemodynamic effects of a single squat (60 s) followed by standing up to investigate the effect of a single hypotensive challenge to cerebral perfusion.

Cerebral Vasomotor Reactivity (CVMR)

Using a previously described method, hypocapnic vasoconstriction and hypercapnic vasodilatation –induced by hyperventilation and rebreathing respectively- were studied in 6 AD patients and 5 aging controls.[16] During hyperventilation and rebreathing, breath-to-breath changes in mean CBFV and BP were recorded and cerebrovascular conductance index (CVCi) was calculated (CBFV/BP). Total cerebral vasomotor reactivity (CVMR) is expressed as the range of percentage changes in CVCi during hyperventilation plus those during rebreathing. In addition, maximum CVMR index was obtained as the linear regression slope of percentage changes in CVCi in response to end-tidal CO2 between 40 - 50 mm Hg.[16]

Statistical Analysis

Comparisons were made between AD and healthy aging. For normally distributed data, two-tailed Student t-test was used. Not all data resulting from transfer function analysis were normally distributed. Here, Student t-test was repeated after log-transformation of the data, and in addition non-parametric testing was used (Wilcoxon rank test). Because of the small number of subjects, in addition to accepting a statistical significance at $p < 0.05$, $p < 0.1$ was interpreted to represent a relevant trend that warrants further exploration. Data are presented as mean and standard deviation (SD).

Results

Patient characteristics

Alzheimer patients had relatively mild cognitive deficits consistent with early stage of the disease (Table 1), but were clearly impaired in episodic memory (only a summary of neuropsychological test results is presented here for brevity). Volumetric analysis of brain MRI's revealed a trend towards higher white matter lesion volumes in AD. Global brain volume was not significantly different as can be expected due to large inter-individual variance in brain volume and the small sample size in this study.

Baseline hemodynamics are presented in Table 2. Ambulatory BP recordings revealed mean (SD) awake blood pressure of 136 (15)/75 (7) mmHg in AD and 124 (13)/71 (8) in aged controls ($p=0.19$ for systolic BP). Participants diagnosed with hypertension were well controlled (mean awake BP < 140/85 mmHg). Two AD patients and one control, who had not previously been diagnosed with hypertension, met criteria for systolic hypertension (mean awake systolic BP of 148, 156 and 146 mmHg respectively).

Table 2 Baseline Hemodynamic Parameters.

	Alzheimer's Disease (N= 9)	Aging Controls (N= 8)	P Value
Systolic blood pressure-mmHg	140.1 (19.8)	125.6 (26.5)	0.23
Diastolic blood pressure-mmHg	73.7 (9.0)	73.1 (9.8)	0.91
Mean arterial pressure-mmHg	101.0 (12.4)	94.7 (15.8)	0.39
Mean arterial pressure-mmHg (from Finapres)	98.4 (12.4)	94.4 (10.9)	0.39
Cerebral blood flow-velocity-cm/s	38.4 (7.1)	54.5 (19.0)	0.05
Cerebrovascular resistance-mmHg/cm/s	2.7 (0.7)	1.9 (0.6)	0.03
End-tidal CO2-mmHg	37.3 (3.0)	37.3 (4.2)	0.96
Total CVMR -%	58 (19)	76 (28)	0.26
Maximum CVMR index-%/mmHg	5.9 (2.6)	5.2(2.0)	0.66

CVMR: cerebral vasomotor reactivity

Despite the early stage of disease, AD patients had a substantially lower CBFV and higher cerebrovascular resistance compared with the controls (Table 1). Normalized for global brain volume, CBFV was 3.6 (0.7) cm/s per 100 ml brain volume in AD versus 5.7 (2.4) cm/s /100 ml in controls ($p = 0.03$).

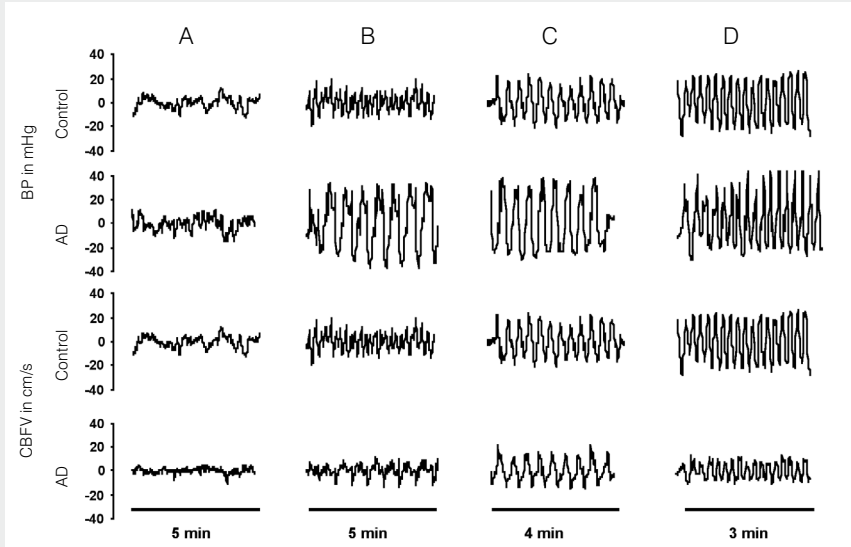
Cerebral hemodynamics

Figure 1 shows an example of spontaneous oscillations in BP and CBFV, as well as the oscillations that were induced by repeated squat-stand maneuvers, in one patient with AD and one control subject. Note that oscillations are larger in AD for BP, but smaller for CBFV, compared to the healthy control.

Figure 2 displays the results of spectral analysis of oscillations in BP and CBFV. Note the larger spectral power in AD for BP oscillations, but not for CBFV.
Indeed, spectral analysis of spontaneous oscillations in BP revealed increased spectral power in the very low frequency range (VLF) in AD (Table 3).

BP oscillations induced by the repeated squat-stand maneuvers were consistently higher in AD in the VLF range (0.025 and 0.05 Hz), and in the LF range (0.1 Hz) (Table 3). Individual differences in the magnitude of BP oscillations could not be

Figure 1 Spontaneous and Induced Oscillations in Blood Pressure and Cerebral Blood Flow Velocity.

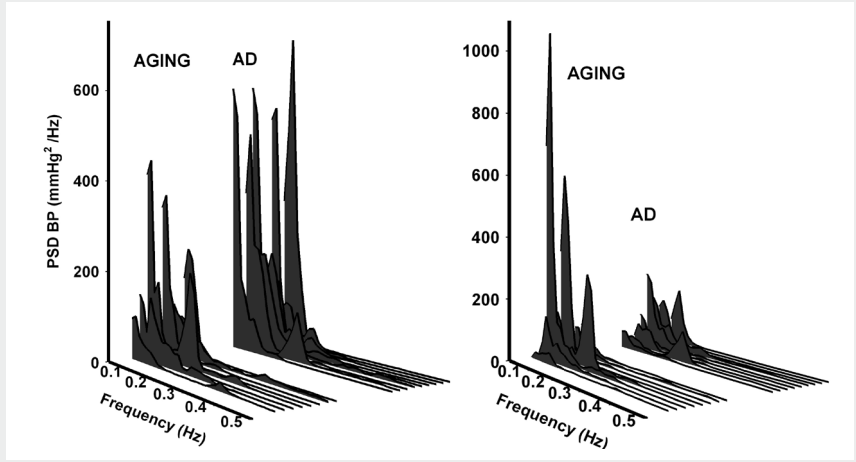


Example from two representative subjects, one with AD and one normal control, demonstrating spontaneous oscillations in blood pressure and cerebral blood flow-velocity over a period of 5 min (panel A). Panel B through D depict the effect of repeated squat-stand maneuvers at different intervals on these oscillations. Recording periods are 5, 4 and 3 min respectively. Panel B shows how repeated squat-stand intervals at 20 s lead to oscillations at 0.025 Hz. Panel C: 10 s intervals with oscillations at 0.05 Hz. Panel D: 5 s intervals with oscillations at 0.1 Hz.

explained by a higher or lower baseline BP, nor by a history of hypertension. The enhanced oscillations in BP suggest that baroreflex function is impaired in patients with AD. Estimates of transfer function gain, phase and coherence are depicted in figure 3. Transfer function gain for induced oscillations at the frequencies of 0.05 and 0.1 Hz was reduced by ~30 % in AD relative to normal controls ($p=0.01$). However, for spontaneous oscillations this difference was not significant ($p=0.18$).

The hemodynamic effects of standing up after a single squat (60 s) are depicted in Figure 4. Whereas BP returned to baseline in controls after the initial rise at the onset of squatting, BP was not stable in AD. The reduction in BP upon standing up after squat was slightly larger (39 mm Hg (SD 16)) in AD than in controls (26 mm Hg (SD 11)), or, expressed as relative change, a reduction of 33 vs. 26 % ($p=0.2$). It is

Figure 2 Spectral Density Plots of Oscillations in Blood Pressure and Cerebral Blood Flow Velocity.



Individual power spectral density plots for aged controls and Alzheimer patients, for blood pressure (left pane) and cerebral blood flow-velocity (right pane). The Y-axis shows the magnitude of the observed oscillations, and the X –axis displays the frequency of these oscillations. Note the much stronger oscillations in blood pressure in the very low frequency range (0.02 – 0.07 Hz) in Alzheimer patients, with reduced oscillations in cerebral blood flow-velocity.

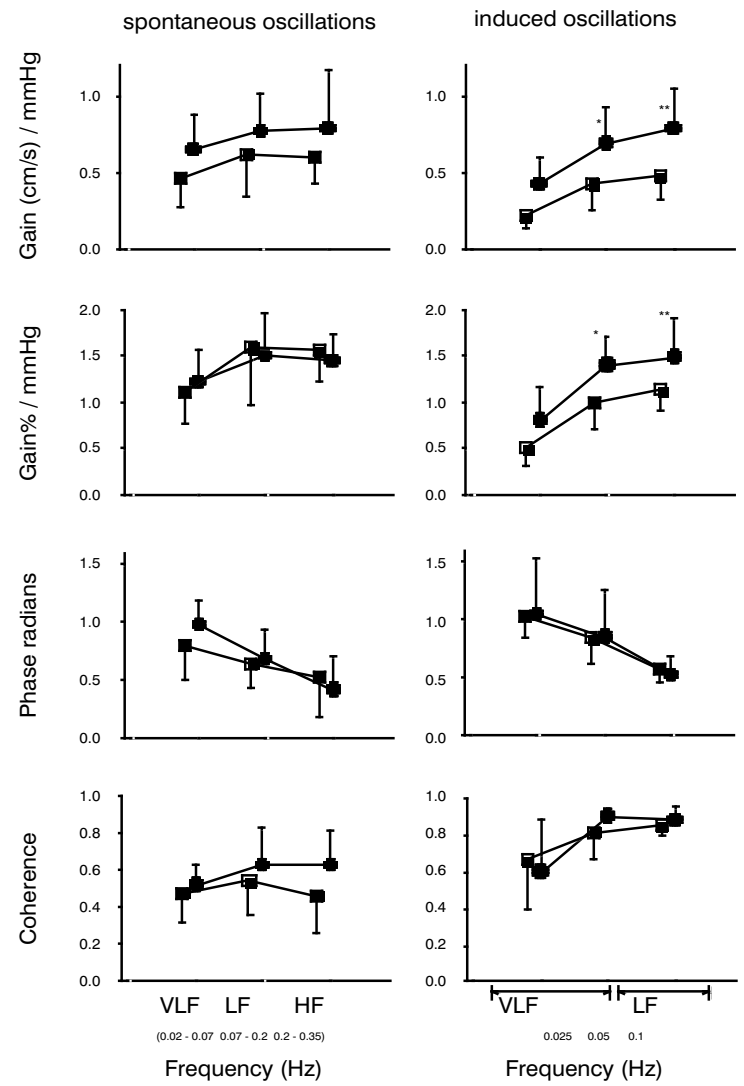
clear in Figure 4 that baseline CBFV was substantially lower in AD (see also Table 2). The orthostatic BP reduction following squat led to a reduction in CBFV of 12 (SD 4) cm/s in AD vs. 21 (SD 26) cm/s in controls, or 26 % and 29 % (p=0.77). AD patients reached very low diastolic flow-velocity values during standing (12 (SD 7) vs. 24 (SD 11) cm/s in controls, p=0.1).

Finally, total cerebral vasomotor reactivity (CVMR) appeared to be lower in AD (Table 2), but this difference was not significant, because of the large individual variability in this small number of patients. Moreover, maximum reactivity index was similar in AD and controls.

MRI and hemodynamics

We performed exploratory analysis of possible relationships between white matter lesion volume, brain volume and hemodynamic parameters. Although the interpretation is limited due to the small sample size, a larger volume of white matter

Figure 3 Transfer Function Analysis of Spontaneous and Induced Oscillations in Blood Pressure and Cerebral Blood Flow Velocity.



Group-averaged data showing results of transfer function analysis of spontaneous oscillations in blood pressure (BP) and cerebral blood flow-velocity (CBFV) as well as of oscillations induced by repeated squat-stand maneuvers. ■: Alzheimer patients. ●: normal controls. Transfer function analysis describes how oscillations in BP are transformed into oscillations in CBFV. Gain is presented as both the absolute and percent changes in CBFV per mmHg change in BP. *: $p < 0.05$; ** $p < 0.01$

Table 3 Spectral Power of Spontaneous and Induced Oscillations in Blood Pressure and Cerebral Blood Flow Velocity.

Spontaneous oscillations	Alzheimer's Disease (N= 9)	Aging Controls (N= 8)	P Value
PS BP - $mmHg^2$			
VLF	9.6 (5.5)	5.6 (2.4)	0.08
LF	2.4 (1.4)	2.6 (3.1)	0.84
HF	0.5 (0.6)	0.6 (0.5)	0.69
PS CBFV – $(cm/s)^2$			
VLF	3.0 (1.7)	5.5 (5.1)	0.18
LF	1.5 (1.4)	2.8 (4.2)	0.43
HF	0.4 (0.5)	0.5 (0.4)	0.68
Induced oscillations at 0.025 Hz	N=4	N=4	
PS BP - $mmHg^2$	239 (150.6)	86.3 (67.3)	0.11
PS CBFV - $(cm/s)^2$	11.8 (7.3)	39.5 (42.9)	0.27
Induced oscillations at 0.05 Hz	N= 9	N=8	
PS BP - $mmHg^2$	460.9 (185.4)	306.8 (131.2)	0.06
PS CBFV - $(cm/s)^2$	84.3 (55.5)	149.6 (109.1)	0.16
Induced oscillations at 0.1 Hz	N=9	N=8	
PS BP - $mmHg^2$	315 (117.8)	195.3 (90.8)	0.03
PS CBFV - $(cm/s)^2$	77.8 (45.4)	126 (58.5)	0.08

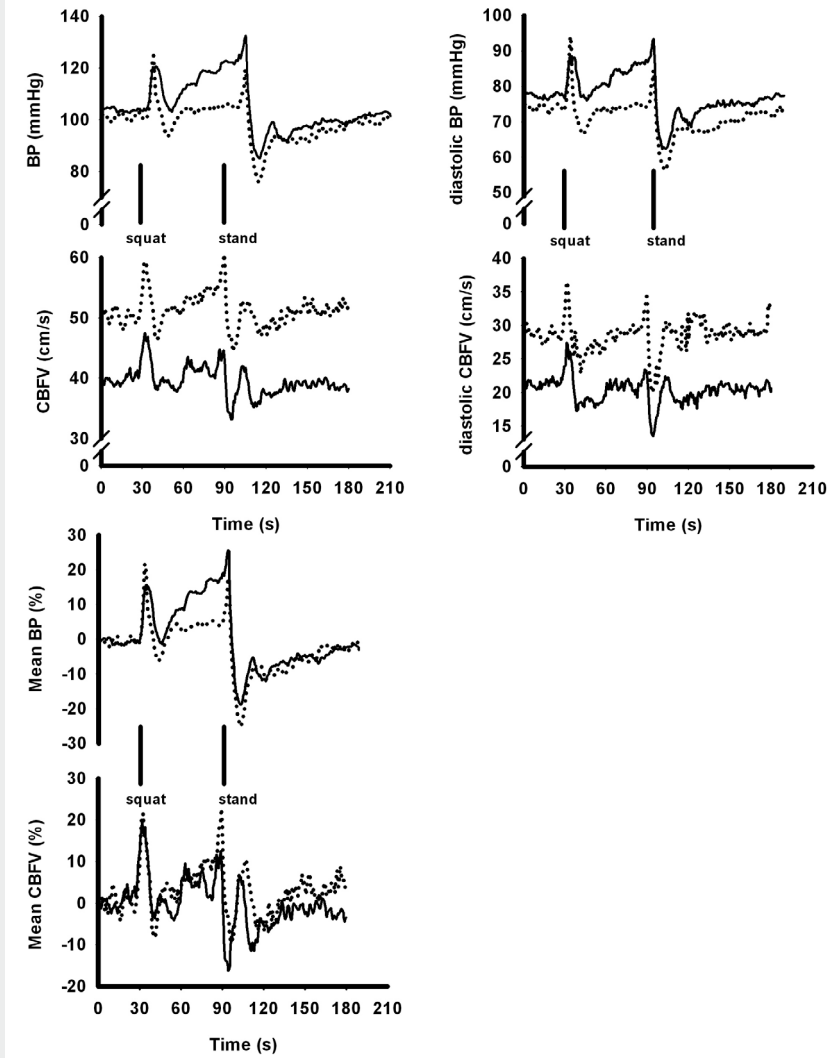
BP: blood pressure. CBFV: cerebral blood flow-velocity. PS: power spectrum, indicating the magnitude of the observed oscillations within the specified frequency range. VLF: very low frequency oscillations, 0.02–0.07 Hz. LF: low frequency, 0.07-0.2 Hz. HF: high frequency, 0.2-0.35 Hz. Spontaneous oscillations were recorded during five minutes of rest in sitting position. Induced oscillations were provoked by repeated squat-stand maneuvers at 20/20, 10/10 and 5/5 s intervals (0.025, 0.05 and 0.1 Hz respectively).

lesions in this study was not associated with higher blood pressure or lower CBFV at baseline, nor with larger blood pressure variability, altered cerebral autoregulation or reduced vasomotor reactivity.

Discussion

With non-invasive monitoring of blood pressure and cerebral blood flow-velocity using TCD, we observed a distinct pattern of changes in cerebral hemodynamics in patients with mild Alzheimer's disease. In summary, these alterations consisted of a reduced CBFV with elevated cerebrovascular resistance that was not explained by

Figure 4 Hemodynamic Effects of Standing after a Single Squat.



Group-averaged beat-to-beat BP and CBFV in AD (solid line) and controls (dotted line) showing the response to a single squat (60 s) followed by standing (90 s). Top left: mean BP and CBFV. Top right: diastolic BP and diastolic CBFV. Bottom left: percentage changes in mean BP and CBFV.

general brain atrophy; and reduced CBFV oscillations, despite increases in oscillations in BP, leading to a reduction in transfer function gain. Although the enhanced damping of flow oscillations in AD might seem beneficial, we found that actually, CBFV was profoundly reduced during an orthostatic challenge. This suggests that intact (or possibly enhanced) pressure autoregulation in AD fails to compensate for the combination of globally reduced cerebral blood flow and instability of blood pressure.

Reduced CBFV in AD

Even when corrected for brain atrophy, baseline CBFV was reduced by 40 % in AD compared with age-matched controls, coupled with a 40% increase in cerebrovascular resistance. Although we measured CBFV only in the MCA (providing blood flow to ~70% of the brain), our study is consistent with findings of a reduction in total CBF of ~20 % early in the disease process of AD,[1,5,17-19] assuming that there were no group differences in the MCA diameters between patients with AD and their normal controls. It has been argued that reduction in CBF in AD could be explained by the loss of brain tissue due to the neurodegenerative process, or by a reduction in brain metabolic demand that parallels the cognitive decline.[20] However, in this study, differences in individual whole brain volume between AD and controls could not explain the differences in CBFV, suggesting that changes in CBF may not be related exclusively to brain atrophy, consistent with previous perfusion studies using MRI.[19,21] In addition, a reduction in brain metabolic demand in the mild stage of AD (CDR 0.5 - 1) is not likely to explain a 40 % reduction in CBF under resting conditions.[17]

Enhanced BP Oscillations in AD

The finding of increased oscillations in BP in patients with AD was unexpected, and has not been reported previously. These oscillations were identified in the very low frequency range between 0.02 and 0.07 Hz. BP oscillations in this frequency range are characterized by periods of 14 -50 s, which translate into intervals of spontaneously elevated BP that last ~7 –25 s, alternated with intervals of reduced BP of that same approximate length. The high BP spectral power in AD patients signifies the magnitude of these changes in BP. The repeated squat-stand maneuvers induced oscillations in BP that were much stronger in AD patients. Instability in BP in AD was also observed in the single squat-stand maneuver

(Figure 4). In this study, we cannot determine the cause of these enhanced oscillations. Most likely, these data indicate that in early AD, alterations occur in BP regulation which may be related to the central impairment of baroreflex function.[22] The right insular region has an important role in the regulation of autonomic function.[23,24] As this region is affected by Alzheimer neuropathology early in the course of disease, it has been suggested that AD may be associated with autonomic dysfunction in baroreflex control of arterial pressure.[23] In a similar vein, pathology of the insula and amygdala has been implicated in central autonomic disturbances including sudden cardiac death.[24]

Reduced CBFV Variability and Dynamic Pressure-Flow Relationship

Our study expands the earlier observations of reduced CBF by showing that oscillations in CBFV are reduced in AD compared to age-matched controls. This finding is striking considering that, because of the stronger oscillations in BP, oscillations in CBFV were expected to be stronger, not weaker, in AD. As we will discuss below, these observations could point towards an enhanced cerebral vasoconstriction or other vascular abnormalities in AD, such as arteriolar luminal obstruction, microvascular deformation or alterations in endothelial function associated with amyloid angiopathy.[7,9,10,25]

It was demonstrated recently that vascular smooth muscle cells in small intracerebral and pial arteries of AD patients displayed hypercontractililty and enhanced vasoconstriction, and that these changes were associated with overexpression of serum response factor (a transcription factor that regulates neurovascular gene expression) and myocardin-regulated contractile proteins, independent of the vascular effects of amyloid-beta.[10] Moreover, endothelial cells of cerebral blood vessels in AD were shown to have reduced expression of the MEOX2 homeobox gene, associated with a reduction in brain capillary density and reduced CBF.[9] Finally, vascular corrosion cast visualization of the brain vasculature in AD mice identified “holes” – areas of vessel elimination – with microvascular distortion, and deposits of amyloid-beta to the microvessels.[7]

Conceivably, these vascular abnormalities may lead to significant increases in cerebrovascular resistance and/or vascular impedance to attenuate CBFV variability in response to oscillations in BP (of note, whereas vascular resistance describes the

relationship between steady-state pressure and flow, impedance describes the total oppositions to oscillatory blood flow entering into a vascular bed and is determined by both vascular resistance and stiffness).[26]

This interpretation may be supported by observations in studies using functional magnetic resonance imaging (fMRI). FMRI measures neuronal activity indirectly through the blood oxygen level dependent (BOLD) hemodynamic response function, and it is interesting to note that the amplitude of this hemodynamic response is reduced in early AD during brain activation.[27] Although this is usually taken to represent altered neural activity and/or neurovascular coupling, in the light of the present study it may well be driven primarily by increases in vascular resistance or impedance in AD.

This is the first study to assess the dynamic pressure–flow relation of the cerebral circulation in patients with AD, and the observation of reduced transfer function gain needs to be further elaborated. Quantification of the dynamic pressure-flow relation of the cerebral circulation based on the transfer function analysis of beat-to-beat changes in BP and CBFV has been employed to assess dynamic cerebral autoregulation.[11,28] It has been suggested that autoregulation effectively reduces the impact on CBF of slow (low frequency) changes in BP, but becomes progressively ineffective for more rapid changes in BP (high frequency), consistent with a "high-pass filter". Consequently, reduction in transfer function gain (and increase in phase) at lower frequencies of BP oscillations is interpreted as normal autoregulation.[12] According to this model, dynamic autoregulation was not impaired in AD, as this would have resulted in an increased rather than decreased transfer function gain, as well as reductions in phase lead. This is in contrast with the findings in an AD animal model where cerebral autoregulation was impaired even before vascular deposition of amyloid-beta had occurred.[8] However, direct comparisons between studies in humans and animals may be difficult. In addition, only static, not dynamic autoregulation was assessed in animal studies.[8]

Finally, the lower gain in AD vs. controls might also be interpreted to suggest that cerebral vasoconstrictive and/or vasodilatory responses to changes in BP were enhanced, thus leading to reductions in CBFV variability (enhanced autoregulation). This interpretation, however, is not supported by the observations during orthostatic hypotension (Figure 4), where it was clear that cerebral autoregulation could not prevent a substantial and prolonged reduction in CBFV in AD. We therefore conclude that the reduced transfer function gain in AD reflects increased cerebrovascular resistance or impedance as discussed above, consistent with recent findings of reduced cerebrovascular compliance measured using perfusion MRI.[19]

Study Limitations

Certain limitations of this study need to be addressed. First, this is an explorative study in a small number of subjects. Second, all patients with AD were treated with the cholinesterase-inhibitor donepezil and the NMDA-receptor antagonist memantine, and it cannot be excluded that either or both of these drugs was responsible for the increased oscillations in BP and the decreased oscillations in CBFV, although there is no evidence to suggest such an effect. On the contrary, there is evidence to suggest that cholinesterase-inhibitors may augment CBF and improve neurovascular function.[29] If so, this could mean that either reduction in transfer gain and hence improved autoregulation reflects the drug effects or that the deleterious neurovascular effects of AD have been underestimated in this study. Third, some patients and controls were hypertensive or used antihypertensive drugs. Previous research has shown that neither mild to moderate hypertension per se, or the use of antihypertensive medication, affects dynamic cerebral autoregulation.[14] Furthermore, exclusion of these patients would seriously hamper the generalizability of this study. Finally, TCD measures flow-velocity - in cm/s - in a (large) segment of the cerebral circulation, and can not directly be compared with CBF in the classic sense - expressed in ml/min per 100 gram of brain tissue. We have addressed this issue in part by expressing CBFV in cm/s per 100 ml of brain volume. Flow velocity will be affected by any change in the diameter of the insonated vessel. Previous studies have demonstrated that it is unlikely that changes in the MCA diameter will occur to the extent that they would influence the estimation of cerebral autoregulation using TCD.[12]

Summary

Patients with early AD had a distinct pattern of altered cerebral hemodynamics including reduced cerebral blood flow and increased cerebral vascular resistance.

Induced blood pressure fluctuations such as might be encountered during daily life were markedly exaggerated, consistent with impaired baroreflex function. Although these BP fluctuations induced smaller changes in cerebral blood flow velocity in AD patients (consistent with either improved dynamic autoregulation or increased cerebrovascular impedance), cerebral blood flow was severely compromised during dynamic standing, demonstrating impaired vasomotor reserve in patients with AD.

References

1. de la Torre JC. Alzheimer disease as a vascular disorder: nosological evidence. *Stroke. 2002;33:1152-1162.*
2. Suter OC, Sunthorn T, Kraftsik R, Straubel J, Darekar P, Khalili K, Miklossy J. Cerebral hypoperfusion generates cortical watershed microinfarcts in Alzheimer disease. *Stroke. 2002;33:1986-1992.*
3. Kalaria RN. Vascular factors in Alzheimer's disease. *Int Psychogeriatr. 2003;15 Suppl 1:47-52.*
4. Aliev G, Smith MA, Obrenovich ME, de la Torre JC, Perry G. Role of vascular hypoperfusion-induced oxidative stress and mitochondria failure in the pathogenesis of Azheimer disease. *Neurotox Res. 2003;5:491-504.*
5. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nat Rev Neurosci. 2004;5:347-360.*
6. van Oijen M, de Jong FJ, Witteman JC, Hofman A, Koudstaal PJ, Breteler MM. Atherosclerosis and risk for dementia. *Ann Neurol. 2007;61:403-410.*
7. Meyer EP, Ulmann-Schuler A, Staufenbiel M, Krucker T. Altered morphology and 3D architecture of brain vasculature in a mouse model for Alzheimer's disease. *Proc Natl Acad Sci U S A. 2008;105:3587-3592.*
8. Niwa K, Kazama K, Younkin L, Younkin SG, Carlson GA, Iadecola C. Cerebrovascular autoregulation is profoundly impaired in mice overexpressing amyloid precursor protein. *Am J Physiol Heart Circ Physiol. 2002;283:H315-H323.*
9. Wu Z, Guo H, Chow N, Sallstrom J, Bell RD, Deane R, Brooks AI, Kanagala S, Rubio A, Sagare A, Liu D, Li F, Armstrong D, Gasiewicz T, Zidovetzki R, Song X, Hofman F, Zlokovic BV. Role of the MEOX2 homeobox gene in neurovascular dysfunction in Alzheimer disease. *Nat Med. 2005;11:959-965.*
10. Chow N, Bell RD, Deane R, Streb JW, Chen J, Brooks A, Van NW, Miano JM, Zlokovic BV. Serum response factor and myocardin mediate arterial hypercontractility and cerebral blood flow dysregulation in Alzheimer's phenotype. *Proc Natl Acad Sci U S A. 2007;104:823-828.*
11. Zhang R, Zuckerman JH, Giller CA, Levine BD. Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol. 1998;274:H233-H241.*
12. van Beek AH, Claassen JA, Rikkert MG, Jansen RW. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab. 2008;doi:10.1038.*

13. Sammons EL, Samani NJ, Smith SM, Rathbone WE, Bentley S, Potter JF, Panerai RB. Influence of noninvasive peripheral arterial blood pressure measurements on assessment of dynamic cerebral autoregulation. *J Appl Physiol. 2007;103:369-375.*
14. Zhang R, Witkowski S, Fu Q, Claassen JA, Levine BD. Cerebral hemodynamics after short- and long-term reduction in blood pressure in mild and moderate hypertension. *Hypertension. 2007;49:1149-1155.*
15. Marquez de la Plata C, Ardelean A, Koovakkattu D, Srinivasan P, Miller A, Phuong V, Harper C, Moore C, Whittemore A, Madden C, Diaz-Arrastia R, Devous M, Sr. Magnetic resonance imaging of diffuse axonal injury: quantitative assessment of white matter lesion volume. *J Neurotrauma. 2007;24:591-598.*
16. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol. 2007;102:870-877.*
17. Nagata K, Buchan RJ, Yokoyama E, Kondoh Y, Sato M, Terashi H, Satoh Y, Watahiki Y, Senova M, Hirata Y, Hatazawa J. Misery perfusion with preserved vascular reactivity in Alzheimer's disease. *Ann N Y Acad Sci. 1997;826:272-281.*
18. Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci. 2005;28:202-208.*
19. Bateman GA, Levi CR, Schofield P, Wang Y, Lovett EC. Quantitative measurement of cerebral haemodynamics in early vascular dementia and Alzheimer's disease. *J Clin Neurosci. 2006;13:563-568.*
20. Shih WJ, Ashford JW, Coupal JJ, Ryo YU, Stipp VV, Magoun SL, Gross K. Consecutive brain SPECT surface three-dimensional displays show progression of cerebral cortical abnormalities in Alzheimer's disease. *Clin Nucl Med. 1999;24:773-777.*
21. Spilt A, Weverling-Rijnsburger AW, Middelkoop HA, van Der Flier WM, Gussekloo J, de Craen AJ, Bollen EL, Blauw GJ, van Buchem MA, Westendorp RG. Late-onset dementia: structural brain damage and total cerebral blood flow. *Radiology. 2005;236:990-995.*
22. Szili-Torok T, Kalman J, Paprika D, Dibo G, Rozsa Z, Rudas L. Depressed baroreflex sensitivity in patients with Alzheimer's and Parkinson's disease. *Neurobiol Aging. 2001;22:435-438.*
23. Royall DR, Gao JH, Kellogg DL, Jr. Insular Alzheimer's disease pathology as a cause of "age-related" autonomic dysfunction and mortality in the non-demented elderly. *Med Hypotheses. 2006;67:747-758.*
24. Cheung RT, Hachinski V. The insula and cerebrogenic sudden death. *Arch Neurol. 2000;57:1685-1688.*
25. de la Torre JC. Cerebromicrovascular pathology in Alzheimer's disease compared to normal aging. *Gerontology. 1997;43:26-43.*
26. O'Rourke MF, Taylor MG. Input impedance of the systemic circulation. *Circ Res. 1967;20:365-380.*
27. Rombouts SA, Goekoop R, Stam CJ, Barkhof F, Scheltens P. Delayed rather than decreased BOLD response as a marker for early Alzheimer's disease. *Neuroimage. 2005;26:1078-1085.*
28. Panerai RB, White RP, Markus HS, Evans DH. Grading of cerebral dynamic autoregulation from spontaneous fluctuations in arterial blood pressure. *Stroke. 1998;29:2341-2346.*
29. Claassen JA, Jansen RW. Cholinergically mediated augmentation of cerebral perfusion in Alzheimer's disease and related cognitive disorders: the cholinergic-vascular hypothesis. *J Gerontol A Biol Sci Med Sci. 2006;61:267-271.*

The gold standard: not a golden standard *(BMJ 2005;329:1121)*

Studies that evaluate a new diagnostic test, procedure, or method should do so by comparing it with a time honoured alternative that is considered to be the current standard in the field. In this context the meaning of the word standard is "authoritative or recognised exemplar of quality or correctness." "Gold standard" is the popular term to describe this test; but "golden standard" is sometimes used as well. In fact, almost all medical publications in Dutch use the term "gouden standaard" which is a translation of "golden standard." Apparently, medical scientists have become confused about the true meaning of the term gold standard.

Inspired by the Olympic Games, where the best athlete wins the gold medal, people who use "golden standard" think the term denotes the best standard in the world. Not bronze, not silver, but gold. Of course, this is incorrect. Gold standard is a historical term borrowed from economists. It signifies a monetary standard, under which the basic unit of currency was defined by a stated quantity of gold. The analogy should be clear: the value of each country's method of payment (currency) was weighed against the gold standard, which made it possible to compare these different currencies for international trading. In a Medline search from 1955 onwards, the first emergence of the term -albeit in a different meaning- was in 1962, in an anonymous commentary in the Lancet. Entitled "Towards a gold standard," it pleaded to set a standard for the use of gold salts in patients with rheumatoid arthritis. It may well have been Rudd who first introduced the "gold standard" in medicine in its current sense in 1979.1 In the following years, the number of publications that employed the term grew rapidly. This was much to the dismay of one biochemist, who thought the term was "presumptuous" for a biological test, since "the subject is in perpetual evolution [and] gold standards are by definition never reached."2 He proposed abolishing the term "because the phrase smacks of dogma …After all, the financiers gave up on the idea of a gold standard decades ago." He failed in his mission, however: since 1995, over 10 000 publications mentioned "gold standard." So why is there also a "golden standard," a term used in over 600 publications since 1995 in English and in many more in foreign languages? I think this is because it is tempting to interpret the word "gold" in gold standard as an adjective, as in gold medal, and then to replace it with "golden," which simply sounds better if English is not your first language. In addition, "golden standard" is a persuasive term that makes sense: if a standard is the one test by which all others are judged, then the golden standard must be perfect. Herein lies, I think, the importance of this discussion. The concept of a "golden standard" implies a level of perfection that can never be attained by any biological test, and will provoke criticism like that ventilated by Duggan.2 In contrast, a gold standard in its true meaning, derived from the monetary gold standard, merely denotes the best tool available at that time to compare different measures. Even in its glory days, the monetary gold standard was never considered perfect. It was subject to endless debate, and in the end it was abandoned for a better system. Similarly, today's gold standard tests will be replaced by better ones. As was eloquently stated by Versi: "It is the absolute truth that is never reached; gold standards are constantly challenged and superseded when appropriate"3

1 Rudd P. In search of the gold standard for compliance measurement. *Arch Intern Med 1979;139:627-8.*

2 Duggan PF. Time to abolish "gold standard". *BMJ 1992;304:1568-9.*

3 Versi E. "Gold standard" is an appropriate term. *BMJ 1992;305:187.*

Chapter 9

Summary and general discussion

Introduction

Advances in measurement techniques have made it possible to study dynamic changes in brain blood flow. In this thesis, these techniques have been further explored, with specific attention for their potential use in elderly subjects. First, we have considered that measurements of cerebral perfusion, complimenting measurements of blood pressure, will provide valuable insight in disorders of blood pressure regulation. Second, we have proposed that measurements of blood pressure, cerebral blood flow, and their interaction, are important in dementia research.

This chapter will provide a summary of the findings in this thesis, followed by a general discussion.

Summary

Part 1. Clinical and methodological background

Chapter 2. This chapter introduces the concept of cerebral autoregulation. It provides a clinical background to indicate its relevance in several conditions that are prevalent in aging subjects. A comprehensive literature review in this chapter shows that there is relatively little information on the effects of aging on cerebral autoregulation, and that there are virtually no data available for subjects older than 75 years. This chapter also describes several methods to analyze and quantify cerebral autoregulation. A suggestion is made for methods that would seem well suited for application in elderly subjects. One such method is the analysis of spontaneous oscillations in blood pressure and cerebral blood flow. These measurements can be performed under resting conditions, for example with the subject in seated position, and require little time (5-10 minutes). A disadvantage of this method is that spontaneous oscillations have small amplitudes, which may limit analysis and interpretation. As a solution, this chapter proposes methods to enhance oscillation amplitudes using repeated maneuvers such as sit-stand or squat-stand.

Chapter 3. In the third chapter we consider the interaction between dementia and cerebral perfusion. One of the hypotheses in Alzheimer's disease is the cholinergic hypothesis, developed 30 years ago, when post mortem studies revealed a severe loss of cholinergic innervation in brains of Alzheimer patients, which was linked to the decline in memory. The treatment of Alzheimer's disease with cholinesterase inhibitors is based on this cholinergic hypothesis, however, this hypothesis fails to account for the lack of specificity of the clinical effects of cholinesterase inhibitors (e.g. improvements in other cognitive domains than memory, such as attention), for the replication of these effects in other dementias, and for the strong and unpredictable intra-individual variation in response to treatment. These findings may be better explained by the premise that cholinesterase inhibitors primarily act by augmenting cerebral perfusion: the cholinergic–vascular hypothesis. This chapter reviews the evidence from preclinical and clinical investigations on the vascular role of the cholinergic neural system. The clinical relevance of this hypothesis is discussed with respect to its interactions with the vascular and amyloid hypothesis of Alzheimer's disease. It is proposed that the role of the cholinergic system in neurovascular regulation and functional hyperemia may elucidate how the cholinergic deficit in Alzheimer's disease contributes to the clinical and pathological features of this disease. Dynamic measurements of cerebral perfusion will be important to further pursue this topic.

Part 2. Tools and methods to investigate cerebral hemodynamics

Chapter 4. This chapter evaluates near infrared spectroscopy (NIRS). NIRS is a non-invasive method to monitor cerebral hemodynamics. Used either alone or in combination with transcranial Doppler sonography, this technique would be well suited for use in cerebrovascular research in aging. Reproducibility of NIRS has primarily been determined in neonates and adults, and only limited information is available in aging subjects. In this chapter, controlled desaturation (the O_2-method) was applied to measure the cerebral blood volume (CBV) with NIRS in 16 healthy subjects aged 65 to 88. This method uses deoxygenated hemoglobin (the concentration of which is manipulated by controlled desaturation) as an intravascular tracer for NIRS. Repeatability (between tests interval: 2 min), short-term reproducibility (intervals of 20 and 40 min) and long-term reproducibility (interval >2 weeks) were determined. We found a coefficient of variation (CV) of 12.5% for repeatability and a CV of 11.7% for short-term reproducibility. The CV for long-term reproducibility

was 15%. We conclude that NIRS can reproducibly measure CBV in subjects aged 65 and older, using the O_2-method. In this group of healthy subjects, this method was well tolerated.

Chapter 5. Cerebral blood vessels strongly respond to changes in CO_2. This phenomenon is termed vasomotor reactivity, and has been used clinically and in research to evaluate cerebrovascular function. Often, transcranial Doppler ultrasonography (TCD) is used to measure changes in cerebral blood flow-velocity together with changes in end-tidal CO_2 (the latter reflecting arterial CO_2-pressure, pCO_2. Blood pressure, however, is also affected by changes in CO_2, and the independent effects of blood pressure on cerebral blood flow will confound interpretation of vasomotor reactivity to CO_2. This chapter proposes a method to analyze vasomotor reactivity by looking at changes in conductance (flow divided by pressure), in addition to flow alone. Moreover, it is demonstrated that by using breath-by-breath analysis of changes in end-tidal CO_2 and conductance, the non-linear dynamics of the relationship between CO_2 and cerebral blood flow are revealed. This may provide a more sensitive method than previously used techniques that have assumed a linear relationship.

Chapter 6. This chapter further investigates how analysis of spontaneous oscillations in blood pressure and cerebral blood flow can be used to quantify the dynamic relationship between pressure and flow. As discussed in chapter 2, these measurements can be performed under resting conditions, and would therefore seem very suitable for application in elderly subjects. However, spontaneous oscillations often have small amplitudes, and therefore their clinical relevance may be questioned. Spontaneous oscillations have different frequencies or periods, ranging from the very low frequency range (< 0.07 Hz, which equals oscillation periods of 15 seconds or longer) to the high frequency range (0.2 - 0.35 Hz, or periods of 3-5 seconds). As described in chapter 2, cerebral autoregulation is particularly effective for very low frequency oscillations in pressure, making it important to study this frequency range. Unfortunately, it is often difficult to demonstrate beyond doubt that all observed very low frequency oscillations in cerebral blood flow are causally related to oscillations in blood pressure. In this chapter, we investigated if it would be possible and feasible to use repeated squat-stand maneuvers in order to induce large oscillations in blood pressure at

frequencies of 0.025 and 0.05 (in the very low frequency range) and 0.1 Hz (in the low frequency range). Indeed, large oscillations at these specific frequencies were induced. We conclude that using repeated squat-stand maneuvers, we are able to study dynamic cerebral autoregulation in the low frequencies under conditions of hemodynamically strong and causally related oscillations in pressure and flow. This improves the estimations of dynamic cerebral autoregulation, and strengthens the clinical relevance of this method since induced oscillations mimic hemodynamic changes associated with postural changes in daily life.

Part 3. Application in clinical research

Chapter 7. In this chapter, we have applied the method described in chapter 5 to investigate cerebral vasomotor reactivity to CO_2 in middle-aged patients with hypertension (mean age 52 years, range 40 -66). In hypertension, cerebral vasomotor reactivity to CO_2 may be reduced as a consequence of cerebrovascular remodeling and endothelial dysfunction. In newly diagnosed untreated patients, blood pressure reduction following treatment may further impair CO_2 reactivity, because compensatory cerebral vasodilatation will be induced by cerebral autoregulation. In this chapter, we studied 11 patients with newly diagnosed, untreated mild to moderate hypertension and 8 controls. At baseline, hypertension was not associated with reduced CO_2 reactivity. In patients, treatment with losartan/hydrochlorothiazide effectively lowered blood pressure. After 1-2 weeks of treatment, and after 3 months of treatment, cerebrovascular resistance was reduced (indicating vasodilatation) and cerebral blood flow velocity remained stable, however, CO_2 reactivity was not affected. In a previous study, we have shown that dynamic cerebral autoregulation was not affected by mild hypertension, nor by rapid or prolonged blood pressure reduction.[1] This study adds to these findings by demonstrating that reactivity to CO_2 is equally unaffected. Previous suggestions that hypertension is associated with impaired vasomotor reactivity appear to be explained by cerebrovascular and/or diabetic co-morbidity.

Chapter 8. Here, the methods described in chapter 5 and chapter 6 have been employed to explore the cerebral hemodynamic characteristics in patients with mild Alzheimer's disease compared with age-matched controls. In this chapter, the

Table 1 Overview of instruments and main measurements used in this thesis.

	Domain	Sample frequency/ Temporal resolution	Main measures	Optional additional measures
Finapres	Blood pressure	200 Hz, measures complete pressure wave within each cardiac cycle	Beat-to-beat changes in systolic, diastolic, and mean blood pressure	Heart rate. Estimation of changes in a.o.: peripheral resistance, stroke volume
TCD	Cerebral blood flow-velocity	200 Hz, measures complete flow-velocity wave within each cardiac cycle	Beat-to-beat changes in systolic, diastolic, and mean flow-velocity (MCA)	Pulsatility index, (micro)embolism detection
NIRS	Tissue oxygenation	200 Hz, measures changes in concentration of hemoglobin (total, and oxygenated/ deoxygenated fractions) within each cardiac cycle	Beat-to-beat changes in oxyHb, HHb and tHb	Estimation of cerebral blood volume (O_2-method). Rapid detection of cortical activation (within msec) using oxyHb signal (cf fMRI BOLD).

Finapres: finger arterial pressure measurement using photoplethysmography. TCD: tanscranial Doppler sonography. NIRS: near infrared spectroscopy. OxyHb: oxygenated hemoglobin.HHb: deoxygenated hemoglobin. tHb: total hemoglobin

emphasis is on the method of transfer function analysis to quantify dynamic cerebral autoregulation. Transfer function analysis uses specific parameters (gain, phase shift and coherence) to quantify the relationship between blood pressure and cerebral perfusion. In this pilot study, we demonstrated that the application of these methods was feasible. Even though we studied a small number of subjects, and the Alzheimer patients were in an early stage of the disease, we noted remarkable alterations in blood pressure regulation and in cerebral perfusion in these patients. Cerebrovascular resistance was increased, and cerebral blood flow was reduced, even when corrected for differences in total brain volume. In addition, oscillatory changes in cerebral blood flow were reduced despite increased blood pressure oscillations in Alzheimer's disease. These findings indicate that already in an early clinical stage, Alzheimer's disease is associated with hemodynamic changes, which affect blood pressure and cerebral perfusion. Moreover, these changes could be described using the methods proposed in this thesis.

Table 2 Summary of the essential features of cerebral autoregulation and reactivity.

	Cerebral autoregulation	Vasomotor reactivity
Equipment used	Finapres, TCD, capnograph, ECG	
Input measures	Beat-to-beat BP, CBFV	Breath-to-breath BP, CBFV, $etCO_2$
Physiological basis	Cerebrovascular adaptation to changes in perfusion pressure	Hypocapnia causes vasoconstriction Hypercapnia causes vasodilatation
Data preparation and analysis	- Conversion to detrended time series - Spectral analysis BP and CBFV time series - Transfer function analysis	- Y-axis: % changes in BP, CBFV, CVC - X-axis: $etCO_2$ - linear regression - exponential function
Output measures	Spectral power of oscillations in BP and CBFV Transfer function phase, gain and coherence	Total reactivity; separate hypocapnic and hypercapnic reactivity Reactivity index
Interpretation	Spectral power: magnitude of the hemodynamic oscillations Gain: damping of BP oscillations to CBFV Phase shift: adaptation of flow to pressure Coherence: (linear) correlation between BP and CBFV oscillations	Reduced vasomotor reactivity indicates limited capacity for vasodilatation and / or constriction
Normal obervations	Lower gain and higher phase-shift for lower frequency oscillations, indicating that slower changes in BP are efficiently "filtered".	Hypocapnia induces ~30% decrease, hypercapnia induces ~70% increase in flow or conductance
Examples of abnormal findings	Carotid stenosis, lacunar stroke, malignant hypertension, diabetes	Carotid artery stenosis, cerebral small-vessel disease

BP: mean blood pressure CBFV: mean cerebral blood flow-velocity CVC: conductance-index (CBFV/BP) $etCO_2$: end-tidal CO_2 (partial pressure of CO_2 in expired air)

Overlooking these results, we will now address some relevant methodological issues regarding the techniques to measure cerebral perfusion. This will be followed by a discussion on the implications of these findings for future research. We will highlight what valuable insights in disorders of blood pressure regulation can be

learned from the measurements of cerebral perfusion described in this thesis, and why measurements of blood pressure, cerebral blood flow, and their interaction, are important in dementia research.

General discussion

Methodological considerations

Measurements of cerebral perfusion

In chapter 2 and 3, we have indicated that there is a need to enhance our knowledge of cerebral hemodynamics in many conditions and diseases that affect the aging population.

Over the past years, there have been important advances in techniques to evaluate cerebral perfusion.[2] These include, but are not limited to, transcranial Doppler ultrasonography (TCD), near infrared spectroscopy, (functional) magnetic resonance imaging (MRI) and positron emission tomography (PET). MRI and PET have seen important improvements in spatial resolution (indicating in what detail regional perfusion differences can be separated) and temporal resolution (indicating in what detail changes in perfusion over time can be captured). For a detailed review comparing different techniques to measure cerebral perfusion, which is beyond the scope of this discussion, we refer to the excellent review of Wintermark *et al.* [2] In short, at this moment, both MRI and PET have limitations and disadvantages, which reduce their potential usefulness in the field of research described in this thesis. First, availability is limited. For PET, this is determined by its high cost, but also depends on the speed of decay of the radio-active tracer, which means that the PET center must be in proximity to the tracer production site. Access to MRI for research purposes, especially to MRI machines with high magnetic field strength (3 Tesla) is also limited due to factors including high clinical demand for MRI and budget limitations. Second, temporal resolution remains too low at this moment for both PET and MRI to capture fast changes in perfusion in response to rapid changes in perfusion pressure. Third and perhaps most importantly, MRI and PET scanning require that the subject is and remains as motionless as possible in the supine position. This makes it impossible to investigate the hemodynamic effects of postural hypotension, and poses important limitations to the evaluation of vasomotor reactivity or hemodynamic responses to cognitive activation.[3-5]

Both TCD and near infrared spectroscopy (NIRS) provide an answer for these three limitations. Although at present, only a few centers world-wide combine these two techniques to measure cerebral hemodynamics in research settings, TCD alone is in fact widely available and is used in clinical practice and research. Both instruments have low cost for acquisition and operation. Temporal resolution is excellent (< 1 s), and as demonstrated in this thesis (chapter 6 and 8), and in previous work,[6] body position and motion pose no limitations. Therefore, we have chosen to evaluate and further develop these techniques in our research into cerebral hemodynamics in aging.

TCD and NIRS have their own specific disadvantages that will be discussed below. For TCD, methodological issues are also discussed extensively in the literature review in chapter 2, and in chapters 5-8. For NIRS, a brief discussion is presented in chapter 4.

NIRS

NIRS measures changes in brain tissue hemoglobin oxygenation. The technique is limited to superficial areas of the brain, and it is not possible to know exactly the size and location of the tissue that is examined. This means that absolute values for hemoglobin concentrations cannot be obtained. In addition, it is possible that the signal is affected by extra-cranial sources (e.g. hemoglobin in scalp, muscle, or bone). With optimal inter-optode distance, the relative contribution of these sources can be minimized (chapter 4). Relative to the brain, perfusion in these extra-cranial tissues is small. Moreover, previous validation studies in our lab have included comparison with fMRI. The physiological constituent of the BOLD signal that is measured with fMRI is in fact the change in oxyhemoglobin that is measured by NIRS. It was shown in our lab that brain cortical oxygenation changes measured by NIRS correlated well with BOLD signal changes measured by fMRI.[7] Since it is certain that the fMRI signal is purely composed of brain tissue oxygenation, this correlation suggests that extra-cranial contamination of the NIRS signal may be negligible.

Age-related changes in brain perfusion and brain atrophy may affect reliability of NIRS in research in elderly subjects. Therefore, we examined reproducibility of NIRS

in aging (chapter 4). We examined short-term and long-term reproducibility of estimations of cerebral blood volume. This technique requires controlled deoxygenation (progressive hypoxia) to reduce in parallel peripheral oxygen saturation (SaO_2) and cerebral oxygenation (oxyhemoglobin). Reproducibility was good, and comparable to other techniques to measure cerebral hemodynamics.[8] These results, together with previous reproducibility results for changes in cerebral oxygenation with orthostatic hypotension[6,9] and correlation with fMRI7 (Mehagnoul-Schipper, thesis 2003), all evidence of sufficient concurrent validity, indicate that NIRS can be confidently used in aging research.

TCD

The limitations of TCD have been discussed in detail in chapter 2. In summary, it is important to realize that TCD provides an indirect measure of flow, namely flow-velocity, which is determined by flow and by vessel diameter. Intra-individual changes in flow-velocity reliably reflect changes in flow, as it has been demonstrated that vessel diameter does not change significantly during tests to assess autoregulation or vasomotor reactivity.[1,4,5] In contrast, inter-individual differences in flow-velocity can importantly be determined by differences in middle cerebral artery (MCA) diameter.[10] Therefore, comparisons of individual values for absolute flow-velocity should not be performed; instead, relative changes in flow-velocity (e.g. % change form baseline) must be used in this case. Comparisons of absolute flow-velocity values between two groups, as performed in part in chapter 8, are acceptable provided that it can be assumed that the distribution of individual differences in MCA diameters is equal in both groups. With this in mind, most comparisons in chapter 8 were based on relative changes in flow-velocity, which eliminates the effect of vessel diameter. Moreover, flow in the MCA does not equal global brain blood flow; however, the MCA does provide blood flow to ~70% of the brain. Also, estimates of flow measured with TCD can not be compared to brain blood flow in the classic sense, expressed as flow per 100 g of brain tissue ("nutrient flow"). In chapter 8, we have addressed this issue in part by expressing TCD flow-velocity relative to whole brain volume as measured by MRI.

Finally, TCD requires a "temporal window", to get a reliable signal. In most subjects, the structure and density of the temporal bone allows sufficient passage of ultrasound waves to create a good quality flow-velocity signal. This small area of the skull where the TCD signal can be obtained is known as the temporal window. Unfortunately, advancing age and female gender are associated with a higher risk of an "inadequate window". This means that in older women, in up to 30% of subjects it will be impossible to obtain an adequate TCD signal.

Advantages of the combination of TCD and NIRS

In this thesis, TCD and NIRS have been evaluated separately. Because these techniques measure quite different aspects of cerebral hemodynamics, we propose that their combination will provide relevant complementary information. To exemplify this, we will briefly discuss which aspects of cerebral hemodynamics are addressed by TCD and NIRS.

Even though TCD provides an estimate of flow changes in the MCA, these changes reflect the dynamics of downstream, smaller vessels, rather than changes in larger caliber vessels such as the MCA itself (chapters 2 and 5-8).[11-15] Thus, in a simplified depiction of complex hemodynamic physiology, the flow changes derived from TCD measurements are a reflection of downstream vasomotor changes (vasodilatation and vasoconstriction) in small vessels, in response to changes in CO_2 (vasomotor reactivity) or changes in perfusion pressure (cerebral autoregulation).

NIRS on the other hand can be regarded as an estimation of the "end result" of these hemodynamic changes at the level of the brain tissue. NIRS parameters indicate whether cortical total hemoglobin levels are stable, increase or decrease, and provide information on the supply of oxygen at the tissue level. For example, a reduction in oxygen-rich hemoglobin (O_2Hb) measured using NIRS suggests that the balance between supply and demand for oxygen has shifted.[7,16,17]

In this way, combined measurements of TCD and NIRS together with Finapres or other devices that measure beat-to-beat blood pressure, will provide the following information, for example during orthostatic hypotension: the amount of blood pressure reduction, its effect on cerebral blood flow, and its effect on oxygen delivery at tissue level.

Sample size- a matter of perspective

The studies in this thesis were performed in small numbers of subjects. In recent

years, medical research has seen a growing interest in randomized controlled trials and epidemiological studies with very large sample sizes. Studies in such large numbers of subjects can provide important new perspectives, as they may identify associations that would have been missed with observations made in smaller numbers of subjects.[18] For comparison, studies with large sample sizes provide the equivalent of an aerial view of an archeological site, revealing a pattern of soil discoloration, consistent with an ancient building complex, that would have gone unnoticed when looked at from ground level.[19] However, such studies with large sample sizes must make sacrifices to the level of detail that can be studied, as they can only obtain a limited number of measurements in individual subjects for obvious reasons of practicality and costs. Therefore, complementary studies in smaller numbers of subjects are warranted to provide the detailed perspective. Using the same comparison, such studies can analyze the soil contents in the identified patterns of soil discoloration to discover the building materials that were used.

In this thesis, we have chosen to study the (patho) physiology of the cerebral circulation, in order to provide more detailed information on the associations between blood pressure, brain perfusion, and brain disease that have been identified by epidemiological research. Such detailed studies are limited to small numbers of subjects, due to the extensive and time-consuming data-analysis that is required. Nevertheless, we think that our findings, which we abstracted from a small group of subjects, potentially affect the diagnostics and management in Alzheimer's disease in larger populations.

Implications

Having described these methodological issues, we will now turn to the implications of the findings in this thesis for clinical research in geriatrics.

Hypertension

We have used a new, and more sensitive, method to measure cerebral vasomotor reactivity in patients with hypertension (chapter 5 and 7). We have investigated middle-aged patients with newly diagnosed mild-to-moderate hypertension. Prior to the study investigating vasomotor reactivity, we had already demonstrated that

dynamic cerebral autoregulation was not altered by the presence of hypertension in these patients.[1] Moreover, rapid normalization of blood pressure (to ≤ 140/90 mm Hg within 1-2 weeks) did not reduce cerebral perfusion, nor did it affect cerebral autoregulation. In chapter 7, we have shown that vasomotor reactivity to CO_2 equally was not affected by hypertension. Moreover, rapid blood pressure reduction did not impair vasomotor reactivity. Together, these findings indicate that the capacity of cerebral vessels for vasoconstriction and vasodilatation, in response to either a change in perfusion pressure or a change in CO_2, was not affected by the presence of hypertension, nor by rapid reductions in perfusion pressure brought about by antihypertensive treatment. These two studies are complimentary. For example, a reduction in perfusion pressure may be compensated for by cerebral vasodilatation, thus maintaining a stable cerebral perfusion. This would suggest that autoregulation is unimpaired. However, if substantial, nearly maximal vasodilatation is required to compensate for the reduced perfusion pressure, it will be clear that any further reductions in perfusion pressure can no longer be compensated for. However, it may not be feasible or even ethical to reduce perfusion pressure to such extents. Here, vasomotor reactivity testing provides a safe and complimentary test. It has been demonstrated, in patients with carotid artery stenosis, that (near-)maximum vasodilatation to compensate for reduced perfusion pressure exhausts the capacity for further CO_2-induced vasodilatation, leading to impaired vasomotor reactivity.[20] In this way, the addition of vasomotor reactivity testing to a test of pressure autoregulation could unmask a compromised cerebral circulation.

These studies address the concern, described in the introduction of this thesis, that blood pressure reduction in hypertensive patients may lower cerebral perfusion. Here, we have shown that in the patient category that has been studied, this concern could be dismissed.

This leads us to the following caveats. We have studied a limited age range (≈ 40-65 years), and we can not assume that these results can be extrapolated to much older subjects. In addition, we studied newly diagnosed patients, and therefore it is unknown how long hypertension had already been present prior to treatment. Finally, these subjects did not have clinical signs or a history of cerebrovascular disease.

Chapter 2 provides a further discussion on cerebral autoregulation. In a review of the literature to date, studies of cerebral hemodynamics in patients with hypertension aged up to ≈ 75 years showed no evidence for impaired autoregulation, regardless of the method that was used to quantify cerebral hemodynamics. Although this supports the findings in chapter 7, further study is required to investigate the effects of hypertension and blood pressure reducing treatment on brain perfusion in much older hypertensive patients, and in patients with co-existing cerebrovascular disease. The methods proposed in this thesis are well-suited to perform this type of research.

Hypotensive syndromes

Disorders of blood pressure regulation are often referred to as the "hypotensive syndromes", because hypotensive episodes are a prominent feature in all of these conditions. These disorders share the fact that the manner in which they affect cerebral perfusion strongly determines their clinical consequences.[21,22] Cerebral autoregulation thus plays an important role, for example, it has been suggested that certain patients with neurogenic orthostatic hypotension have augmented or expanded cerebral autoregulation, which could explain how these patients can tolerate substantial falls in blood pressure.[23]

Thus far, management of hypotensive syndromes has focused on limiting the reduction in blood pressure. The methods described in this thesis make it possible to study cerebral perfusion in conjunction with blood pressure.[24] This research in patients with disorders of blood pressure regulation can help to answer some of the following questions:

1. Are there inter-individual differences in autoregulation or vasomotor reactivity that explain the variation in clinical symptoms, and if so, which patient characteristics determine these differences?
2. How are autoregulation and vasomotor reactivity affected by different classes of medication? For example, antihypertensive agents may be required to reduce hypertension, but could aggravate orthostatic hypotension, in a patient with a disorder of blood pressure regulation. Angiotensin II receptor blocking (ARB) agents potentially enhance cerebral vasodilatation, and may augment autoregulation and hence orthostatic tolerance. Therefore, in theory, thiazide diuretics and ARB's may have similar blood pressure lowering properties, but different effects on cerebral perfusion. Using the methodology laid out in this thesis, it is possible to investigate such differential drug effects on both blood pressure and cerebral circulation.
3. In neurogenic orthostatic hypotension, treatment has aimed at volume expansion, but recent "smart drug" development includes treatment with cholinergic agents.[23] The effect of cholinergic agents (e.g. physostigmine) on postural blood pressure reduction has been studied, however, how do these drugs affect cerebral perfusion (see also the discussion on cholinergic –vascular innervation in chapter 3)? This also can be studied using the methodology laid out in this thesis.

Dementia

Despite its first description more than 100 years ago, Alzheimer's disease essentially remains a disease of unknown cause and without treatment.[25] For comparison, AIDS, a comparably devastating disease, was first described less than 30 years ago; its cause has been unraveled, and several treatment options are available that effectively delay disease progression. Why is it that we have been able to solve the HIV/AIDS puzzle, but not the Alzheimer puzzle? Research publications on HIV/AIDS in medical literature clearly outnumber those on Alzheimer's disease, suggesting that part of the explanation may lie in differences in research priority and effort. Furthermore, it is clear that HIV/AIDS is a monocausal, exogenous (infectious) disease, with a relatively short delay between infection and disease onset. If these are characteristics that speed up the unraveling of a disease, it can be argued that Alzheimer's disease has not yet been unraveled because it has precisely the opposite characteristics. However, it has also been suggested that research in Alzheimer's disease has been looking in the wrong direction,[26] or has been trapped in mono-causal thinking, ignoring the evidence for multicausality in this disease.[25] It is true that most research to date has focused on the amyloid hypothesis.[27] This hypothesis regards Alzheimer's disease as a disease of increased production and/or decreased clearance of beta-amyloid, leading to aggregation of neurotoxic beta-amyloid oligomers.[27,28] Treatment strategies along the lines of this hypothesis are constantly developing, and several promising drugs are underway. However, human trials with several recently developed "promising drugs" have all been disappointing. It is this disappointment that has led some to consider this

amyloid-hypothesis as a “wrong direction” for Alzheimer research, one that neglects the evidence for a vascular origin of this disease- the vascular hypothesis of Alzheimer’s disease.[26] In short, this vascular hypothesis suggests that vascular alterations lead to increased susceptibility to hypoxia and oxidative stress, which in turn lead to Alzheimer pathology. In this hypothesis, beta-amyloid is considered a product, not a cause, of Alzheimer’s disease.

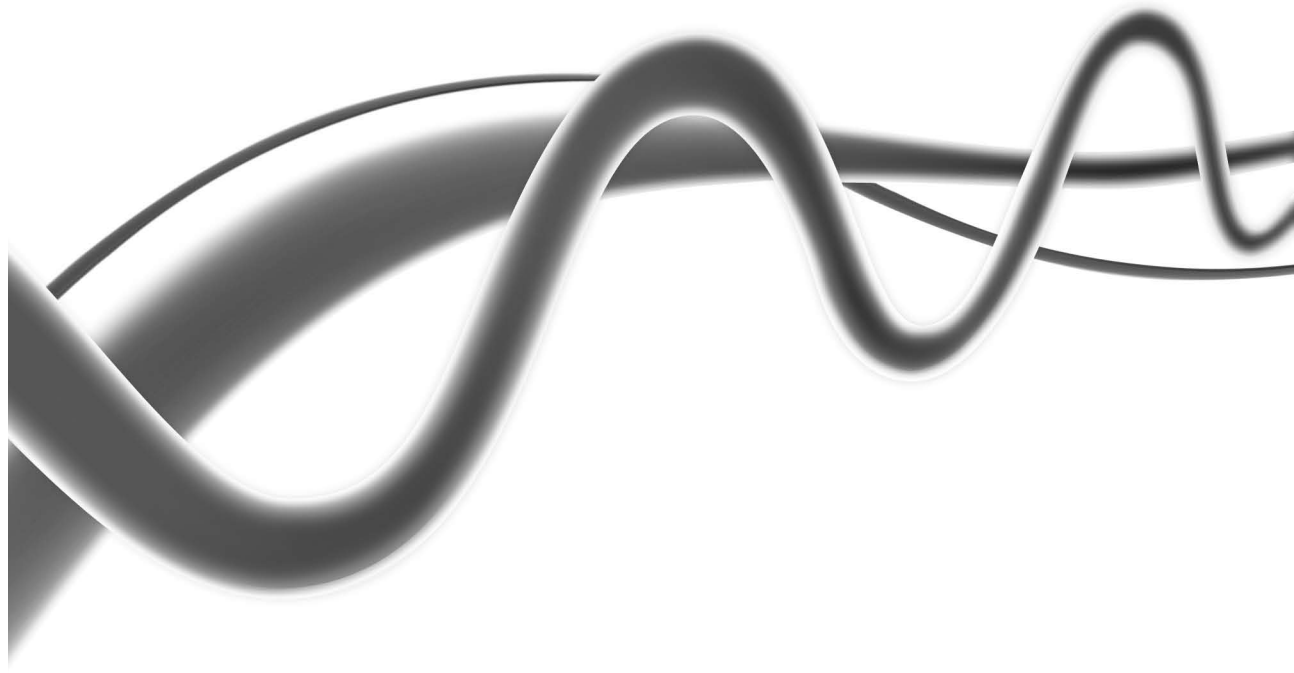
Recent advances however suggest that the vascular hypothesis and the amyloid hypothesis are not mutually exclusive.[3,29] For example, mounting evidence demonstrates vascular effects of beta-amyloid, and implicates vascular alterations as a source of impaired beta-amyloid clearance.[30, 31]

This clearly illustrates the importance of further investigations into the aspects of vascular alterations in Alzheimer’s disease. In chapter 8, we have shown that, using the methods proposed in this thesis, it is feasible to measure cerebral hemodynamics in aging subjects with Alzheimer’s disease. In this study, we found indications that several aspects of hemodynamics were affected in patients with early Alzheimer’s disease. Blood pressure regulation was altered, which could be consistent with alterations in baroreflex function as a result of brain pathology, and/or with functional vascular effects of circulating beta-amyloid. Cerebral perfusion was reduced, with elevated cerebrovascular resistance and reduced oscillations in cerebral blood flow. This could be consistent with altered endothelial or smooth-muscle function, or with structural alterations in the vascular (capillary) bed. Such alterations have been demonstrated in animal studies and in limited in vitro studies with human material (see chapter 8). Further research is warranted to confirm these observations of hemodynamic alterations in a larger number of patients with Alzheimer’s disease, and to elucidate the mechanisms behind these changes. Both detailed small scale and more global large scale studies are needed to clarify the position of both the amyloid and the vascular factors in the complex, probably multifactorial, and slowly progressive pathophysiology of Alzheimer’s disease.[25,29] In our lab, for example, detailed research, using the methods validated in this thesis, is currently underway to investigate hemodynamic changes in a larger number of older patients with Alzheimer’s disease and age-matched cognitively normal controls. Moreover, the effects of treatment with cholinesterase-inhibitors on cerebral hemodynamics will be addressed, in line with the cholinergic-vascular hypothesis.

References

1. Zhang R, Witkowski S, Fu Q, Claassen JA, Levine BD. Cerebral hemodynamics after short- and long-term reduction in blood pressure in mild and moderate hypertension. *Hypertension 2007; 49(5):1149-1155.*
2. Wintermark M, Sesay M, Barbier E et al. Comparative overview of brain perfusion imaging techniques. *Stroke 2005; 36(9):e83-e99.*
3. Claassen JA, Jansen RW. Cholinergically mediated augmentation of cerebral perfusion in Alzheimer's disease and related cognitive disorders: the cholinergic-vascular hypothesis. *J Gerontol A Biol Sci Med Sci 2006; 61(3):267-271.*
4. Claassen JA, Zhang R, Fu Q, Witkowski S, Levine BD. Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing. *J Appl Physiol 2007; 102(3):870-877.*
5. van Beek AH, Claassen JA, Rikkert MG, Jansen RW. Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab 2008; 28(6):1071-1085.*
6. Mehagnoul-Schipper DJ, Vloet LC, Colier WN, Hoefnagels WH, Jansen RW. Cerebral oxygenation declines in healthy elderly subjects in response to assuming the upright position. *Stroke 2000; 31(7):1615-1620.*
7. Mehagnoul-Schipper DJ, van der Kallen BF, Colier WN et al. Simultaneous measurements of cerebral oxygenation changes during brain activation by near-infrared spectroscopy and functional magnetic resonance imaging in healthy young and elderly subjects. *Hum Brain Mapp 2002; 16(1):14-23.*
8. Claassen JA, Colier WN, Jansen RW. Reproducibility of cerebral blood volume measurements by near infrared spectroscopy in 16 healthy elderly subjects. *Physiol Meas 2006; 27(3):255-264.*
9. Mehagnoul-Schipper DJ, Colier WN, Jansen RW. Reproducibility of orthostatic changes in cerebral oxygenation in healthy subjects aged 70 years or older. *Clin Physiol 2001; 21(1):77-84.*
10. Bishop CC, Powell S, Rutt D, Browse NL. Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke 1986; 17(5):913-915.*
11. Aaslid R, Lash SR, Bardy GH, Gild WH, Newell DW. Dynamic pressure--flow velocity relationships in the human cerebral circulation. *Stroke 2003; 34(7):1645-1649.*
12. Atkinson JL, Anderson RE, Sundt TM, Jr. The effect of carbon dioxide on the diameter of brain capillaries. *Brain Res 1990; 517(1-2):333-340.*

13. Hurn P, Traystman RJ. Changes in arterial gas tension. In: Edvinsson L, Krause D, editors. Cerebral blood flow and metabolism. *2 ed. Philadelphia: Lippincott Williams & Wilkins; 2002: 384-394.*
14. Wei EP, Kontos HA, Patterson JL, Jr. Dependence of pial arteriolar response to hypercapnia on vessel size. *Am J Physiol 1980; 238(5):697-703.*
15. Zhang R, Witkowski S, Fu Q, Claassen JA, Levine BD. Cerebral hemodynamics after short and long-term reduction in blood pressure in mild and moderate hypertension. *Hypertension 2007; 49(5):1149-1155.*
16. Mehagnoul-Schipper DJ, Vloet LC, Colier WN, Hoefnagels WH, Jansen RW. Cerebral oxygenation declines in healthy elderly subjects in response to assuming the upright position. *Stroke 2000; 31(7):1615-1620.*
17. Obrig H, Villringer A. Beyond the visible--imaging the human brain with light. *J Cereb Blood Flow Metab 2003; 23(1):1-18.*
18. Claassen JA. Epidemiology: friend or foe? *BMJ 2004; 329:467.*
19. Wilson DR. Air Photo Interpretation for Archaeologists. *Tempus; 2000.*
20. Blaser T, Hofmann K, Buerger T, Effenberger O, Wallesch CW, Goertler M. Risk of stroke, transient ischemic attack, and vessel occlusion before endarterectomy in patients with symptomatic severe carotid stenosis. *Stroke 2002; 33(4):1057-1062.*
21. Claassen JA. Cerebral perfusion in neurogenic orthostatic hypotension. *Lancet Neurol 2008; 7(7):573-574.*
22. Low PA, Singer W. Cerebral perfusion in neurogenic orthostatic hypotension - Authors' reply. *Lancet Neurol 2008; 7(7):574.*
23. Low PA, Singer W. Management of neurogenic orthostatic hypotension: an update. *Lancet Neurol 2008; 7(5):451-458.*
24. Schondorf R, Stein R, Roberts R, Benoit J, Cupples W. Dynamic cerebral autoregulation is preserved in neurally mediated syncope. *J Appl Physiol 2001; 91(6):2493-2502.*
25. Olde Rikkert MG, Teunisse JP, Vernooij-Dassen M. One hundred years of Alzheimer's disease and the neglected second lesson of Alois Alzheimer on multicausality in dementia. *Am J Alzheimers Dis Other Demen 2005; 20(5):269-272.*
26. De La Torre JC. Alzheimer disease as a vascular disorder: nosological evidence. *Stroke 2002; 33(4):1152-1162.*
27. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. *Lancet 2006; 368(9533):387-403.*
28. Kukar TL, Ladd TB, Bann MA et al. Substrate-targeting gamma-secretase modulators. *Nature 2008; 453(7197):925-929.*
29. Olde Rikkert MG, van der Flier WM, de Leeuw FE et al. Multiple diagnostic tests are needed to assess multiple causes of dementia. *Arch Neurol 2006; 63(1):144-146.*
30. Claassen JA. Alzheimer's disease: trade-off for increased survival with atherosclerosis? *Ann Neurol 2007;DOI 10.1002/ana.21285.*
31. Deane R, Zlokovic BV. Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. *Curr Alzheimer Res 2007; 4(2):191-197.*



Chapter 10

Samenvatting in het Nederlands

Inleiding

Ontwikkelingen op het gebied van meettechnieken maken het nu mogelijk om snelle, dynamische veranderingen in hersendoorbloeding waar te nemen. In dit proefschrift zijn deze technieken verder verkend, met specifieke aandacht voor hun mogelijke gebruik bij ouderen. Het proefschrift heeft twee hoofdthema's: 1) stoornissen in bloeddrukregulatie en 2) eigenschappen van het hersenvaatbed bij dementie.

Het nauwkeurig kunnen meten van dynamische veranderingen in hersendoorbloeding biedt een waardevolle aanvulling op het meten van alleen de bloeddruk, wanneer onderzoek wordt gedaan bij patiënten met stoornissen in de bloeddrukregulatie. Dit zijn bijvoorbeeld patiënten met ernstige duizeligheid bij opstaan, of patiënten met veelvuldige "wegrakingen".

Recente ontwikkelingen op het gebied van dementie geven aan dat metingen van bloeddruk, cerebrale bloeddoorstroming, en hun interactie, van belang zijn bij onderzoek bij patiënten met cognitieve stoornissen en dementie.

Dit hoofdstuk bevat een samenvatting van de bevindingen in dit proefschrift.

Deel 1. Klinische en methodologische achtergrond

Hoofdstuk 2. Dit hoofdstuk introduceert het concept cerebrale autoregulatie: het systeem waarbij hersenbloedvaten reageren op schommelingen in de bloeddruk om zo de hersendoorbloeding zo constant mogelijk te houden. Het hoofdstuk biedt een klinische achtergrond voor dit begrip, en toont de relevantie ervan aan bij verschillende aandoeningen die vaak voorkomen bij ouderen. Uit een uitgebreide literatuurstudie in dit hoofdstuk blijkt dat er relatief weinig informatie is over de effecten van veroudering op cerebrale autoregulatie, en dat er vrijwel geen gegevens beschikbaar zijn voor patiënten ouder dan 75 jaar. Dit hoofdstuk beschrijft ook verschillende methoden om cerebrale autoregulatie te analyseren en te kwantificeren. Er worden suggesties gedaan voor methoden die zeer geschikt lijken voor toepassing bij oudere patiënten. Een van deze methoden is de analyse van spontane schommelingen in bloeddruk en cerebrale bloeddoorstroming. Deze metingen kunnen namelijk worden verricht in rust, bijvoorbeeld als de patiënt rustig zit, en duren niet lang (5-10 minuten). Een nadeel van deze methode is dat spontane schommelingen (oscillaties) zwak zijn (ze hebben kleine amplitudes), en dit beperkt de analyse en interpretatie. Als een mogelijke oplossing hiervoor wordt voorgesteld om door herhaalde bewegingen, zoals zitten-staan, of kniebuigen-staan, sterkere schommelingen in bloeddruk en hersendoorbloeding op te wekken.

Hoofdstuk 3. In het derde hoofdstuk kijken we naar de interactie tussen dementie met name de ziekte van Azheimer en de doorstroming van de hersenen. Een van de hypothesen over het ontstaan van de ziekte van Alzheimer is de cholinerge hypothese, 30 jaar geleden ontwikkeld toen bij post-mortem onderzoek bleek dat er een ernstig verlies was van cholinerge innervatie in de hersenen van Alzheimerpatiënten. Dit verlies leek gekoppeld aan de achteruitgang in het geheugen. De behandeling van de ziekte van Alzheimer met cholinesteraseremmers (in Nederland zijn dit de geneesmiddelen rivastigmine (Exelon) en galantamine (Reminyl)) is gebaseerd op deze cholinerge hypothese. Deze hypothese biedt echter geen goede verklaring voor het gebrek aan specificiteit van de klinische effecten van cholinesteraseremmers (bijvoorbeeld verbetering van de cognitieve andere domeinen dan het geheugen, zoals aandacht), voor het feit dat vergelijkbare behandeleffecten optreden bij andere dementievormen, en voor de sterke en onvoorspelbare intra-individuele variatie in de respons op de behandeling. Deze bevindingen kunnen beter worden verklaard door de veronderstelling dat cholinesteraseremmers vaatverwijding veroorzaken in de hersenen, en daarmee een verbetering van hersendoorbloeding bewerkstelligen. We hebben deze hypothese de cholinerge-vasculaire hypothese genoemd. Dit hoofdstuk geeft een overzicht van de gegevens uit het preklinisch en klinisch onderzoek op de vasculaire rol van het cholinerge neurale systeem. De klinische relevantie van deze hypothese wordt besproken, evenals de verhouding met andere hypothesen voor de ziekte van Alzheimer: de vasculaire- en de amyloid- hypothese. We bespreken de mogelijke rol van het cholinerge systeem in de neurovasculaire koppeling: de locale regulatie van hersenactiviteit en doorbloeding. Dit mechanisme kan verduidelijken hoe het cholinerge tekort bij de ziekte van Alzheimer bijdraagt aan de klinische en pathologische kenmerken van deze ziekte. Dynamische metingen van de cerebrale perfusie zijn van belang om dit onderwerp verder te bestuderen.

Deel 2. Instrumenten en methoden voor onderzoek naar de cerebrale hemodynamiek

Hoofdstuk 4. Dit hoofdstuk evalueert nabij-infrarood spectroscopie (NIRS). NIRS is een niet-invasieve methode om cerebrale hemodynamiek te meten. Alleen, of in combinatie met Transcraniële Dopplermetingen, kan deze techniek zeer geschikt zijn voor cerebrovasculair onderzoek bij veroudering. Reproduceerbaarheid van NIRS is onderzocht bij pasgeborenen en volwassenen, maar er is slechts beperkte informatie beschikbaar voor reproduceerbaarheid bij ouderen.

In dit hoofdstuk werd gecontroleerde desaturatie (de O_2-methode) toegepast om het cerebrale bloed volume (CBV) te meten met NIRS in 16 gezonde personen met een leeftijd van 65 tot 88 jaar. Deze methode maakt gebruik van veranderingen in gedeoxygeneerd hemoglobine in het hersenbloed. De concentratie van dit zuurstofarme hemoglobine wordt veranderd door de gecontroleerde desaturatie, en daarmee kan het worden gebruikt als een intravasculaire indicator voor NIRS. Herhaalbaarheid (interval tussen de tests: 2 min), korte termijn reproduceerbaarheid (intervallen van 20 en 40 min) en lange termijn reproduceerbaarheid (interval> 2 weken) werden vastgesteld. We vonden een variatiecoëfficiënt (CV) van 12,5% voor de herhaalbaarheid en een CV van 11,7% voor de korte termijn reproduceer-baarheid. De CV voor de lange termijn reproduceerbaarheid was 15%. We concluderen dat NIRS reproduceerbaar het CBV kan meten bij personen van 65 jaar en ouder, met behulp van de O_2-methode. In deze groep van gezonde proefpersonen werd deze methode bovendien goed verdragen.

Hoofdstuk 5. Cerebrale bloedvaten reageren sterk op veranderingen in CO_2. Dit verschijnsel heet vasomotore reactiviteit, en is al uitvoerig gebruikt in de klinische praktijk en in onderzoek om de hersenvaatfunctie te evalueren. Vaak wordt Transcraniële Doppler echografie (TCD) gebruikt voor het meten van veranderingen in de cerebrale bloeddoorstroming-snelheid, samen met veranderingen in het eind-expiratoire CO_2 (als maat voor de hoeveelheid arterieel CO_2 (de partiële druk of pCO_2). Echter niet alleen de hersendoorbloeding, maar ook de bloeddruk wordt beïnvloed door veranderingen in CO_2. De effecten van deze bloeddrukveranderingen op de cerebrale bloeddoorstroming zullen de interpretatie verstoren van de directe effecten van CO_2 op de hersenvaten (de vasomotore reactiviteit van CO_2).

Dit hoofdstuk beschrijft een methode voor het analyseren van vasomotore reactiviteit door te kijken naar veranderingen in zowel de stroomsnelheid in de hersenvaten als de bloeddruk. Door te kijken naar veranderingen in deze parameters in opeenvolgende ademteugen komt de niet-lineaire dynamica van de relatie tussen CO_2 en cerebrale bloeddoorstroming aan het licht. Hierdoor is deze methode mogelijk meer gevoelig dan eerder gebruikte methoden, die zijn uitgegaan van een lineair verband.

Hoofdstuk 6. Dit hoofdstuk onderzoekt hoe de verdere analyse van spontane schommelingen in bloeddruk en cerebrale bloeddoorstroming kan worden gebruikt voor de kwantificering van de dynamische relatie tussen druk en doorbloeding (flow). Zoals besproken in hoofdstuk 2 kunnen deze metingen worden verricht in rust, waardoor ze vooral geschikt lijken voor toepassing bij oudere patiënten. Echter, spontane oscillaties hebben vaak kleine amplitudes, en aan de klinische relevantie van deze schommelingen kan dus worden getwijfeld. Spontane oscillaties hebben verschillende frequenties (of periodes), van het zeer lage frequentie bereik (<0,07 Hz, wat gelijk is aan een schommeling met een periode van 15 seconden of langer) naar het hoge frequentiebereik (0,2 - 0,35 Hz, of een periode van 3-5 seconden). Zoals beschreven in hoofdstuk 2, is de cerebrale autoregulatie bijzonder effectief voor zeer laag frequente drukoscillaties, waardoor het van belang is dat dit frequentie bereik goed wordt gemeten. Helaas is het vaak moeilijk om aan te tonen dat alle waargenomen zeer laagfrequente schommelingen in de cerebrale bloeddoorstroming gerelateerd zijn aan schommelingen in de bloeddruk. In dit hoofdstuk hebben we onderzocht of het mogelijk en haalbaar zou zijn om herhaalde hurk-sta bewegingen (bv. 10 seconden hurkstand afgewisseld door 10 seconden staan) te gebruiken om grote schommelingen in de bloeddruk in een frequentie van 0,025 en 0,05 (in het zeer lage frequentie bereik) en 0,1 Hz (in het lage frequentiebereik) te veroorzaken. Inderdaad bleek het mogelijk om grote oscillaties op deze specifieke frequenties teweeg te brengen. We concluderen dat het gebruik van herhaalde hurk-sta bewegingen ons in staat stelt om dynamische cerebrale autoregulatie te bestuderen in de lage frequenties, onder condities van hemodynamisch sterke en causaal gerelateerd oscillaties in druk en flow. Dit verbetert de nauwkeurigheid van de metingen van dynamische cerebrale autoregulatie, en versterkt de klinische relevantie van deze methode omdat de sterkte van de veroorzaakte schommelingen in bloeddruk lijkt op die van

hemodynamische veranderingen die gepaard gaan met houdingsveranderingen in het dagelijks leven.

Deel 3. Toepassing in klinisch onderzoek

In dit hoofdstuk hebben we de methode beschreven in hoofdstuk 5, toegepast, om cerebrale vasomotore reactiviteit voor CO_2 te onderzoeken bij patiënten van middelbare leeftijd met hypertensie (gemiddelde leeftijd 52 jaar, spreiding 40 -66). Bij hypertensie kan de cerebrale vasomotore reactiviteit voor CO_2 verminderd zijn als gevolg van cerebrovasculaire “remodeling” (veranderingen in structuur, wanddikte en diameter van hersenvaten) en endotheeldysfunctie. Bij nieuw gediagnosticeerde nog onbehandeld patiënten met hoge bloeddruk, zou verlaging van de bloeddruk door behandeling een nadelige invloed kunnen hebben op de CO_2-reactiviteit. In dit hoofdstuk hebben we 11 patiënten onderzocht met nieuw gediagnosticeerde, onbehandelde lichte tot matige hypertensie en 8 controles. Bij de uitgangsmeting bleek hypertensie niet geassocieerd met verminderde CO_2-reactiviteit. Bij patiënten werd door de behandeling met losartan / hydrochloorthiazide de bloeddruk effectief verlaagd. Na 1-2 weken van de behandeling, en na 3 maanden van de behandeling, was de cerebrovasculaire weerstand afgenomen (duidend op vasodilatatie) en de cerebrale bloeddoorstromingssnelheid bleef stabiel, maar de CO_2-reactiviteit werd niet beïnvloed. In een eerdere studie hebben we aangetoond dat dynamische cerebrale autoregulatie niet wordt aangetast door een milde hypertensie, noch door een snelle danwel een langdurige verlaging van de bloeddruk. Deze studie vormt een aanvulling op deze bevindingen door aan te tonen dat de reactiviteit van CO_2 evenmin is aangetast. Eerdere suggesties waarbij hypertensie in verband wordt gebracht met een verminderde vasomotore reactiviteit lijken te worden verklaard door cerebrovasculaire comorbiditeit of een tevens bestaande diabetes.

Hoofdstuk 8. Hier zijn de methoden, beschreven in hoofdstuk 5 en hoofdstuk 6, toegepast om na te gaan of er verschil is in cerebrale hemodynamische eigenschappen tussen patiënten met de ziekte van Alzheimer en gezonde controles van vergelijkbare leeftijd. In dit hoofdstuk wordt de nadruk gelegd op de overdrachtsfunctieanalyse (transfer function analysis) als methode om dynamische cerebrale autoregulatie te kwantificeren. Deze analyse maakt gebruik van specifieke parameters zoals demping (gain), faseverschuiving en coherentie voor de kwantificering van de relatie tussen de bloeddruk en de hersenendoorbloeding. In deze pilotstudy hebben we aangetoond dat de toepassing van deze methoden haalbaar is. Hoewel wij een klein aantal personen bestudeerden en de Alzheimer patiënten onderzocht werden in een vroeg stadium van de ziekte, vonden we opmerkelijke veranderingen in bloeddrukregulatie en hersenendoorbloeding bij deze patiënten. De cerebrovasculaire weerstand was verhoogd, en de cerebrale bloeddoorstroming was verminderd, zelfs als werd gecorrigeerd voor verschillen in het totale hersenenvolume. Bovendien waren de spontane oscillaties in de cerebrale bloeddoorstroming verlaagd bij de ziekte van Alzheimer ondanks versterkte bloeddruk oscillaties. Deze bevindingen wijzen erop dat al in een vroeg-klinische fase, de ziekte van Alzheimer geassocieerd is met hemodynamische veranderingen. Bovendien konden deze veranderingen worden beschreven met behulp van de methoden die in dit proefschrift zijn beschreven.

Acknowledgements/Dankwoord

Mijn promotor en co-promotor in Nijmegen, prof. Marcel Olde Rikkert en dr. René Jansen, wil ik danken voor hun uitstekende coaching bij dit project. Er was veel te regelen: investeringen in nieuwe apparatuur, een onderzoeksverblijf in de VS, afstemming van onderzoekswerk met de klinische taken en onderwijs, om maar enkele voorbeelden te noemen. Zonder jullie hulp had ik dit niet kunnen doen. Beste Marcel, je spreekt en denkt graag in voetbalmetaforen. Je bent een getalenteerde coach met ambitie en gedrevenheid, en jij weet de Nijmeegse Geriatrie te laten scoren, nationaal en internationaal, en het beste uit je medewerkers naar boven te halen. Beste René, jij hebt een zeldzame combinatie van talenten: clinicus, docent, onderzoeker, en manager. Ik heb veel te danken aan die vaardigheden, en veel geleerd van je managementvaardigheden voor mijn onderzoek. Daarnaast kwam je ervaring met wonen en werken in de VS goed van pas.

I am indebted to my supervisor and co-supervisor in the USA, prof. Benjamin Levine and dr. Rong Zhang. Dear Ben, you kindly accepted my request to temporarily become part of your research institute. You are an experienced researcher in cardiovascular physiology, and you direct an institute that is highly competitive in its research but maintains an atmosphere of friendly collegiality. I have been fortunate to have learned so much from you and your co-workers. Dear Rong, much of the knowledge found in this thesis I have learned from you or through you. You are a true scientist, and a most kind human being with a broad interest. What initially started out as a plan for a brief visit, has grown into a well-spent 7 months of research with two published papers and four underway. I look forward to our continued collaboration.

For their participation in the thesis defense, I am most grateful to professors Jacques Lenders, Maria Hopman, Philip Scheltens, Flavio Nobili, Monique Breteler, and doctors Jaco Pasman and Mirek Latka.

Many people in Dallas, TX were involved in this doctoral thesis. I am grateful for the participation of the patients with Alzheimer's disease, their caregivers, and the healthy controls, all from the ADC at UT Southwestern Medical Center. I was touched by the patients' fervor to contribute to this research, and by the pride with

which they bear their disease. I am much obliged to Kristin Martin-Cook for her help with recruitment, and to Ramon Diaz-Arrastia for his support. At the IEEM, many thanks to Cynthia, Phyllis and Sherry for getting me settled at the IEEM, and Robin, Collin, Jeff, David, Qi and Katja for research assistance and fun. And last but not least, many thanks to the wonderful people at Calvary (Jim, Patti, John, Robin, John, Becky, and our kindergarten teacher Mrs J.) for providing a school for Oliver and helping my family to get settled in the US.

In Nijmegen ben ik dank verschuldigd aan de groep van 30 proefpersonen, die mij trouw gedurende deze promotie heeft gevolgd, en aan onderzoeksverpleegkundige Francien Jannink en physician assistant Anja Bos. De afdeling neurofysiologie was een belangrijke steun bij het opzetten van de transcraniele Doppler metingen, in de personen van Jaco Pasman, Yvonne, Wilma en Lucia. Willy Colier (Artinis) was een belangrijke hulp bij het ontwikkelen van de meetopstelling, waarin uiteraard “zijn” NIRS. Dank aan de afdeling Geriatrie in haar volle breedte (a(n)ios, collegae klinisch geriaters, paramedici, secretariaat, verpleeghulpen, verpleegkundigen, en voedingsassistenten) voor belangstelling en ondersteuning op diverse manieren, begrip voor mijn soms conflicterende agenda’s, en ook voor het –althans door sommigen…- jaarlijks meerennen in mijn Geriatric Giants zevenheuvelenloopteam.
Met het starten van mijn promotieonderzoek stapte ik met één been in een andere wereld; de onderzoekswereld. Gedurende mijn promotietraject is de onderzoeksgroep van de afdeling geriatrie sterk gegroeid. Mijn collega-onderzoekers en deels oud kamergenoten, waarvan enkelen onlangs gepromoveerd of benoemd to hoogleraar, wil ik hartelijk danken voor hun bijdragen om onze geriatrische onderzoeksgroep te laten uitgroeien tot een omgeving waarin met veel plezier op hoog niveau onderzoek wordt gedaan: Arenda, Miriam, Maud, Marianne, Liesbeth, Roy, René, Olga, Els, Lia, Marieke, Miriam, recent gestart Madelijn, Diane en Petra, en als brug naar de patiëntenzorg Anja en William. René, inmiddels post-doc, is een belangrijke steun en drijvende kracht voor ons onderzoek. Arenda is door haar combinatie van enthousiasme, gedrevenheid, talent en gezelligheid de ideale onderzoeker om te begeleiden en een aanwinst voor mijn onderzoekslijn: de lat ligt hoog voor de opvolgers! Miriam (ontdekt in Dallas) zorgde voor een succesvolle onderzoeksbespreking en reanimeerde samen met Marieke en Arenda de borrelcultuur. Mijn afgelopen promotiejaren kon ik voor ontspanning en humor altijd rekenen op die “harde borrelkern” Arenda, Miriam en Marieke: jullie zijn echt ontzettend leuke meiden.

Suzanne, coole meid en al jaren onze vriendin, en Louis, “running mate” waarmee ik wekelijks al rennend de belangrijke zaken uit het leven (en soms onderzoek) bespreek, leuk dat jullie mijn paranimfen zijn.

Tot slot mijn familie: veel dank aan mijn ouders en mijn schoonouders voor hun steun, aan Mireille voor een heleboel, waaronder het mogelijk maken dat ook mijn “vrije tijd” grotendeels in dit proefschrift gestoken kon worden, en het meeverhuizen naar het idyllische Texas, en aan mijn drie boefjes Olivier, Laura en Izzy, voor het regelmatig onmogelijk maken om tijd aan onderzoek te besteden.

Publications

Dynamic cerebral autoregulation during repeated squat-stand maneuvers
JA Claassen, BD Levine, R Zhang
J Appl Physiol. 2008; in press

Cerebral perfusion in neurogenic orthostatic hypotension
JA Claassen
Lancet Neurol. 2008;7:573-4

Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly
AH van Beek, JA Claassen, MG OldeRikkert, RW Jansen
J Cereb Blood Flow Metab. 2008;28:1071-85

Alzheimer's disease: trade-off for increased survival with atherosclerosis?
JA Claassen
Ann Neurol. 2007; 10.1002/ana.21285

Cerebral hemodynamics after short and long-term reduction in blood pressure in mild and moderate hypertension
R Zhang, S Witkowski, Q Fu, JA Claassen, BD Levine
Hypertension 2007;49:1149-55

Transcranial Doppler estimation of cerebral blood flow and cerebrovascular conductance during modified rebreathing
JA Claassen, R Zhang, S Witkowski, Q Fu, BD Levine
J Appl Physiol. 2007;102:870-720

Cholinergically mediated augmentation of cerebral perfusion in Alzheimer's disease and related cognitive disorders: the cholinergic-vascular hypothesis
JA Claassen, RW Jansen
J Gerontol A Biol Sci Med Sci. 2006;61:267-71

Reproducibility of cerebral blood volume measurements by near infrared spectroscopy in 16 healthy elderly subjects
JA Claassen, WJ Colier, RW Jansen
Physiol Meas. 2006;27:255-64

Carotid sinus syndrome: looking sideways is sufficient cause for syncope
JA Claassen, RW Jansen
J Am Geriatr Soc. 2006;54:188-9

The serotonin syndrome
JA Claassen, HP Gelissen.
N Engl J Med. 2005;352:2454-6

Life-threatening serotonin syndrome following a single dose of a serotoninreuptake inhibitor during maintenance therapy with a monoamine oxidase inhibitor
AM Zonneveld, M Hagenaars, NC Voermans, HP Gelissen, JA Claassen.
Ned Tijdschr Geneesk 2006;150:1081-4

'Gold standard', not 'golden standard'
JA Claassen
Ned Tijdschr Geneeskd. 2005;149:2937

Diagnostic bias in (supra) centenarians
JA Claassen
Age Ageing 2006;35:326

Tellymedicine
JA Claassen.
BMJ 2005;331:1185

The gold standard: not a golden standard
JA Claassen
BMJ 2005;330:1121

Personal View. Epidemiology: friend or foe?
JA Claassen
BMJ 2004;329:467

Immunodeficiency in adult patients as the first sign of a primary underlying condition
JA Claassen, RA de Vries, C Richter
Ned Tijdschr Geneeskd. 2003;147:93-5.

Validation of the supine venous pump function test: a new non-invasive tool in the assessment of deep venous insufficiency
MC Janssen, JA Claassen, WN van Asten, H Wollersheim, MJ de Rooij, T Thien
Clin Sci 1996;91: 483-88

The emperor's pointer
JA Claassen
BMJ 2002;324:418

Vasculaire dementie
JA Claassen
Dementie Actueel 2007

Hypertensiebehandeling bij ouderen
JA Claassen
Patient Care 2008

Bloeddrukregulatie bij ouderen
JA Claassen
Patient Care 2007

Curriculum Vitae

Jurgen Claassen was born on April 20 1969 in Maastricht, the Netherlands. He entered Medical School at Radboud University Nijmegen in 1987. His interest in cardiovascular research was initiated by a research fellowship at the departments of nuclear medicine and cardiology at the CHUV, Lausanne, Switzerland in 1992, for which he was awarded by the Netherlands Heart Foundation. In 1995 he obtained his medical degree from Radboud University Nijmegen, as well as the United States Medical License (USMLE I and II). He then worked in internal medicine (general internal medicine and subspecialties) in the United Kingdom and the Netherlands, followed by geriatrics fellowship training at Radboud University Nijmegen Medical Center. He became board certified in 2004 and joined the staff of the department of Geriatric Medicine. Preparations for the research presented in this thesis were made in 2005. During seven months in 2005-2006 he was a visiting scientist at the Institute for Exercise and Environmental Medicine, Presbyterian Hospital and University of Texas Southwestern Medical Center at Dallas, TX, in collaboration with the department of Neurology. His continued research is supported by grants from Internationale Stichting Alzheimer Onderzoek (2006) and the Netherlands Heart Foundation (2008).

He is married to Mireille Huigens. They have three children: Olivier, Laura and Isabelle.

Jurgen Claassen werd geboren op 20 april 1969 te Maastricht, en groeide op in de omgeving van Nijmegen. In 1987 behaalde hij het eindexamen aan het Stedelijk Gymnasium Nijmegen, waarna hij Geneeskunde studeerde aan de Radboud Universiteit Nijmegen. In 1992 ontving hij een beurs van de Nederlandse Hartstichting voor cardiovasculair onderzoek aan de CHUV in Lausanne. In 1995 behaalde hij het Nederlandse en Amerikaanse artsexamen, waarna hij voor enkele maanden als senior house officer in Engeland werkte, waar hij kennis maakte met het vak Geriatrie. Na terugkeer in Nederland werkte hij in het Canisius Wilhemina Ziekenhuis op de afdelingen cardiologie, longziekten, en interne geneeskunde, waarna hij in 1999 startte met de opleiding tot klinisch geriater in Ziekenhuis Rijnstate (interne geneeskunde), UMC St. Radboud (klinische geriatrie) en GGZ Oost Brabant (ouderenpsychiatrie). In 2004 volgde registratie als klinisch geriater en trad hij toe tot de staf van de afdeling klinische geriatrie van het UMC St Radboud. In dat jaar werden ook de voorbereidingen getroffen voor dit proefschrift. In 2005 en 2006 werkte hij zeven maanden als visiting scientist aan het Institute for Exercise and Environmental Medicine in Dallas, USA. Hier verrichtte hij een deel van de in dit proefschrift opgenomen onderzoeken op het gebied van cardiovasculaire en cerebrovasculaire fysiologie. Ook werd hier een belangrijke basis gelegd voor het vervolgonderzoek, dat hij na terugkeer in Nijmegen verrichte naast zijn werkzaamheden als klinisch geriater. In 2006 ontving hij een beurs van Internationale Stichting Alzheimer Onderzoek waarmee de onderzoekslijn naar vasculaire aspecten bij de ziekte van Alzheimer kon worden opgezet. In 2008 werd een beurs toegekend door de Nederlandse Hartstichting, waarmee de onderzoekslijn naar de interactie tussen hypertensie, cerebrale perfusie en cognitie verder kan worden voortgezet.

Jurgen Claassen is sinds 1997 getrouwd met Mireille Huigens en is vader van Olivier (1999), Laura (2003) en Isabelle (2005).

Age and Ageing 2006; **35:** 326
doi:10.1093/ageing/afj004

Diagnostic bias in (supra-)centenarians

There is a risk of positive bias in our judgement of how well centenarians are doing. We may want to demonstrate to our medical students that ageing is not synonymous with cognitive decline or frailty, and are eager to find role models for successful ageing. When we portray these individuals, there is the danger that we—subconsciously—fall victim to ageism, by lowering our standards of expectation for those who have reached the magical age of 100.

To test this hypothesis, we designed a study that enabled us to obtain an unbiased geriatric assessment in a well-known supra-centenarian.

Given the scarcity of well-known (supra-)centenarians, we decided to enroll Mr Claus. Mr Claus is a publicly known figure who pays seasonal visits to the UK. The general opinion on Mr Claus is that he is a fine example of successful ageing. We were unable to obtain his informed consent; however, it is generally felt to be unnecessary to obtain informed consent from public figures before publicly revealing, discussing and interpreting any of their potentially relevant medical conditions. Two geriatricians participated in this study. One geriatrician carefully studied the existing extensive media coverage of Mr Claus, and analysed relevant aspects of his behaviour that could assist in making a comprehensive geriatric diagnosis. Special attention was paid to Mr Claus' pattern of speech, his social interaction, his attitude towards others (including children), his driving skills, as well as direct and indirect evidence for his visuospatial orientation. These observations were presented to the second geriatrician in a way that would not reveal either the age or identity of the subject. This blinded geriatrician then established a geriatric diagnosis on the basis of these observations.

Mr Claus' speech was interpreted as being inappropriately jovial and loud, with obvious repetition (for example his use of the utterance 'Ho'). His social behaviour demonstrated a reduced sense for social constraints (obstructing the entrance to busy warehouses, scaring children or putting them on his lap). There was evidence for disinhibition bordering on mania (an almost obsessive habit to buy presents for a large number of people). In contrast, memory was relatively preserved (remembering to visit each year, remembering for whom presents were bought) as were visuospatial capabilities (advanced driving skills including passing the Reindeer test, box-in-chimney test and fill-the-stocking test). Finally, a positive family history was noted (Mr Claus has a cousin who lives on the continent and displays very similar behaviour). On the basis of these findings, the blinded geriatrician diagnosed a probable frontotemporal dementia (FTD) in Mr Claus. The key features of FTD that were observed in this subject are presented below.

Key clinical features in Mr Claus supporting a diagnosis of FTD

- Extraordinary joviality and verbal output, with repetitive speech
- Disinhibition
- Lack of judgment, lack of respect for social constraints (may include inappropriate behaviour)
- Change in ingestive behaviour, with excessive eating leading to weight gain
- Preservation of orientation and visuospatial functioning
- Preservation of memory
- Stereotypic and perseverative behaviour
- Positive family history in some patients

This preliminary study demonstrated that a blinded and unbiased analysis of cognitive function in a well-known centenarian, who is generally perceived as an example for successful ageing, can reveal serious but hitherto unrecognised disturbances compatible with a diagnosis of neurodegenerative disease [1].

JURGEN A. H. R. CLAASSEN
Department of Geriatric Medicine, Radboud University Medical Centre, Internal mail code 931, Postbox 9101, 6500 HB Nijmegen, The Netherlands
Fax: (+31) 24 3617408
Email: j.claassen@ger.umcn.nl

1. Knopman DS, Boeve BF, Parisi JE *et al.* Antemortem diagnosis of frontotemporal lobar degeneration. Ann Neurol 2005; 57: 480–8.