UNIVERSITY OF SOUTHAMPTON

RECOVERY OF CEREBRAL AUTOREGULATION DURING THE FIRST THREE MONTHS AFTER ACUTE ISCHAEMIC STROKE - A Longitudinal Study Using Transcranial Doppler Ultrasonography

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ABSTRACT

FACULTY OF MEDICINE – DEPARTMENT OF GERIATRIC MEDICINE

Master of Philosophy

Recovery of cerebral autoregulation during the first three months after acute ischaemic stroke - A longitudinal study using transcranial Doppler ultrasonography

by Dr Joseph Shiu Kwong Kwan

Cerebral autoregulation (CA) is a mechanism that maintains a constant cerebral blood flow over wide ranges of blood pressure (BP). CA is impaired after stroke and changes in cerebral blood flow may become passive to changes in BP. Manipulation of BP after stroke may worsen regional cerebral blood flow with possible clinical implications. However, little is known about the recovery of CA soon after stroke. In this study, we used transcranial Doppler (TCD) to examine the recovery of CA after ischaemic stroke.

We studied ten stroke patients and eleven controls. Each patient was examined within seven days, at six weeks, and at three months. For each examination, cerebral blood flow velocity (CBFV) and BP were continuously recorded by bilateral TCD and Finapres, respectively. We examined the relationship between spontaneous oscillations of BP and CBFV, and between induced BP oscillations (by rhythmic handgrip) and CBFV, using cross-spectral transfer function analysis (calculation of phase, gain, and coherence).

We demonstrated that, using rhythmic handgrip, CA changes over the first three months after stroke, although no difference was found between patients and controls. Moreover, these changes appear to affect the hemisphere contralateral to the ischaemia, which suggests that disturbances in cerebral haemodynamics after stroke may be global in nature. We also found that the competence of CA was dependent on the frequency of BP oscillations. This indicates that CA behaves as a high-pass filter system, even after ischaemic stroke. The combination of TCD and rhythmic handgrip appear to be a useful non-invasive method of assessing CA after stroke. This technique may become a useful clinical tool in the future.

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PREFACE

Stroke is a devastating disease that accounts for considerable mortality and morbidity. It is also a common disease, with at least 130,000 patients suffering their first ever stroke per year. The majority of these are ischaemic strokes. However, despite enormous efforts to discover effective treatments that would improve clinical outcome, there are only very few pharmacological agents that have been shown to make a difference. Research has also started to concentrate on ways to limit the damage to the ischaemic areas and encourage neuronal recovery. The important discovery of the ischaemic penumbra is just an example of the progress that has been made. Each step of the biochemical cascade that take place within the ischaemic penumbra may be a target for an effective therapy.

Another area that deserves much attention is the disturbances in cerebral haemodynamics and autoregulation of blood flow after ischaemic stroke. There is inadequate knowledge of both the impairment and recovery of these disturbances, which may have important clinical consequences. For example, trials evidence is awaited on whether blood pressure lowering soon after stroke is associated with clinical improvement. Physiological studies in these areas, which there are few, are vital in order to provide physicians with a sound scientific background on which to design the appropriate trials.

This thesis describes a physiological study that was conducted between October 1998 and August 1999. The recovery of cerebral autoregulation during the first three months after acute ischaemic stroke was explored, using transcranial Doppler ultrasonography, which has become the most popular technique for pathophysiological studies of this kind. For this purpose, the relationship between oscillations of blood pressure (spontaneous and induced) and cerebral blood flow velocity was examined using cross-spectral transfer function analysis. The present study has also evaluated the use of rhythmic handgrip as a new and non-invasive technique of stimulating blood pressure oscillations. It is our aim that the findings of this study can provide a basis for future research on this important disease.

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LIST OF ABBREVIATIONS

General Abbreviations

OCSP	Oxford Community Stroke Project
CT	Computerized Tomography
MRI	Magnetic Resonance Imaging
MRA	Magnetic Resonance Arteriography
PET	Positron Emission Tomography
SPECT	Single-Positron Emission Computerized Tomography
TACS	Total Anterior Circulation Stroke
PACS	Partial Anterior Circulation Stroke
LACS	Lacunar Stroke
POCS	Posterior Circulation Stroke
AVM	Arterio-Venous Malformation
SCOI	Small Centrum Ovale Infarction
MCA	Middle Cerebral Artery
BP	Blood Pressure
CBF	Cerebral Blood Flow
CBFV	Cerebral Blood Flow Velocity
CPP	Cerebral Perfusion Pressure
CVR	Cerebral Vascular Resistance
$CMRO_2$	Cerebral Metabolic Rate for Oxygen
CMR_{gl}	Cerebral Metabolic Rate for Glucose
OEF	Oxygen Extraction Fraction
Sjo_2	Jugular Bulb Saturation of Oxygen
Po_2	Partial Pressure for Oxygen
Pco_2	Partial Pressure for Carbon Dioxide
rt-PA	Recombinant Tissue Plasminogen Activator
ATP	Adenosine Tri-Phosphate

Transcranial Doppler & Analysis-Related Abbreviations

TCD	Transcranial Doppler
TCDM	Transcranial Doppler Mapping
CC-TCD	Colour-Coded Transcranial Doppler
CE-TCCS	Colour-Enhanced Transcranial Colour-Coded Sonography
LDF	Laser Doppler Flow
Vs	Peak Systolic Velocity
Vd	End-Diastolic Velocity
Vm	Time-Averaged Mean (TAM) Velocity
Vmean	Instantaneous Mean Velocity
Vmax	Maximum Velocity
Vmin	Minimum Velocity
PI	Pulse Index
HITS	High Intensity Signal
BWE	B-Wave Equivalent
FFT	Fast Fourier Transformation
sCA	Static Cerebral Autoregulation Index
MSC	Mean Squared Coherence
SD	Standard Deviation
SEM	Standard Error of Mean
RHG	Rhythmic Handgrip

CHAPTER 1 INTRODUCTION

1.1 Stroke

In this section, the definitions, burden, pathological types, and clinical subtypes of stroke will be briefly discussed to provide background to the subject of cerebrovascular disturbances after acute stroke. The present study specifically concentrates on ischaemic stroke in the middle cerebral artery territory, which will be described in detail.

1.1.1 Definitions of Stroke & Transient Ischaemic Attack

The definition of stroke is a clinical one. According to the World Health Organisation, stroke is defined as:

"A syndrome characterised by rapidly developing clinical symptoms and/or signs of focal, and at times global, loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin" [1]

This World Health Organisation definition includes stroke due to cerebral infarction, primary intracerebral haemorrhage, intraventricular haemorrhage and symptomatic subarachnoid haemorrhage. It, however, does not include subdural haemorrhage, epidural haemorrhage, and intracerebral haemorrhage or infarction caused by infection or tumour.

The definition of transient ischaemic attack differs chiefly in the time domain, being "a clinical syndrome characterised by an acute loss of focal cerebral or monocular function with symptoms lasting less than 24 hours and which is thought to be due to inadequate cerebral or ocular blood supply as a result of arterial thrombosis or embolism" [2].

The differentiation between these two conditions is essentially arbitrary and not particularly informative when considering the pathophysiology, management, and prognosis. However, the distinction is of greater significance in differential diagnosis, case-control studies and measurement of stroke incidence.

1.1.2 Burden of Stroke

Stroke is the third most common cause of death in most Western countries, after coronary heart disease and cancer. In the United Kingdom (UK), it accounts for 12% of all deaths and is the most important cause of severe disability [3]. Every year at least 130,000 people in the UK have a stroke for the first time. A fifth die within the first month and a significant proportion of survivors remains permanently disabled. In the UK, stroke accounts for around 5% of all National Health Service costs and this is likely to rise with an increase in both the elderly population and the incidence of stroke [4].

Although the costs related to acute hospital and community care of stroke are substantial, it is mainly the costs related to long-term care of disabled survivors that account for most of the overall lifetime expenditure. The financial burden on the society as a whole is enormous but is extremely difficult to estimate because it is mostly born by the patients' families and social services. Approximately one in four people who have a stroke is of working age and so its indirect costs, such as the impact on national productivity, also need to be taken into consideration [5].

1.1.3 Pathological Types of Stroke

Diagnosis of the pathological type of stroke is clinically important since treatment, prognosis and secondary prevention are different for ischaemic and haemorrhagic stroke. In the UK, community-based studies, such as the Oxfordshire Community Stroke Project ('OCSP', 1990), have provided invaluable insight into the causes of first-ever stroke according to the different pathological types. The OCSP demonstrated that around 80% of strokes were ischaemic in origin, secondary to the complications of atherothrombosis, intracranial small vessel disease or embolism from the heart. Around 10% were due to primary intracerebral haemorrhage and 5% to subarachnoid haemorrhage. The remaining 5% had an unusual underlying cause, such as venous infarction, or an uncertain cause [6].

From the point of view of clinical practice, the first step to classification is the distinction of cases of ischaemic stroke from those due to primary intracerebral haemorrhage. This distinguishes groups of stroke with different aetiology, prognosis and rate of recurrence. It also influences decisions about medical and possible neurosurgical treatments. The standard method for making this distinction is by performing computerised tomography (CT) or magnetic resonance imaging (MRI) of

the brain. A CT brain scan can reliably detect intracranial haemorrhage immediately and it should therefore be performed as soon as practically possible, preferably within a few days of onset of stroke. The necessity for promptness is due to a reducing sensitivity and specificity of the CT brain scan over time (weeks), as the haematoma evolves in appearance [5].

1.1.4 Clinical Subtypes of Stroke

Apart from the ability to identify intracranial haemorrhage, a CT or MRI brain scan can also help to provide information on the size and site of the lesion, confirming the diagnosis from clinical findings. Stroke can be classified into different clinical subtypes according to the clinical signs exhibited by the patient [7]. The clinical subtypes of stroke include total anterior circulation syndrome (TACS), partial anterior circulation syndrome (PACS), lacunar syndrome (LACS), and posterior circulation syndrome (POCS) [7]. This classification, devised by Bamford *et al.* (1991), also provides estimation of prognosis for each subtype of stroke in terms of rate of recurrence, probability of being alive and independent, and alive and dependent, at one year. Mortality rate can also, therefore, be readily calculated. For instance, a patient with TACS (e.g. from a total occlusion of the internal or middle cerebral artery) has a 4% chance of being alive and independent, 36% chance of being alive and dependent, and a 6% chance of recurrence, at one year. The risk of mortality is, therefore, 60%. This classification, together with individual patient characteristics, enables the clinician to plan appropriate investigations, better predict the outcome, and communicate more informatively with the patient and relatives. Data from the OCSP (1990) revealed that 51% of cerebral infarction were of the anterior circulation syndromes (total and partial), which were also associated with the poorest prognosis; 96% of patients with TACS, and 43% of patients with PACS, were dead or dependent at one year [6].

1.1.5 Ischaemic Stroke in the Middle Cerebral Artery Territory

The clinical syndrome arising from ischaemia or infarction affecting the middle cerebral artery (MCA) depends on the segment of MCA that is occluded. The MCA has four main segments that are susceptible to pathology; they include the main stem, deep perforating (lenticostriate) arteries, cortical branches and the medullary perforating arteries. The anatomy of the MCA will first be described in detail.

The first segment of the MCA tracks laterally between the upper surface of the temporal lobe and the inferior surface of the frontal lobe until it reaches the Sylvian fissure. From the proximal part of the MCA main stem a variable (usually 6 to 12) number of lenticostriate arteries branch at right angles to the parent artery and enter the anterior perforated substance. The areas supplied by the lenticostriate arteries, which are functional end-arteries, include the lentiform nucleus, caudate nucleus, internal capsule, and globus pallidus. In the Sylvian fissure, the MCA bifurcates to give superior and inferior divisions, which in turn give rise to cortical branches, supplying various areas of the cortex. MCA medullary perforating arteries, which are also functional end-arteries, arise from the cortical arteries of the surface of the hemispheres. They supply the subcortical white matter (i.e. the centrum ovale) [5].

Occlusion of the MCA main stem, either from impaction of an embolus or extension of a more proximal thrombus (e.g. from the internal carotid artery), often occurs in the proximal portion, thereby involving the lenticostriate arteries. Consequently, there is ischaemia of both the deep and superficial territories of the MCA. Typically, this presents clinically with a contralateral hemi-motor and sensory deficit, hemianopia and disturbance of higher cortical functions (e.g. dysphasia, sensory inattention), i.e. signs of a total anterior circulation syndrome. It may, however, only present with two of these three signs, i.e. a partial anterior circulation syndrome.

Occlusion of a single lenticostriate artery results in a lacunar syndrome. A lacune appears as a small and deep infarct on CT brain scan. Clinically, it may present with a pure motor or sensory deficit, or ataxic hemiparesis. Fisher *et al.* (1965) showed that up to 80% of lacunes are clinically silent [8]. Lacunar syndromes are usually caused by local vasculopathies, a consequence of chronic conditions such as hypertension and diabetes mellitus, rather than embolism.

Occlusion of the superior division of the MCA (cortical branches) produces a clinical syndrome similar to that from occlusion of the MCA main stem. Occlusion of the inferior division (cortical branches) usually causes a hemianopia or dysphasia, with or without inattention. The two main occlusive mechanisms are embolism and low flow secondary to more proximal vascular lesions [9].

Lastly, occlusion of a single MCA medullary perforating artery causes 'small centrum ovale infarct' (SCOI), which presents with clinical signs similar to those of a lacunar syndrome. Nevertheless, larger infarcts may rarely occur and present with

signs of total or partial anterior circulation syndromes. Although SCOI have traditionally been regarded as a lacunar syndrome, Lammie *et al.* (1999) showed that "the lesions of SCOI were pathologically and pathogenetically heterogeneous, and although SCOI presents clinically with signs of lacunar syndrome, they might represent conditions of different aetiologies" [10].

1.2 Cerebral Blood Flow and Ischaemic Stroke

1.2.1 Technological Advances

In the last half of the century, there have been tremendous developments in the technology for studying cerebral haemodynamics and imaging of the brain. Methods of measuring cerebral blood flow have become more accurate and reliable and have had a major impact on our understanding of the regulation of cerebral blood flow and the pathophysiology of cerebral ischaemia. Until ten years ago, the only regional physiological measurements in the human brain that were available for routine clinical investigations have been cerebral blood flow and to a lesser extent, cerebral blood volume [11]. Older techniques of measuring regional cerebral blood flow in two dimensions utilised the inert and diffusible radio-tracer, xenon 133 (^{133}Xe), either by intracarotid injection or inhalation [12]. The former approach is now abandoned due to its invasiveness, while the latter has been largely replaced by various tomographic approaches, which include positron emission tomography (PET), single-photon emission computed tomography (SPECT), and xenon-computed tomography [12].

During the last six years or so, efforts have been concentrated in developing techniques that are able to measure cerebral blood flow or blood flow velocity non-invasively and continuously. There is now an increased diversity of physiological variables, such as cerebral metabolic rate of oxygen ($CMRO_2$) and brain glucose utilisation (CMR_{gl}), which are available for measurement with the use of new tracers and PET. Other techniques, including transcranial Doppler (TCD) ultrasonography, near infrared spectroscopy, laser flowmetry, and magnetic resonance angiography (MRA) are increasingly used in both research and clinical settings [13]. Some of these techniques enable cerebral blood flow and blood flow velocity to be assessed continuously for considerable periods of time, with reasonable accuracy, acceptable costs and outstanding temporal or (not *and*) spatial resolution [13]. However, clinical applications have been complicated by difficulties in the interpretation of measurements and incomplete understanding of the determinants of cerebral blood flow in health and disease. More research is therefore needed before these techniques can be used in routine clinical practice.

1.2.2 Study of Cerebral Blood Flow after Stroke

Research in stroke has revolutionised the traditional belief that stroke treatment and prevention are of little value. It has been clearly shown that organised treatment for stroke makes a genuine difference. Four major examples of discovery are briefly mentioned here. Firstly, for every twenty patients managed by a multidisciplinary team in a stroke unit, one extra patient survives without disability. Secondly, very early treatment with recombinant tissue plasminogen activator (rt-PA) can substantially improve the chance of a good recovery after an ischaemic stroke for highly selected patients. Thirdly, modification of vascular risk factors such as blood pressure, early use of antiplatelet therapy, and anticoagulation are all helpful in preventing stroke recurrence. Lastly, open carotid artery surgery can prevent recurrent stroke in patients with recent symptoms due to a severe internal carotid artery stenosis [4].

These recent breakthroughs in stroke research have raised the enthusiasm of scientists and researchers around the world. The search for new treatment strategies has been associated with an expansion of knowledge in stroke pathophysiology. There are, however, still many areas in stroke where knowledge is relatively deficient. For example, there is a need for a deeper understanding of the cellular and molecular biological processes, disturbances of cerebral haemodynamics, and genetic adaptation after acute ischaemic stroke.

The increase in the study of cerebral blood flow and haemodynamics after stroke has been aided by the technological advances that have taken place. Much research has been carried out to explore the pathophysiology of cerebral ischaemia. For example, the relationship between cerebral ischaemia and bioenergetic failure (with mitochondrial dysfunction and loss of ion homeostasis), ischaemic penumbra, and the disturbance of brain microcirculation and blood-brain barrier are some of the areas that have been extensively reviewed.

1.2.3 Borderline Ischaemia

Ischaemia is defined as a reduction in cerebral blood flow of sufficient severity to cause functional or metabolic deficits, or overt brain damage [14]. The reduction of cerebral blood flow is usually due to reduced inflow, secondary to a fall in arterial pressure or to vascular obstruction, but it may also be caused by raised intracranial or tissue pressure. The term 'ischaemia' therefore covers a wide variety of conditions

with cerebral blood flow values ranging from those that are borderline for maintenance of cellular functions to those that lead to cessation of flow.

'Borderline' or 'moderate' ischaemia is considered to exist when cerebral blood flow is reduced to less than 50% of control [5]. The signs and symptoms of moderate ischaemia resemble those observed in severe hypoxia. The resultant reduction in oxygen delivery is sufficiently severe to disturb metabolic activities that sustain normal neuronal transmission, but too mild to threaten cellular survival. A similar situation probably exists in tissues at the periphery of a focal ischaemic lesion. In this area, a moderate reduction in perfusion pressure would readily affect the metabolic capacity of the tissue because of the inability to respond to a demand for increased energy production [15]. Borderline ischaemia also initiates compensatory mechanisms which in turn sacrifice electrophysiological activity (hence, suppression of electroencephalogram activity) in order to reduce energy expenditure. This enables near-normal adenosine tri-phosphate (ATP) concentrations and membrane ion gradients to be maintained and cell viability to be preserved, at least temporarily [5]. If moderate ischaemia persists for several hours, however, cell death is inevitable.

This penumbral zone of a focal ischaemic lesion with borderline ischaemia is commonly termed the 'ischaemic penumbra'. The cells within this area are at risk of permanent damage from a further fall in cerebral blood flow to critical level [15]. The pathophysiology of the ischaemic penumbra will now be discussed in detail.

1.2.4 Ischaemic Penumbra

The working brain normally consumes about one-third of its energy for maintenance of synaptic transmission, one-third for transport of sodium and potassium, and one-third for preserving structural integrity. In a state of hypoxia and ischaemia, there is a hierarchy of energy-dependent cellular functions that results in a characteristic sequence of events [16]. It has been shown that a gradual reduction of oxygen delivery produced cerebral functional deficits when oxygen partial pressure (Po_2) fell below 60% of control; with a further sustained fall below 50%, structural damage occurred [16]. Astrup *et al.* (1997) established well defined thresholds of cerebral blood flow needed to support neuronal electrical function (at the upper flow range) and cellular ion homeostasis (at the lower flow range) [17]. The intermediate zone with blood flow rates between these two thresholds is termed the 'penumbra'. The penumbra is characterised as an area of reduced cerebral blood flow with suppressed

neuronal electrical function but without structural damage or abnormal membrane potential [18]. This 'viable but non-functional' collaterally perfused penumbral zone has been demonstrated by Olsen *et al.* (1983) in acute stroke patients [19].

It has been shown that in the ischaemic penumbra surrounding the densely ischaemic core, protein synthesis is inhibited at a flow threshold of about 0.55 ml/g/min, followed by stimulation of anaerobic glycolysis at 0.35 ml/g/min. The release of neurotransmitters and disturbance of energy metabolism occur when cerebral blood flow decreases below 0.2 ml/g/min. When cerebral blood flow is reduced to below 0.15 ml/g/min, complete electrical failure occurs with anoxic depolarisation, but membrane function is still preserved. A massive release of potassium, indicating irreversible loss of membrane function and subsequent cell death, occurs when cerebral blood flow falls below 0.06 to 0.1 ml/g/min [17, 20]. The degree of ischaemia thus represents a threshold for loss of neuronal electrical function (i.e. electrical failure).

There is considerable variation in the absolute cerebral blood flow values that define the thresholds of neuronal transmission and membrane failure. This variation is dependent on the technique of flow measurement, ischaemic model, anaesthesia used, and the animal species under study. An alternative to using absolute flow values, a better expression of thresholds may be to use percentages of control values to describe critical flow rates. For example, the upper threshold can be regarded as 40% and lower threshold as 12 to 15% of control, respectively [16].

In addition to the degree of ischaemia, the duration of ischaemia also determines whether the thresholds are crossed. With prolonged reductions in cerebral blood flow below 0.1 ml/g/min, cellular transport mechanisms and neurotransmitter systems fail, potentially toxic transmitters are released (e.g. L-glutamate), free radicals and lipid peroxides are formed, all of which may damage cells further. Substances such as platelet activating factor, which is released by neurones, may also be neurotoxic. Consequently, infarction occurs and, even if flow is restored, function cannot recover [5].

At the cellular level, three mediators of ischaemic cell death have been found. They are unregulated increases in intracellular calcium concentration, acidosis, and the production of free radicals. The induction of early genes and expression of heat shock proteins may also modulate the process [5]. These processes will be discussed in detail in Section 1.2.5.

Much of the work that has provided knowledge of the localisation and spatial extent of penumbral regions has used models of middle cerebral artery occlusion as the prototype of focal ischaemia. Olsen *et al.* (1983) investigated the ischaemic penumbra in stroke patients using the xenon injection technique for measuring regional cerebral blood flow. Collaterally perfused areas were presumed to be the ischaemic penumbra; the size and location of these areas were found to depend on the site and extent of middle cerebral artery occlusion [21]. The advent of new MRI techniques, namely diffusion-weighted imaging and perfusion-weighted imaging, has enabled repeated non-invasive monitoring of ischaemic brain lesions and rapid detection of the extent of ischaemic injury after stroke.

In patients with acute cerebral infarction, it is unclear how large the ischaemic penumbra is likely to be, how long it is likely to remain in this state and how much recovery is likely if blood flow is restored. Therefore, it is difficult to predict just how long the time window is to allow successful therapeutic intervention in any individual. Accurate information about the ischaemic penumbra can best be obtained from PET scanning. Marchal *et al.* (1996) used PET scanning to show that there was persistence of substantial volumes of potentially viable brain tissue after ischaemic stroke in humans and the therapeutic window might be as long as 17 hours after the onset [22]. Different therapeutic interventions for stroke probably have different therapeutic time windows. For example, the time window for effective thrombolytic therapy may be shorter than for neuroprotective agents. These interesting hypotheses remain to be tested and explored.

1.2.5 Ischaemic Cascade

In cerebral tissue, changes occur in neurones, glial cells, and other structural components in differing degrees and at different times after the onset of ischaemia, which means that in humans cerebral infarction is a dynamic and highly unstable process rather than a discreet event. In other words, infarction is not an 'all-or-nothing' episode [5]. As cerebral blood flow approaches the critical threshold of electrical and ionic failure, at around 0.06 to 0.1 ml/g/min, a sequence of biochemical events takes place at the cellular level and irreversible cell death rapidly occurs. The biochemical events that occur during ischaemia are multifactorial and are commonly known as the 'ischaemic cascade' [23]. Each step along this cascade may potentially be a target for therapeutic intervention.

Several variables may affect the ischaemic cascade and consequently the severity of stroke. These include the severity of cerebral blood flow reduction, its duration, its distribution, and the adequacy of its reperfusion where possible. Many of the events that constitute the steps of the ischaemic cascade follow a highly predictable order [20].

The initial reduction of cerebral blood flow is followed rapidly by inhibition of protein synthesis, depletion of intracellular energy stores, membrane depolarisation, and release of extracellular potassium. These events are accompanied by initial increases in oxygen extraction fraction (OEF), glucose metabolism (CMR_{gl}) and lactic acidosis [see Section 1.3.1]. Membrane depolarisation causes opening of voltage-operated calcium channels, allowing disturbance of tightly regulated neuronal calcium homeostasis. Glutamate is released from pre-synaptic stores, and in the presence of glycine activates *N*-methyl-D-aspartate (NMDA) receptor. The immediate consequences are increased sodium permeability and cellular swelling, leading to cytotoxic oedema. Calcium influx, through the NMDA-associated ion channels, greatly increases intracellular calcium. Further disturbances in ionic influx occur as a result of glutamate's effect on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and metabotropic receptors. Increases in other neurotransmitters, such as γ-aminobutyric acid (GABA), dopamine, and noradrenaline, may also contribute to the damage.

Increased intracellular calcium is the pivotal event of the ischaemic cascade because it activates a large number of subsequent damaging enzymatic pathways [24]. For example, calcium (through calmodulin) activates protein kinases such as Cam-kinase II and protein kinase C, which may cause imbalance in neuronal homeostasis by causing protein phosphorylation. Calcium also activates lipases, such as phospholipase A, which lead to the production of arachidonic acid and free radicals [24]. Activation of nitric oxide synthetase results in increased level of damaging nitric oxide.

Increased gene expression in ischaemic regions, which is likely to be induced by spreading depolarisations after ischaemia, has many damaging consequences. Cytokines such as tumour necrosis factor (TNF) and interleukin-1 (IL-1) cause tissue inflammation. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) enhance white blood cell interaction with the vascular endothelium to produce

blood-brain barrier damage and occlusion of the microcirculation, causing a 'no flow' state. The production of growth factors such as nerve growth factor (NGF) may also result in increased level of nitric oxide [23].

1.2.6 Other Cerebral Blood Flow Disturbances in Ischaemic Stroke

Changes in cerebral blood flow on the side contralateral to the ischaemic lesion have been repeatedly demonstrated since first described by Kempinsky *et al.* (1961) and Høedt-Ramussen and Skinhøj (1964) [25]. This phenomenon, which is also known as diaschisis, is characterised by reduction in blood flow, reduced cerebral metabolism and suppressed neuronal activity in the non-ischaemic hemisphere after a unilateral ischaemic stroke. Høedt-Ramussen and Skinhøj described it as a "transneural depression of cerebral metabolism" [26]. Several theories have been proposed to explain this phenomenon, including neurogenic, vasogenic and chemical mechanisms. It has been suggested that the flow changes in the non-ischaemic hemisphere are caused by a combination of the immediate effects of decreased neuronal stimulation modified by loss of cerebral autoregulation, release of vasoactive substances into the cerebrospinal fluid, cerebral oedema, and other factors. It is, however, still unclear whether these flow reductions in diaschisis precede the onset of stroke, or are the consequence of it [27]. The exact mechanisms and clinical relevance of diaschisis remain to be clearly elucidated.

Ischaemic lesions are responsible for most of the neurological deficits in patients with stroke. In 1966, Lassen *et al.* found focal areas of cerebral hyperaemia (or 'hyperperfusion') in the acute stage of stroke [28]. An overabundant cerebral blood flow relative to the metabolic needs of the brain tissue was denoted the 'luxury perfusion syndrome'. The incidence of focal cerebral hyperaemia in acute stroke is unknown and probably varies with time from onset of stroke. The incidence is higher in studies investigating patients soon after stroke; focal cerebral hyperaemia was found in 50% of patients within 3 days of stroke (Paulson *et al.*, 1970) compared with 10% of patients within 16 months of stroke (Uemura *et al.*, 1978) [29]. In 1981, Olsen *et al.* found that infarcts involving the cortical surface as demonstrated on CT scan were consistently (100%) associated with areas of focal cerebral hyperaemia, but infarcts localised in the deep grey and white matter were associated with hyperaemia in only a small proportion (16%) of patients [29].

In general, focal cerebral hyperaemia can be divided into three separate groups according to the location:

1. *Borderzone Hyperaemia* – areas of hyperaemia surrounding the ischaemic lesion. This is likely to be a result of accumulation of acid metabolites in the borderzone of the acute infarct.
2. *Post-ischaemic Hyperaemia* – areas of hyperaemia in brain tissue without arterial occlusion but localised to areas of infarction (on CT brain scan), or areas of marked 'capillary blush' and early venous filling (on cerebral angiography). This is likely to be due to recanalization of a previously occluded artery and metabolically uncoupled vascular dilatation due to lactic acidosis, adenosine vasoactive ions, and free radicals.
3. *Remote Hyperaemia* – areas of hyperaemia in non-ischaemic and apparently normal brain tissue, remote from the ischaemic lesion. The mechanism of this phenomenon remains unknown.

The hyperaemic areas are often extensive and co-exist with the ischaemic focus. It has been suggested that treatment aimed at reducing blood flow in hyperaemic areas might reduce the risk of cerebral oedema and haemorrhage, and even improve clinical prognosis [30]. Therapeutic interventions such as hyperventilation, use of vasoconstricting and dilating agents, induced hypotension, and hypertension, have all been proposed as potentially beneficial measures [31]. The possible clinical implications of focal cerebral hyperaemia will be discussed in detail in Section 1.4.1.

1.3 Control of Cerebral Blood Flow after Stroke

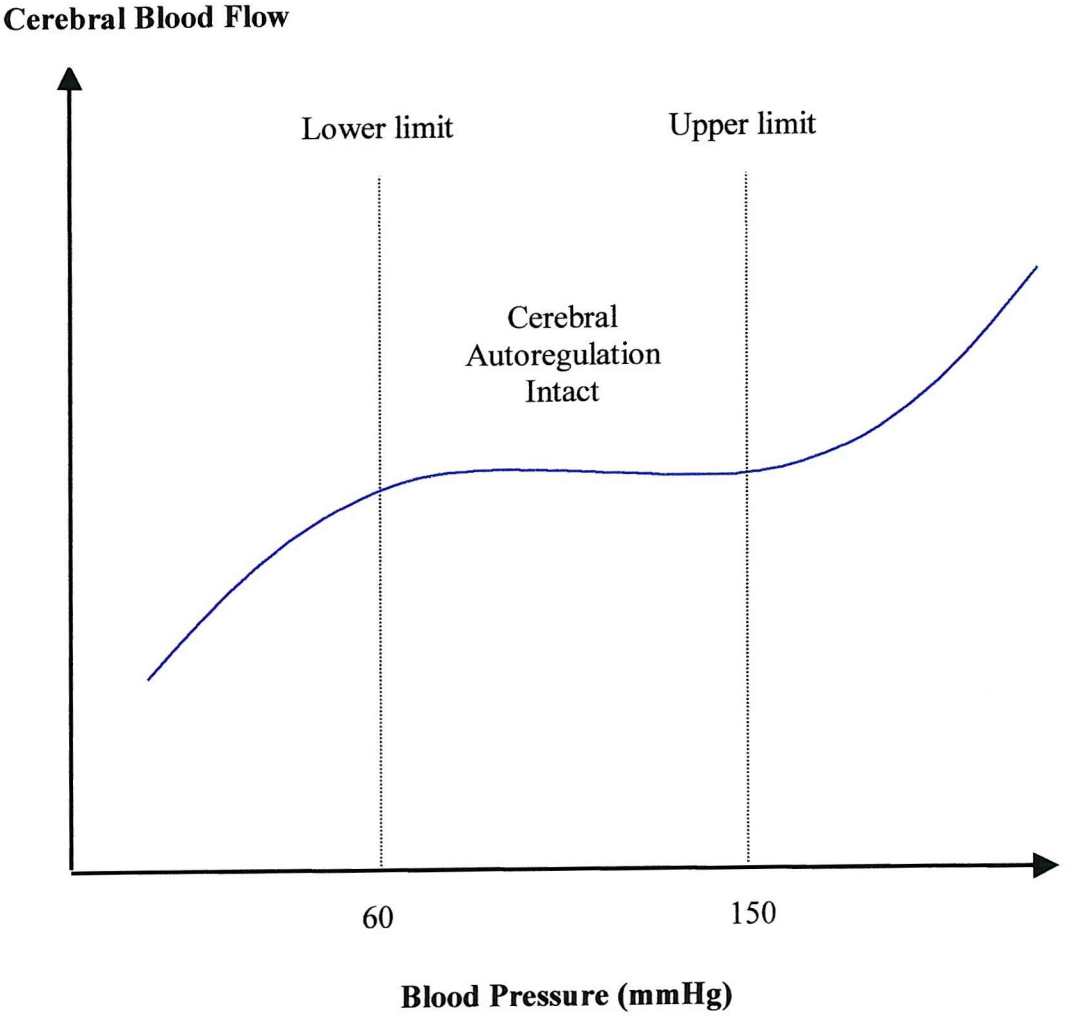
1.3.1 Cerebral Autoregulation & Ischaemia

Autoregulation of blood flow is defined as the physiological regulatory mechanism that maintains a constant flow over wide ranges of arterial blood pressure and perfusion pressure [32]. For the brain, the cerebral perfusion pressure is defined as the mean arterial pressure minus the intracranial pressure. In humans, this autoregulatory mechanism is not unique to the brain but can be found in many other vascular beds, including the retina (first demonstrated in 1941), kidneys (1946), coronary circulation (1959), intestine (1989) and the peripheral circulation (1949) [32].

In normal conditions, the haemodynamics of the cerebral circulation are closely regulated to ensure a constant cerebral blood flow. The first observations of autoregulation of cerebral blood flow were made by Fog in 1930. By observing the cat's pial vessels through a cranial window and manipulating the blood pressure, he found that pial vessel diameter altered according to blood pressure, and that pial autoregulatory vasomotor responses were independent of neurogenic stimuli [33]. In 1959, Lassen reviewed a large number of studies on cerebral blood flow in man during controlled hypotension and established the concept of cerebral blood flow autoregulation and its lower limit [34]. In his review, Lassen introduced the term 'autoregulation', which was a term widely used in renal physiology, to the field of cerebral haemodynamics. He was also responsible for the development of the 'autoregulation curve' that has been the model on which much of subsequent research in cerebral autoregulation is based [see Figure 1 on page 15]. The upper blood pressure limit of cerebral autoregulation was first demonstrated in hypercapnic dogs in 1971; the existence of such an upper limit had been reported in the renal circulation as early as 1951 [32].

Cerebral autoregulation can also be considered, at the level of the resistance vessel, as the occurrence of vasodilatation in response to a decrease in cerebral perfusion pressure, and the occurrence of vasoconstriction in response to increased cerebral perfusion pressure [32]. The maintenance of cerebral blood flow is therefore activated by proportional changes in cerebral vascular resistance through changes in vessel diameter of the resistance pial arterioles. In other words, in the situation where completely effective cerebral autoregulation exists, cerebral vascular resistance (CVR) changes proportionally and precisely to changes in cerebral perfusion pressure

Figure 1. The classic 'autoregulation curve'. It shows the static relationship between cerebral blood flow and blood pressure. The dotted lines represent the approximate blood pressure values for the upper and lower limits of autoregulation in normal humans. This diagram has been adapted from Lassen *et al.* (1959).



(CPP), and the cerebral blood flow (CBF) thus remains constant [32]. The following equations describes the above relationship:

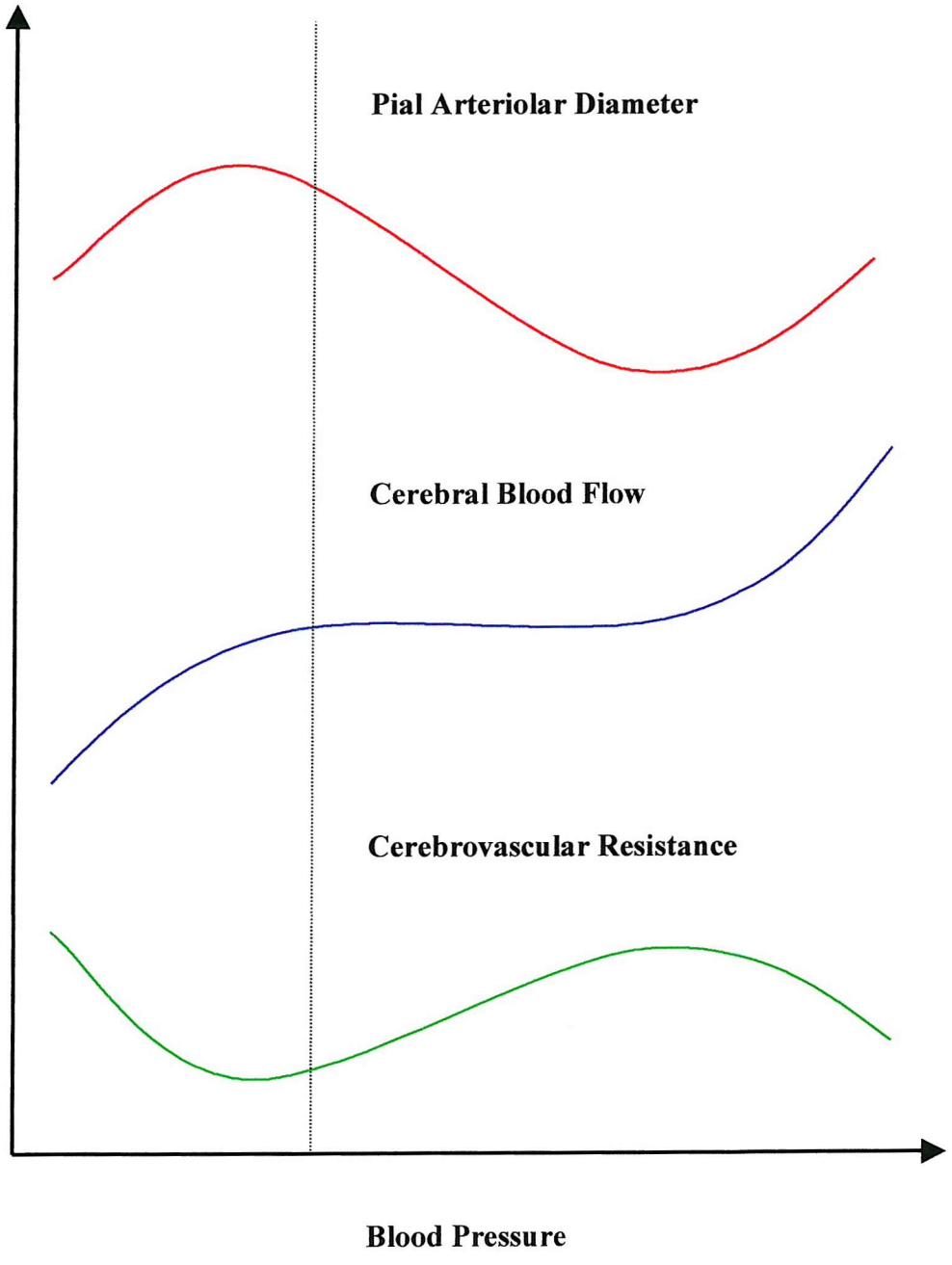
$$\mathbf{CBF = CPP / CVR} \quad \textit{or alternatively,}$$

$$\mathbf{CVR = CPP / CBF}$$

The limits of autoregulation reflect the points at which vasomotor adjustments are exhausted and cerebral vascular resistance cannot either increase (upper limit) or decrease (lower limit) further to regulate cerebral blood flow [13]. The upper and lower limits for the capacity of the resistance pial arterioles to dilate and constrict, and flow changes beyond the limits of cerebral autoregulation, have been extensively investigated. Cerebral vascular resistance reaches a minimum (i.e. with maximum vasodilatation) beyond the lower limit of autoregulation and cerebral blood flow falls passively with a falling mean arterial pressure [33]. At still lower pressures, a steep rise in cerebral vascular resistance is seen as the arterial vessels collapse; this arterial pressure threshold is known as the 'critical closing pressure' [35]. Conversely, beyond the upper limit of autoregulation, forced vasodilatation and a fall in cerebral vascular resistance lead to a passive rise in cerebral blood flow [see Figure 2 on Page 17].

Thus, cerebral autoregulation is effective over a wide range of arterial blood pressures, but has lower and upper pressure limits. In healthy humans, under normal conditions, these limits have been shown to be about 60 mmHg and 150 mmHg, respectively [32]. If blood pressure falls moderately below the lower limit of autoregulation (e.g. to < 50%, with a fall in cerebral blood flow of > 30%), then oxygen uptake by the brain is maintained at its normal level by an increase in the oxygen extraction fraction from the blood [11]. At this stage, clinical symptoms are insignificant. If blood pressure drops further, the increase in oxygen extraction fraction becomes inadequate and symptoms of cerebrovascular insufficiency, such as fainting, dizziness, pallor, sweating and yawning, develop. When the increase in oxygen extraction fraction from the blood can no longer counterbalance the decrease in cerebral blood flow, there is a fall in the cerebral oxygen uptake which corresponds to a fall in the cerebral metabolic rate, i.e. the cerebral metabolic rate of oxygen ($CMRO_2$) and glucose (CMR_{gl}). With further blood pressure reduction, consciousness is lost and ischaemic brain damage occurs, initially reversible and then irreversible. The measurement of cerebral metabolic rate and blood flow can be useful in providing information on the balance of energy supply and demand.

Figure 2. The relationship of pial arteriolar diameter, cerebral blood flow, and cerebral vascular resistance, with blood pressure. It shows that pial arteriolar dilatation reaches a maximum *below* the lower limit of autoregulation (represented by vertical dotted line). This diagram has been adapted from Paulson *et al.* (1990).



Cerebral ischaemia can therefore be regarded as the state in which cerebral energy metabolism becomes limited by an inadequate blood supply, that is, when blood flow and energy metabolism become linearly related to each other, so that metabolism becomes flow-rate dependent [11]. In the human brain, there is normally a considerable excess of energy substrates that act as a reserve, protecting against significant variations in cerebral perfusion pressure. For instance, the brain receives two to three times its normal requirement of oxygen per minute and seven times that of glucose [11]. Studies involving PET scanning have shown that the supply and demand of oxygen and glucose are closely matched regionally throughout the brain. Thus, metabolically more active regions of the brain receive proportionally more blood than those that are less active. In other words, there is regional coupling of flow to metabolism. Cerebral ischaemia, therefore, cannot be defined solely in terms of adequacy of cerebral blood flow, but also in terms of the corresponding cerebral metabolic activity.

The brain, therefore, is usually extremely well equipped to prevent hypoxia and ischaemia. A whole cascade of compensatory mechanisms is operative once blood supply begins to decrease. Apart from reducing cerebral vascular resistance, collateral blood supply also increases around and beyond the circle of Willis. There is augmented blood flow toward the ischaemic region from other cerebral arteries, transcortical leptomeningeal anastamoses, and the ophthalmic artery. The increase in oxygen extraction fraction, as mentioned above, is only a 'last resort' before ischaemic damage occurs [36].

1.3.2 Modulators of Cerebral Autoregulation

The lower and upper limits of cerebral autoregulation are not completely fixed but can be modulated by many factors. These include sympathetic nervous activity, the renin-angiotensin system, any factor that decreases or increases cerebral blood flow (e.g. arterial carbon dioxide and oxygen tension), and certain pharmacological agents (e.g. acetazolamide, angiotensin converting enzyme inhibitors and calcium antagonists) [37]. Furthermore, Hartl *et al.* (1995) showed that, in normal controls, cerebral vasomotor reactivity was significantly dependent on age, but not sex [38].

Carbon dioxide is one of the most potent vasodilator stimuli for the cerebral circulation. Thus, hypercapnia decreases cerebral vascular resistance by vasodilatation and cerebral blood flow increases as a consequence [39]. The inverse is true for

hypocapnia. With a high arterial carbon dioxide tension (Pco_2) and an already dilated resistance circulation, the pial vessels are less able to further vasodilate in response to a fall in blood pressure or cerebral perfusion pressure [32]. The upper and lower limits of cerebral autoregulation are therefore shifted toward lower blood pressure, and may ultimately be abolished altogether. The capacity of the resistance vessels to vasodilate is known as 'cerebral vasomotor reactivity' or 'cerebrovascular reserve'. It denotes the percentage increase of regional cerebral blood flow or blood flow velocity after application of a vasodilatory stimulus, such as carbon dioxide (mixture of 5% carbon dioxide and 95% oxygen) or acetazolamide (intravenous 1 g), as compared to pre-stimulus values [36]. In normal conditions, there is a 2 to 4% change in cerebral blood flow for each mmHg change in Pco_2. In certain clinical situations, such as before carotid endarterectomy for internal carotid artery stenosis, assessment of cerebral vasomotor reactivity can indicate the extent of exhaustion of the cerebrovascular reserve (i.e. abundance of collateral flow) and the potential risk of ischaemic damage from cerebral insufficiency [40].

There is evidence that activation of sympathetic nerves may shift the upper limit of autoregulation toward higher blood pressure whereas acute denervation may shift both the lower and upper limits of autoregulation toward lower blood pressure [41]. Chronic sympathetic denervation, however, does not shift the limits of autoregulation. This shift of the upper limit toward higher blood pressure is probably a physiological mechanism, protecting the brain against blood pressure increases during activation. The sympathetic nervous system predominantly exerts its vasomotor function in the larger cerebral resistance vessels (i.e. the inflow tract), whereas cerebral autoregulation is predominantly a function of the smaller resistance vessels. During sympathetic activation with vasoconstriction of the larger vessels, the smaller vessels further downstream will dilate as an autoregulatory response to maintain a constant cerebral blood flow, as long as the blood pressure is within the limits of cerebral autoregulation.

Disease states of the brain and vascular systems may impair or abolish cerebral autoregulation and cerebral vasomotor reactivity. Cerebral vasomotor dysfunctions often result in cerebral autoregulation impairment in conditions such as severe head injury [42, 43, 44], ischaemic stroke [45], subarachnoid haemorrhage [46, 47], and carotid artery stenosis [40]. This impairment may or may not be associated with abnormal cerebral vasomotor reactivity [see Section 1.3.3]. Changes in systemic

blood pressure are then followed by similar changes in the cerebral blood flow [37, 33]. In other words, cerebral blood flow becomes 'pressure-passive'. Other neurological diseases in which cerebral autoregulation is impaired include neonatal brain asphyxia, infections and neoplastic lesions of the central nervous system. Furthermore, cerebral autoregulation impairment has been shown, by Bentsen *et al.* (1975) and Kastrup *et al.* (1986), to occur in some patients with long-term type I diabetes mellitus, probably as a result of diabetic microangiopathy ('arteriolar hyalinosis') [48, 49].

In chronic hypertension, cerebral autoregulation is adapted to higher blood pressure. Thus, in animals and humans, the lower and upper limits of cerebral autoregulation are shifted toward higher blood pressure. The reason for this is not entirely clear, but it is possibly the consequence of hypertensive structural vascular adaptation with vessel wall thickening and lumenal narrowing limiting cerebral vasomotor reactivity. At very high blood pressure, there is forced vasodilatation and loss of cerebral autoregulation, and therefore cerebral blood flow rises passively. With worsening hyperperfusion, blood-brain barrier dysfunction and cerebral oedema occur, initially focally and later more generalised [32]. This has been termed 'acute hypertensive encephalopathy' and is a clinical syndrome characterised by headache, convulsions, and deteriorating conscious level. As cerebral oedema continues to develop, the patient lapses into coma and cerebral perfusion pressure decreases to low level due to increasing intracranial pressure. This sequence of pathological events is well documented in many experimental studies that demonstrated the existence of an upper limit of cerebral autoregulation [37]. In acute hypertensive encephalopathy, it is important to reduce and closely monitor the blood pressure, but in the presence of cerebral autoregulation impairment, there is a risk of inducing cerebral ischaemia, especially if blood pressure reduction is too rapid [33].

Angiotensin converting enzyme inhibition, with agents such as angiotensin converting enzyme inhibitor and angiotensin II receptor antagonist, has been shown to shift the limits of cerebral autoregulation toward lower blood pressure values [50, 51]. In the rat model, Paulson *et al.* (1988) found that the renin-angiotensin system affected cerebral autoregulation mainly by influencing the vasomotor function (vasodilatation) of the larger cerebral resistance vessels [52]. There is also evidence that the renin-angiotensin system can influence the regulation of cerebral blood flow

independent of the sympathetic nervous system [53]. The effects of angiotensin converting enzyme inhibitors after stroke will be discussed in Section 1.4.2.

Other pharmacological agents also affect cerebral autoregulation and cerebral vasomotor reactivity. For example, intravenous acetazolamide and inhaled 5% carbon dioxide, as already mentioned, have vasodilator effects on resistance blood vessels and are useful agents for the clinical assessment of cerebral vasomotor reactivity. Dahl *et al.* (1989) showed that nitroglycerin produced vasodilatation of the basal intracranial arteries, causing a reduction in cerebral blood flow velocity without concurrently changing cerebral blood flow [54]. Strebel *et al.* (1995) observed that anaesthetic agents such as isoflurane and desflurane impaired cerebral autoregulation, whereas propofol preserved it [55].

1.3.3 Cerebral Autoregulation Impairment after Stroke

In normal circumstances, an increase in arterial blood pressure (and cerebral perfusion pressure) produces an increase in cerebral vascular resistance from vasoconstriction of resistance arteries and arterioles. This counteracts the increase in cerebral perfusion pressure so that cerebral blood flow remains constant. The recent introduction of newer techniques for measuring cerebral blood flow has led to the realization that this autoregulatory mechanism is significantly disturbed as a result of brain ischaemia. It has been shown that in the first weeks to months after transient ischaemic attacks and ischaemic stroke, there is disruption of cerebral autoregulation and alteration of the vasodilator responses to, for example, hypercapnia (i.e. cerebral vasoreactivity) [11, 27, 56]. This is sometimes collectively termed 'vasomotor paralysis'. This phenomenon results in a passive pressure-flow relationship between the blood pressure and cerebral perfusion pressure. The cerebral perfusion pressure is therefore susceptible to changes in blood pressure and this may alter the balance between substrate delivery and utilisation in the brain. This increased vulnerability of the brain may play a role in the post-ischaemic extension of the damage initiated by cerebral ischaemia.

Vasomotor paralysis with loss of autoregulation is a common and well known phenomenon in acute ischaemic stroke [32]. In humans, impaired cerebral autoregulation in the acute phases of stroke was demonstrated as early as 1968 [57]. In rats with middle cerebral artery occlusion, cerebral autoregulation is completely lost in ischaemic regions with a low flow of less than 30% of the normal and is

partially preserved in tissue with a flow greater than 30% [58]. It has been shown that autoregulation is impaired in the blood vessels supplying the ischaemic lesion but can be fully or partially preserved in collateral vessels. This helps to explain the relative preservation of cerebral autoregulation in the regions surrounding the ischaemic core. The exact mechanism of vasomotor paralysis has not been elucidated fully. It has been suggested that vasodilatation of resistance vessels, which results from tissue ischaemia and acidosis, occurs in an attempt to increase regional cerebral blood flow. In these dilated vessels, the vasoregulatory function is impaired and autoregulation is lost [33]. Furthermore, in the ischaemic areas distal to an arterial occlusion, the arteriolar pressure is much lower than normal and below the lower limit of autoregulation. Thus, the apparent cerebral autoregulation impairment could also simply reflect the passive pressure-flow relationship below the lower limit of autoregulation [32].

With the occurrence of cerebral autoregulation impairment after stroke, a decrease in blood pressure, which in turn decreases cerebral perfusion pressure due to a passive pressure-flow relationship, should theoretically worsen the regional cerebral blood flow within the ischaemic and penumbral regions. Conversely, an increase in blood pressure should theoretically improve the blood flow in those regions. However, loss of cerebral autoregulation may also be present in areas of post-ischaemic hyperaemia [see Section 1.2.6]. This may occur in the acute phase after a spontaneous recanalization of a thromboembolic occlusion, where there is a predominantly increased cerebral blood flow [30]. An increase in blood pressure in this situation may therefore lead to an increased cerebral perfusion pressure accelerate the development of brain oedema, haemorrhagic transformation of the infarction (or rebleeding in intracranial haemorrhage), and blood-brain barrier damage. Such a condition is known as 'hyperperfusion syndrome'. This situation can also occur after carotid endarterectomy for a chronic severe stenosis of the internal carotid artery. The vascular bed distal to the carotid stenosis may have reset the limits of autoregulation toward lower blood pressures or have severely impaired cerebral autoregulation. It is therefore vulnerable to a blood pressure rise above the upper limit of autoregulation.

Chronic threatening ischaemia, also known as 'misery perfusion', is a condition that may be present if an occlusion or stenosis of a major cerebral artery (e.g. middle cerebral artery) results in only a moderate reduction in the local blood pressure distal to the occlusion. In these instances, the local blood pressure may be at

or just below the lower limit of autoregulation, and cerebral blood flow may be normal or slightly reduced in the resting state. Over time, the limits of cerebral autoregulation are likely to be reset toward lower blood pressures, such that even a minor reduction of blood pressure may cause it to fall below the lower limit of autoregulation. This may in turn result in cerebral ischaemia if there is an inadequate rise in the oxygen extraction fraction [see Section 1.3.1]. Lindegaard *et al.* (1985) assessed the intracranial haemodynamics in patients with carotid artery stenosis and found that many had abnormal cerebral autoregulation and an impaired potential for collateral flow formation [59]. In addition, Widder *et al.* (1986) also demonstrated impaired cerebral vasomotor reactivity in patients with haemodynamically critical carotid stenoses and occlusions [60].

Cerebral autoregulation can also be impaired in regions far removed from the areas of ischaemia. It has been shown, for example that the lower limit of cerebral autoregulation was reduced in the rat cerebellum after transient bilateral carotid occlusion. Furthermore, autoregulation to hypertension was impaired in the ipsilateral cerebral cortex in patients with deep hemispheric infarcts [56]. These remote effects suggest that trans-synaptic neural mechanisms can influence cerebral autoregulation.

Studies in animals and humans have demonstrated that focal or global cerebral ischaemia is also associated with a reduction in cerebral vasomotor reactivity to hypercapnia. These studies have shown that in focal cerebral ischaemia by occlusion of the middle cerebral artery, cerebral vasomotor reactivity is virtually abolished within the ischaemic core, and is present but diminished in more peripheral areas [56]. Baumgartner *et al.* (1994) found that patients with low-flow cerebral infarcts had severely reduced cerebral vasomotor reactivity to carbon dioxide on the symptomatic side, as compared to the asymptomatic side [61].

Similar to cerebral autoregulation, cerebral vasomotor reactivity can also be attenuated in areas remote from the ischaemic lesion. These observations again suggest that trans-synaptic effects, perhaps mediated via changes in cerebral metabolism in regions connected with the ischaemic area, are able to influence cerebral vasomotor reactivity. In the ischaemic core and the penumbra, both the cerebral autoregulation and cerebral vasomotor reactivity may be lost. However, in some circumstances, there may be dissociation between the two variables and cerebral autoregulation may be impaired despite a normal cerebral vasomotor reactivity, and vice versa. Olsen *et al.* (1983) clearly demonstrated that such dissociation occurred

within the ischaemic penumbra in acute stroke patients [19]. On the other hand, Diehl *et al.* (1994) found a close correlation between cerebral autoregulation and vasomotor reactivity even in patients with severe carotid and middle cerebral artery stenosis [62]. Other aspects of cerebral autoregulation after stroke, as well as different methods of assessing autoregulation, will be discussed in Section 1.5.

1.4 Clinical Implications

1.4.1 Blood Pressure Changes after Acute Ischaemic Stroke

Hypertension is one of the most important treatable risk factors for stroke. For every 7.5 mmHg rise in usual diastolic blood pressure, there is a doubling in the risk of stroke, and that there is a close relationship between usual diastolic blood pressure and recurrent stroke [5]. Antihypertensive therapy in primary stroke prevention has been extensively evaluated in randomised controlled trials. The results demonstrated a 38% relative risk reduction of stroke amongst patients who received conventional antihypertensive therapy, with the benefits becoming apparent within one and two years of starting treatment. However, there are only a few small trials that have evaluated the effects of antihypertensive therapy in secondary stroke prevention. A meta-analysis (Gueyffier *et al.*, 1997) of these trials demonstrated a substantial 28% relative risk reduction in recurrent stroke in the treatment group [63]. The available evidence, therefore, strongly supports the use of long-term antihypertensive therapy in both primary and secondary prevention of stroke.

There is, however, considerable uncertainty about the relative risk and benefits of lowering the blood pressure in the acute phase of stroke recovery. Antihypertensive treatment may theoretically reduce the risk of rebleeding in intracerebral and subarachnoid haemorrhage, haemorrhagic transformation of infarction, and development of cerebral oedema. However, lowering the blood pressure in a patient with an acute ischaemic stroke, where there may be impaired autoregulation, may further compromise the regional blood flow in the ischaemic areas. The duration of cerebral autoregulation impairment after stroke is also unclear. There is evidence to suggest that it may persist for weeks [31, 64] or even months [65] after stroke.

Hypertension is a common finding after acute stroke but it usually falls spontaneously over the first week, with little change thereafter. Britton *et al.* (1986) examined the natural course of blood pressure after emergency admission in 209 stroke patients and found that 69% of the stroke group (vs. 36% of the controls) had blood pressure greater than 170/100 mmHg. They also showed that during the first four days, there was spontaneous decline of the blood pressure, and the drop was greater the higher the initial value [66]. Interestingly, Harper *et al.* (1994) demonstrated that moderate to heavy alcohol consumption before stroke was

associated with a greater systolic blood pressure decline in the first week after the event compared with stroke patients who were light drinkers or abstainers [67]. Raised blood pressure on admission may also indicate chronic hypertension; about 50% of stroke patients are hypertensive before the onset of stroke. These patients are likely to have higher blood pressures than those without pre-existing hypertension and are more likely to present with end organ damage, including hypertensive retinopathy, renal dysfunction, and left ventricular hypertrophy of the heart. Blood pressure in the acute phase is also generally higher in patients with haemorrhagic than with ischaemic stroke [67].

The pathophysiology of hypertension after stroke has not been fully elucidated. It is likely to be the result of a combination of factors including physical and mental stress of hospital admission, the 'white coat' effect, the neurological sequelae of the acute stroke itself, and the 'Cushing' reflex (i.e. hypertension secondary to raised intracranial pressure). However, it remains unclear whether post-stroke hypertension represents a pathophysiological response to maintain cerebral perfusion of reversibly damaged neuronal tissue, or is a marker of the severity of stroke and the risk of further clinical progression. Harper *et al.* (1994), using 24-hour ambulatory blood pressure monitoring, showed that the spontaneous fall in blood pressure after admission was independent of the stress of hospital admission [67].

Due to cerebral autoregulation impairment, cerebral blood flow is to a major degree dependent on systemic blood pressure levels after acute stroke, so any changes may have significant effects on cerebral blood flow. This may also have clinical and prognostic implications. Some studies have demonstrated an association between poor outcome and both high and low blood pressure after acute stroke, whilst other have found no relationship [68]. Robinson *et al.* (1997) showed that systolic blood pressure variability, which may reflect centrally induced sympathetic nervous system activity, was significantly greater in acute stroke patients than normal controls, but the prognostic significance of this finding required further evaluation [68]. Korpelainen *et al.* (1999) showed that acute hemispheric and medullary brain stem infarctions were associated with dysfunction of the cardiovascular autonomic regulatory system, which manifested as reduction in the magnitude of heart rate variability. It was also suggested that abnormal heart rate variability could be involved in prognostically unfavourable cardiac complications, such as arrhythmias and sudden cardiac death, and other known manifestations of autonomic impairment associated with stroke [69].

1.4.2 Blood Pressure Manipulation after Stroke

Observational data relating blood pressure soon after stroke onset with subsequent outcome do not provide a clear guideline and a systematic review of all the available studies is currently in progress. At least one study has suggested that higher initial blood pressure is associated with a favourable outcome, whereas others have suggested that an increasing level of baseline blood pressure in patients with impaired consciousness is associated with a progressively higher probability of a poor outcome [70, 71]. Evidence from the International Stroke Trial (1997) has demonstrated a U-shaped relationship, with early death and poor long-term outcome being more frequent in patients in the highest and lowest quartiles of systolic blood pressure. However, a poor outcome at 6 months was only associated with low values of systolic blood pressure, not high ones [72, 5]. Therefore, although it is tempting to hypothesize that adjusting the level of blood pressure to an 'optimum' may lead to a better outcome, it is uncertain whether it is better to have blood pressure high and risk breaking through the upper limit of autoregulation (impaired after stroke) and so causes cerebral oedema and haemorrhage, or better to have blood pressure low and risk worsening cerebral ischaemia [70].

To date, there is no large randomised controlled trial that has specifically aimed to compare blood pressure reduction with control in patients with acute ischaemic stroke. However, there are smaller trials in which the intervention, for example intravenous and oral nimodipine, or oral beta-blockers, has reduced the blood pressure. In the trial involving intravenous nimodipine, a fall in blood pressure was associated with an increased risk of early death and a poor long-term outcome. It has been suggested that this deleterious effect was associated with and possibly caused by a reduced cerebral blood flow and increase in cerebral infarct volume [73]. Others have suggested that after stroke, partial occlusion of an arterial distribution may create decreased and turbulent flow in the remaining patent portions of the vessel. Decreasing blood flow by reducing arterial blood pressure may promote thrombus formation and potentially extend the original occlusion [74]. Furthermore, Yatsu *et al.* (1985) postulated that patients with acute ischaemic stroke may have unpredictable vascular lesions that promote further ischaemic damage by antihypertensive therapy. For example, patients with stenotic proximal internal carotid artery may have a greater risk of exacerbation of tissue ischaemia following antihypertensive therapy because the stenosis imposes greater distal reductions in

blood flow [75]. Therefore, most experts recommend that antihypertensive treatment should be avoided in acute stroke patients unless vital organs are compromised, diastolic blood pressure exceeds 130 mmHg, or acute hypertensive encephalopathy develops [76, 77]. However, others such as Spence *et al.* (1985) have suggested that hypertension following acute stroke should be carefully treated to reduce the risk of cerebral oedema, which is a major cause of post-stroke morbidity and mortality [78].

Not all antihypertensive agents affect the cerebral circulation in the same way. Angiotensin converting enzyme inhibitors (ACEI), whether administered acutely or chronically, have been shown to maintain cerebral blood flow despite pronounced lowering of the systemic blood pressure in patients with chronic hypertension and chronic heart failure [79, 80]. A large randomised controlled trial is currently in progress to investigate whether perindopril, a slow-acting ACEI, is effective in lowering blood pressure without adversely affecting cerebral blood flow in patients with acute ischaemic stroke, between two and seven days after onset. Preliminary results of this trial ('Protection against Recurrent Stroke Study' or PROGRESS) showed that perindopril was well tolerated and cerebral blood flow was not significantly affected [81].

Conversely, some investigators have suggested that induced hypertension after stroke may be clinically beneficial. Rordorf *et al.* (1997) retrospectively studied the effects of pharmacologically (phenylephrine) induced hypertension in acute stroke in humans. The results suggested that careful use of phenylephrine-induced hypertension was not associated with an increase in mortality or morbidity in acute stroke. The authors of this study recommended its use in carefully selected patients with multiple stenosis of cerebral arteries [82].

Although the benefits of antihypertensive treatment for secondary stroke prevention are well documented, there is no reliable evidence on the optimal timing of the commencement of antihypertensive therapy after stroke. This in part reflects a lack of knowledge of the timing and nature of recovery of cerebral autoregulation and cerebral vasomotor reactivity after acute ischaemic stroke. Mori *et al.* (1993) showed that cerebral autoregulation may be impaired up to two or three years after stroke, but no other investigators have confirmed this finding [65]. Thirty years ago, Agnoli *et al.* (1968) demonstrated loss of cerebral autoregulation in the majority of acute stroke (within 2 days) patients, whereas only a few of the chronic stroke (over 15 days) patients had impaired autoregulation [83]. Moreover, Dyker *et al.* (1998) speculated

that, because ischaemic injury following stroke evolved over 48 hours in humans, autoregulation and local cerebral perfusion would be deranged for approximately 72 hours in patients with stroke [84]. So far, there are no randomised controlled trials that have compared early and late treatment of hypertension following stroke. Although some clinicians advocate starting antihypertensive treatment soon after stroke onset [29], most experts advise against it [37, 32, 76]. Systematic experience of blood pressure modification in the acute phase of stroke is lacking. Current UK recommendations state that antihypertensive treatment may be commenced for stroke patients with persisting hypertension (i.e. no spontaneous decline) about one to two weeks after stroke [76, 5].

1.4.3 Influences on Acute Stroke Care

The uncertainty over the appropriate management of hypertension in acute stroke is likely to remain until more evidence emerges from large clinical trials. There is also no clear guideline for the management of spontaneous hypotension in acute stroke. Hypotension is less common than hypertension after stroke, but the true incidence is uncertain. Hypotension after acute stroke may be a result of dehydration (especially in dysphagic stroke), inefficient enteral or parenteral rehydration after admission, and overzealous antihypertensive therapy [37]. Orthostatic hypotension, defined as a fall in systolic blood pressure of more than or equal to 20 mmHg after postural change, occurs more frequently in elderly and acute stroke patients, probably because of secondary sympathetic dysfunction [85].

The presence of cerebral autoregulation impairment in acute ischaemic stroke means that a decrease in blood pressure may worsen cerebral blood flow and cause ischaemia. A spontaneous low blood pressure is likely to have similar effects. This therefore suggests that a low blood pressure should theoretically be actively reversed or prevented in order to counteract a fall in cerebral blood flow. However, a pharmacologically induced blood pressure increase may increase the risk of cerebral oedema, intracranial haemorrhage, and hypertensive encephalopathy. There is very little randomised evidence to support active reversal or prevention of spontaneous or iatrogenic hypotension. Some observational evidence, however, may be found in the study that demonstrated an increased risk of poor outcome with intravenous nimodipine in acute stroke, probably as a result of induced hypotension. It was found that the patients who were treated in centres where hypotension was actively reversed

or prevented by intravenous fluid infusion (e.g. Indredavik *et al.*) were more likely to have better outcome [73].

Olsen *et al.* (1983) suggested that both hypertension and hypotension should be avoided. They stated "thus changing the blood pressure in the setting of an acute stroke seems to be a two-edged sword. Elevation benefits the ischaemic penumbra and harms the hyperaemic areas by augmenting local oedema; lowering benefits the hyperaemia and harms the penumbra". Olsen also suggested that increasing cerebral perfusion pressure, not by inducing hypertension but by early restoration of blood flow, in acute stroke patients may be beneficial [19]. Recent evidence from randomised trials indeed suggests that early restoration of blood flow with thrombolytic therapy improves clinical outcome. The most recent systematic review of the randomised trials of thrombolytic therapy in ischaemic stroke showed that thrombolytic treatment, when administered within 3 hours of stroke, was associated with 128 fewer patients dead or dependent per 1000 treated [86].

Hospital-based studies have shown that over 40% of patients admitted to hospital have a reduced conscious level on admission [87]. Generally, these patients are nursed lying down in lateral positions to prevent obstruction of their airway and aspiration. For conscious patients, however, some have suggested that the best position is upright in a chair since this posture is thought to prevent hypoxia by allowing the diaphragm to descend naturally and thereby increase lung volume [88]. An upright posture is also associated with a reduced intracranial pressure and orthostatic hypotension in some patients. Although, in theory, a reduction in intracranial pressure would result in a higher cerebral perfusion pressure, there is a potential risk of lowering cerebral blood flow by an unintentional blood pressure fall with a background of impaired cerebral autoregulation. Although some studies have shown that altering the posture of patients with acute stroke may alter blood pressure, oxygenation and heart rate [89], it remains unclear whether routine alterations of posture, e.g. during nursing care and head-up tilting, produces a significant change in cerebral blood flow or adversely affect prognosis. This may influence acute stroke management, such as nursing care, timing and intensity of mobilisation, as well as intensity of monitoring of blood pressure. Indredavik *et al.* (1999) recently showed that time from admission to mobilisation was the single most important factor in determining discharge to home from the stroke unit [90]. They recommended that mobilisation should be commenced as soon as possible after admission.

Another occasion when hypotension and hypoxia may occur in stroke patients is after eating. Postprandial hypotension, defined as a decrease in systolic blood pressure of 20 mmHg or more after eating, is particularly common in older hypertensive patients [91]. The mechanism of postprandial hypotension is not fully understood. Possible contributors include: inadequate sympathetic nervous system compensation for meal-induced splanchnic blood pooling; impairments in baroreceptor function; inadequate increases in cardiac output; and impaired peripheral vasoconstriction, insulin-induced vasodilatation, and release of various vasodilatory gastrointestinal peptides [91]. In the elderly, this fall in blood pressure has led to cerebral ischaemia in some cases [92]. Furthermore, Krajewski *et al.* (1993) used transcranial Doppler ultrasonography to assess blood flow velocity during postprandial hypotension and found that postprandial hypotension was associated with vasoconstriction of the cerebral arteries, which may lead to ischaemia during periods of marked blood pressure decline [93]. Although there are only few studies this topic, it remains interesting as a potential problem for acute stroke patients.

1.5 Cerebrovascular Pathophysiology

1.5.1 Cerebral Metabolism & Blood Flow in Healthy People

The human brain is a unique organ since it has a high metabolic demand for energy and, unlike other organs, uses glucose (about 75 to 100 mg/min, or 125 g/day) as its sole substrate for energy metabolism. Glucose is metabolised in the brain entirely via the glycolytic pathway and the tricarboxylic acid cycle. Each molecule of glucose is broken down in a series of enzymatic steps, known as glycolysis, to two molecules of pyruvate. In the presence of oxygen (aerobic glycolysis), pyruvate is metabolised first by pyruvate dehydrogenase and then by a series of mitochondrial reactions to carbon dioxide and water with the formation of 36 molecules of adenosine tri-phosphate (ATP). This is the maximum ATP yield. In the absence of oxygen (anaerobic glycolysis), this sequence of events is blocked or retarded at the stage of pyruvate oxidation, leading to the reduction of pyruvate to lactate. This pathway only yields 2 molecules (rather than 36 molecules) of ATP from one molecule of glucose. In addition, lactic acid accumulates in and outside the cells and mitochondria lose their ability to sequester calcium, so any calcium entering or released within the cell will raise the intracellular calcium level [94]. Neurones in the brain require a constant supply of ATP to maintain their integrity and to keep the major intracellular cation, potassium, within the cell and the major extracellular cations, sodium and calcium, outside the cell. As the brain is unable to store energy, it requires a constant supply of oxygenated blood and glucose to maintain its function and structural integrity.

Global cerebral blood flow per unit of brain, in a healthy young adult, is about 5 to 5.5 ml/g/min. The value is significantly higher for people below 20 years of age and lower for those over 60 years. For a brain of average weight (around 1400 g in a 65 kg adult), which is only 2% of total body weight, the total cerebral blood flow at rest is disproportionately large at about 800 ml/min, which is 15 to 20% of total cardiac output of blood. At this level of blood flow, whole brain oxygen consumption, measured as the cerebral metabolic rate of oxygen ($CMRO_2$), is about 0.33 to 0.35 ml/g/min, or 45 ml of O_2/min, which is 20% of the total oxygen consumption of the body at rest [5].

Under normal circumstances, cerebral blood flow is determined by the cerebral perfusion pressure at the base of the brain and by the cerebral vascular resistance, which is governed by blood viscosity and the size of the intracranial

arteries. The cerebral perfusion pressure represents the difference between arterial pressure forcing blood into the cerebral circulation and the venous backpressure. The mean cerebral perfusion pressure is therefore the mean systemic arterial blood pressure minus the intracranial venous pressure. Mean arterial pressure is approximately the diastolic blood pressure plus one-third of the pulse pressure (systolic blood pressure minus diastolic blood pressure).

To illustrate, in a normal adult, the diastolic blood pressure is about 80 mmHg, pulse pressure is about 40 mmHg. The mean arterial pressure is therefore:

$$80 + (1/3 \times 40) = 93 \text{ mmHg.}$$

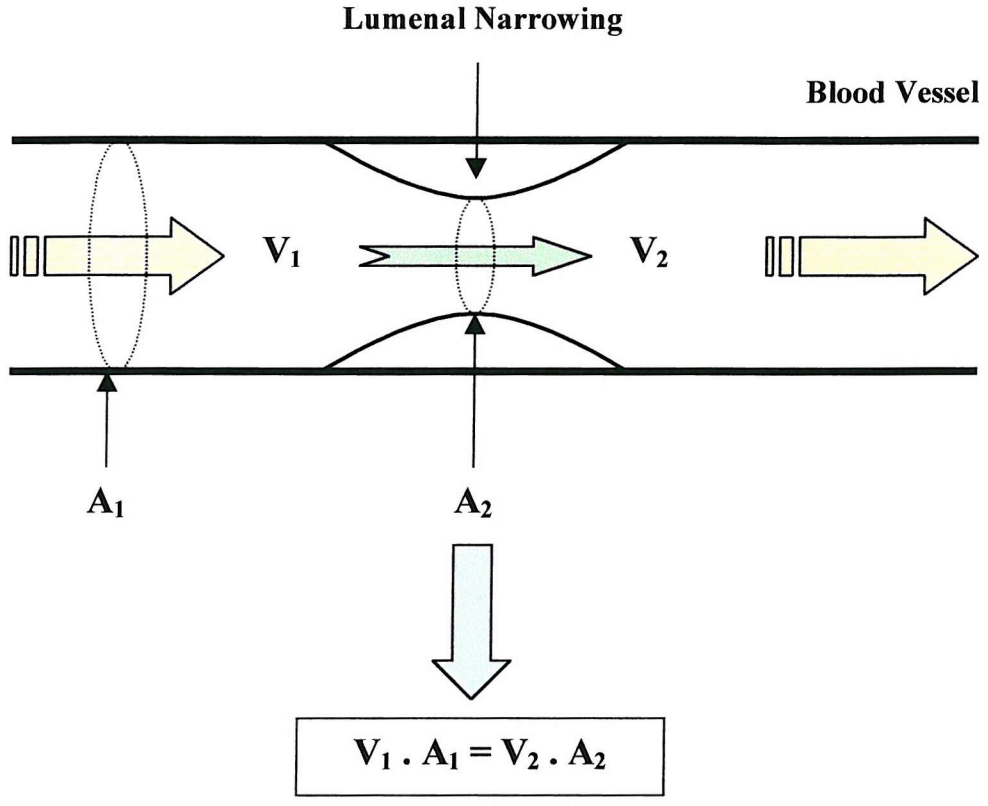
The normal intracranial venous pressure is about 10 mmHg. Therefore the cerebral perfusion pressure is approximately (93 – 10) = 83 mmHg.

When resting cerebral perfusion pressure is constant, any change in cerebral blood flow is caused by a change in cerebral vascular resistance, usually because of alteration in the diameter of small intracranial arterioles or arteries. Under normal circumstances, there is a direct correlation between cerebral blood flow and cerebral blood volume. Blood flow and volume will both increase and decrease as vessels dilate and constrict, respectively. The ratio between cerebral blood flow and cerebral blood volume (CBV: CBF) remains constant over a wide range of cerebral blood flow at normal cerebral perfusion pressure [5].

According to Poiseuille's law, as the luminal cross-sectional area of an artery or arteriole decreases with vasoconstriction, the blood flow velocity in that segment of vessel increases [95]. This paradoxical relationship can be likened to the increased velocity of the water jet from a hosepipe when the nozzle is partially occluded by one's finger. The narrower the lumen, the greater the pressure and flow velocity of the stream of water [see Figure 3 on Page 34]. As the lumen becomes nearly occluded, the flow velocity becomes substantially reduced and water dribbles out of the hosepipe. This relationship underlies the principles governing the interpretation of blood flow velocities in the major basal cerebral arteries by transcranial Doppler (TCD) ultrasonography. TCD ultrasonography will be discussed in detail in Section 1.5.2.

In the resting brain with normal cerebral perfusion pressure, cerebral blood flow is closely matched (or 'coupled') with the metabolic demands of the neurones. Grey matter, which has a higher metabolic rate, therefore has higher regional cerebral

Figure 3. This shows the velocity acceleration that must occur through stenosis in order to maintain constant flow. Under conditions of constant flow, the product of the velocity (V) and the cross-sectional area (A) in the inflow portion of the vessel must equal the product of the velocity and the cross-sectional area at the region of stenosis.



blood flow than white matter, which has a lower metabolic rate. Local blood flow varies directly with local brain function by 10 to 20%, even global cerebral blood flow stays fairly constant under steady state conditions. Lassen *et al.* (1977) showed that during voluntary hand movements, the metabolic activity of the contralateral motor cortex increases over a few seconds, accompanied by rapid vasodilatation of the cerebral resistance vessels, leading to an increase in cerebral blood flow and blood volume. The oxygen extraction fraction and glucose extraction fraction, however, stayed constant [96]. Aaslid *et al.* (1987) also demonstrated that, in response to light stimulation of the retina, cerebral blood flow volume in the posterior cerebral artery, which supplies the visual cortex, increased by 20.2%, while flow velocity increased by 16.4%. It was also shown that the regulation of blood flow was very rapid; only about 2 seconds elapsed from application of the stimulus to 50% full response [97]. Conversely, low flow does not necessarily mean vessel occlusion but can also indicate a low metabolic demand. The physiological mechanism behind this close coupling of metabolism with cerebral blood flow is unknown, although it has been suggested that brain activity produces vasodilator metabolites or neural discharges, or both, which regulate the vasoactivity of the blood vessels.

Cerebral blood flow is inversely related to whole-blood viscosity [98]. The main determinant of whole-blood viscosity is haematocrit and thus cerebral blood flow is inversely related to haematocrit. Ameriso *et al.* (1990) demonstrated this inverse association between mean TCD blood flow velocity and both haematocrit and fibrinogen [99]. However, this relationship is not because the high haematocrit raises whole-blood viscosity and thereby slows the blood flow; rather, the higher oxygen content of high haematocrit blood allows cerebral blood flow to be lower and yet to maintain normal oxygen delivery according to metabolic demands [5]. Other local factors such as red cell and platelet aggregation, and metabolic factors such as carbon dioxide and oxygen concentration, also significantly influences cerebral blood flow and flow velocity [100]. Furthermore, Ackerstaff *et al.* (1990) showed that cerebral blood flow velocity, as measured by TCD ultrasonography, was affected significantly by age and gender; women in general had significantly higher blood flow velocities than men [101]. Martin *et al.* (1994) found that the slowing of cerebral blood flow velocity with age probably reflected a reduction in cerebral perfusion and cerebral arterial compliance, and an increase in diameter of normal cerebral arteries. [102].

1.5.2 Transcranial Doppler Ultrasonography

Transcranial Doppler (TCD) ultrasonography is a relatively new examination method that enables the measurement of cerebral blood flow velocities in the basal intracranial arteries. In 1979, based on earlier work by Satomura and Kaneko, Nornes *et al.* described an intraoperative pulsed Doppler sonographic method to study cerebral haemodynamics [95]. Three years later, Aaslid *et al.* (1982) introduced the PEDOF 2-MHz pulsed Doppler device by Vingmed that enabled non-invasive measurement of the blood flow velocity in large basal intracranial arteries [103]. Since then, new forms of TCD, such as colour-coded TCD (CC-TCD) and contrast-enhanced transcranial colour-coded sonography (CE-TCCS), have been developed with possible clinical applications [104, 105].

There are numerous advantages that TCD ultrasonography offers over other methods of studying cerebral haemodynamics. In particular, it is non-invasive, safe, inexpensive, and repeatable on demand. It can be done at the bedside and results are instantly obtainable. It is also not too difficult to learn and perform with a reasonable accuracy [5]. TCD ultrasonography provides information on velocity of the cerebral blood flow, and its direction in relation to the ultrasound probe, in major basal cerebral arteries [106]. From this information, and various other calculated parameters, it is therefore possible to assess different aspects of cerebrovascular haemodynamics. However, it does not measure the actual rate of cerebral blood flow in volume per unit time. TCD observations, therefore, cannot be interpreted in the same manner as cerebral blood flow images obtained by SPECT or PET studies [107].

TCD ultrasonography uses high-energy bi-directional pulsed Doppler ultrasound for the non-invasive measurement of blood flow within the intracranial arteries and veins. Ultrasound refers to sound frequencies higher than 20,000 cycles per second. The typical ultrasound transducer (i.e. probe) is a piezoelectric crystal that is stimulated to emit pulses of ultrasound of a known frequency (usually 2 MHz). The interface between the probe and the tissue being insonated is provided by a water-based coupling gel.

The probe, in the time between emitting ultrasound pulses, acts also as a receiver and translates frequencies back into electrical signals. A computer performs an automated fast Fourier transformation (FFT) of the received signal and this information is then displayed as an X-Y representation. The X-axis represents time and the Y-axis velocity in cm/s. The intensity of the reflected signal is represented by

the brightness of the displayed velocity [see 4 on Page 38]. In general, the sample volume has a cylindrical shape, with a diameter of about 4 mm, and a length that can adjusted between 4 and 15 mm.

In 1842, Christian Doppler described the physical phenomenon that has been named the Doppler principle [108]. The Doppler technique of TCD ultrasonography is based on the Doppler principle, by which the relative direction and velocity of the moving reflectors can be determined. The Doppler principle states that ultrasound waves (of frequency Fo) directed at a stream of moving red blood cells will be reflected back with a changed frequency. This change or shift in frequency (Fs) is known as Doppler shift and can be determined by the following Doppler formula:

$$\mathbf{Fs} = \frac{\mathbf{2 \,.\, Fo \,.\, V \,.\, Cos\theta}}{\mathbf{C}} \quad \textit{or alternatively} \quad \mathbf{V} = \frac{\mathbf{Fs \,.\, C}}{\mathbf{2 \,.\, Fo \,.\, Cos\theta}}$$

where Fo = original frequency of incident ultrasound beam (2 MHz)

V = velocity of blood flow (cm/s)

θ = angle between ultrasound beam and axis of blood flow

C = velocity of sound in the medium (soft tissues)

The velocity of blood flow is thus proportional to the Doppler shift and can be easily calculated once θ and C (1,540 m/s in human soft tissues) are known. A positive or negative Doppler shift means that the blood is flowing toward or away from the probe, respectively. The change in blood flow velocity during the cardiac cycle is displayed on the screen of the TCD unit. Unless the cross-sectional area (πr^2) is known, absolute flow (F) cannot be estimated but changes in flow will follow those in velocity (V) if the cross-sectional area remains approximately constant. This theory is expressed in the following equation [54]:

$$\mathbf{F = \pi r^2 \times V}$$

In normal circumstances, the highest velocities are found in the middle cerebral artery (mean ± 2 SD = 65 ± 17 cm/s, systolic ± 2SD = 94 ± 23 cm/s, diastolic ± 2SD = 46 ± 12 cm/s) and vary depending on the existence of other factors [107]. The technique of vessel identification and the different calculated derivatives, such as time averaged mean velocity and Gosling pulsatility index, will be discussed in detail in Section 2.2.1.

Figure 4A. A colour TCD trace from a right MCA. Positive signal represents flow *toward* the probe and negative signal represent flow *away* from the probe.

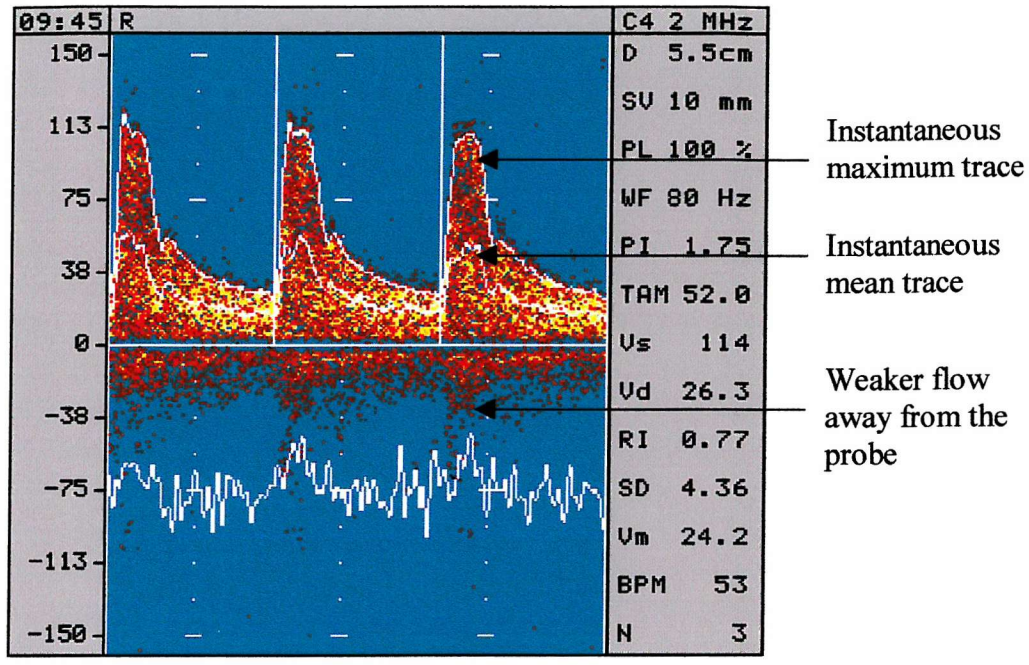
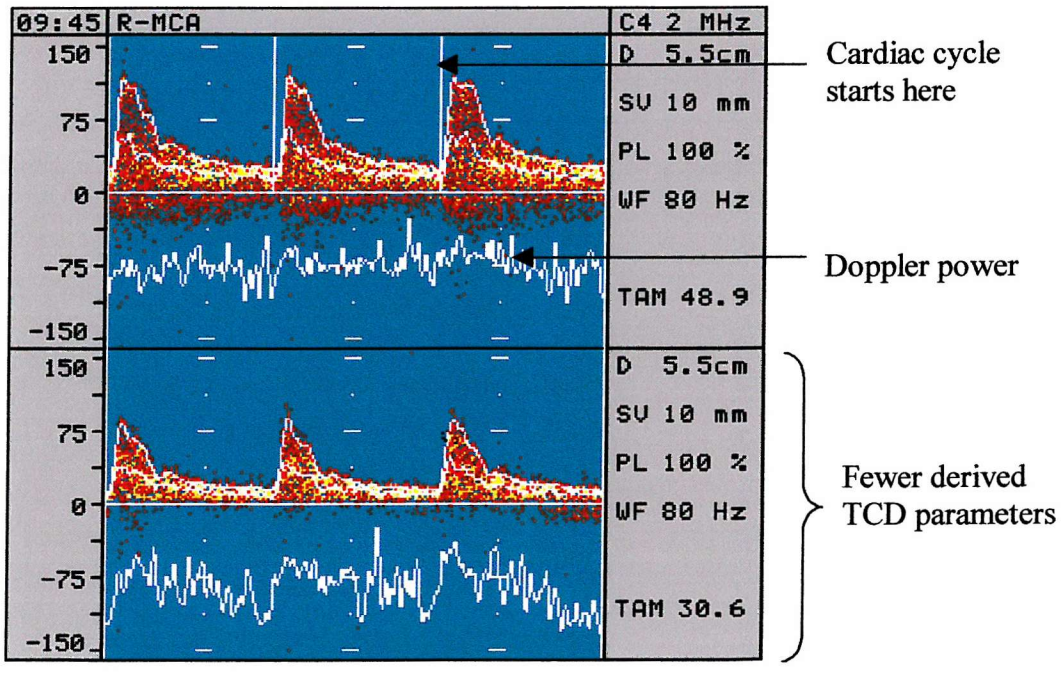


Figure 4B. A colour TCD trace from both MCAs. The beginning of each cardiac cycle is always determined by the upper signal (white vertial markers).



TCD ultrasonography has a large number of clinical applications and more are presently being developed [106]. TCD ultrasonography is a useful tool in many different specialist fields, including neurology and neurosurgery, stroke medicine, adult and paediatric intensive care, cardiology, and cardiothoracic surgery. Some of these are outlined below.

One of the original applications of TCD ultrasonography was in the detection of vasospasm, by Aaslid *et al.* in 1984. Increased blood flow velocity was found in patients with vasospasm, in accordance with the principle that velocity of blood flow in a vessel is inversely proportional to its diameter. Time average velocities of 120 to 230 cm/s in the middle cerebral artery were demonstrated during vasospasm after subarachnoid haemorrhage, with velocities up to 350 cm/s in isolated cases [109]. Subsequently many studies have confirmed its validity and usefulness in detecting vasospasm, especially in the middle cerebral and distal internal carotid arteries, after subarachnoid haemorrhage. It has been shown that TCD ultrasonography has a sensitivity of 94% and a specificity of 90% for detecting angiographic spasm of the middle cerebral artery [100].

TCD ultrasonography can also reliably and accurately detect other causes of focal increases of TCD blood flow velocity including intracranial stenosis from atherosclerosis, or resolving emboli. However, if a large infarction exists distal to the stenosis, there may be a low blood flow velocity due to reduced metabolic demand of the tissue [106]. Lindegaard *et al.* (1986) showed that TCD ultrasonography is useful in the evaluation and diagnosis of intracranial artery occlusive disease [110]. In conditions such as chronic severe extracranial occlusive disease, collateral blood supply can be detected (e.g. via the anterior communicating artery) by demonstrating increased cerebral blood flow in the contralateral anterior cerebral artery and reversed flow in the ipsilateral anterior artery. Giller *et al.* (1993) demonstrated that cerebral vasomotor reactivity to carbon dioxide or acetazolamide in the collateral circulation and in ischaemic regions can be reliably assessed by TCD ultrasonography [111]. In cases of arterio-venous malformation (AVM), TCD ultrasonography may be used to detect elevated blood flow velocity in arterial 'feeders' to the malformation, as well as to follow the effects of therapeutic interventions such as arterial embolisation and radiation treatment. TCD ultrasonography can also be used to demonstrate abnormal cerebral vasomotor reactivity of the feeding artery due to low resting cerebral vascular resistance.

In cases of ischaemic stroke, Toni *et al.* (1998) showed that TCD examination within six hours after stroke can help to predict both early deterioration and early improvement. Serial TCD ultrasonography is also able to detect both early and late recanalization [112]. Recently, Alexandrov *et al.* (1999) showed that emergency bedside single-channel TCD ultrasonography was sensitive and specific in determining arterial occlusion and stenosis in acute ischaemic stroke [113]. Alexandrov *et al.* (1995) also demonstrated that the cerebral perfusion abnormalities in ischaemic stroke, as assessed by a combination of TCD ultrasonography and SPECT, correlated well with the clinical outcome and the volume of brain damage. It was suggested that this combined method might improve the accuracy of prognosis in the 'hyperacute' phase of cerebral ischaemia [114]. Furthermore, Martin *et al.* (1995) demonstrated that a combination of extracranial and transcranial Doppler ultrasonography was useful in identifying major vascular pathology of the anterior cerebral circulation in patients with acute cerebral infarction. It may also be useful for the rapid identification of patients most and least suitable for acute intervention, such as thrombolytic therapy to promote recanalization, and for monitoring their response to the intervention [115].

Raised intracranial pressure is a result of brain injury and by itself can contribute to further, and sometimes irreversible tissue damage [107]. As intracranial pressure increases and cerebral perfusion pressure decreases, TCD ultrasonography exhibits increasing high-resistance signals, with decreasing blood flow velocities, corresponding to a decrease in cerebral blood flow. In cases of extremely high intracranial pressure, there is cessation of cerebral blood flow, which may result in brain death. TCD ultrasonography has been shown to be a useful and reliable (with 91% sensitivity and 100% specificity) technique in determining brain death and other conditions with progressive obstruction of the cerebral circulation [100, 116]. Current methods of monitoring intracranial pressure are invasive and present problems of haemorrhage and infection. Estimation of intracranial pressure from cerebral blood flow velocity using TCD ultrasonography is currently being explored. Klingelhofer *et al.* (1988) found a good correlation between intracranial pressure and TCD flow indices in a small group of patients with "various severe cerebral diseases" [117].

Recent improvements in TCD equipment and computer software have enabled continuous recording of blood flow velocity. Continuous TCD ultrasonography recording is useful for monitoring adequacy of cerebral blood flow during

neuroradiological or surgical procedures that can compromise cerebral perfusion pressure. During surgery, such as coronary bypass operation or carotid endarterectomy, the monitoring of cerebral blood flow velocity by TCD ultrasonography may help to prevent hypoperfusion or hyperperfusion when blood pressure fluctuates significantly [107]. Larsen *et al.* (1994) showed that TCD ultrasonography can reliably evaluate changes in cerebral blood flow during fluctuations in cerebral perfusion pressure [118]. TCD ultrasonography can also be used directly to detect the presence of solid microemboli, such as thrombi and platelet aggregates, by demonstrating high-intensity transient signals ('HITS'). It is a new diagnostic tool that can detect patients who are at high risk of embolic stroke and to whom prophylactic treatment can then be given [108].

Continuous TCD ultrasonography provides continuous information on relative cerebral blood flow velocity changes, which enables the study of the haemodynamics of the cerebral circulation. This has allowed the assessment of cerebral autoregulation and cerebral vasomotor reactivity, as well as blood flow velocity changes induced by other means such as pharmacological administration and cortical (e.g. visual) stimulation [119]. The non-invasive determination of cerebral autoregulation may be useful in evaluating strategies to improve cerebral autoregulation as well as aid in the optimal management of intracranial pressure control and preservation of optimal cerebral circulation [100]. In a review of 68 reports of studies of static cerebral autoregulation in humans, Panerai *et al.* (1998) found that the most frequently used methods for estimating changes in cerebral perfusion were TCD ultrasonography (36 cases) and ^{133}Xe clearance or other indicator-dilution methods (24 cases). With TCD ultrasonography, the most common artery insonated was the middle cerebral artery (26/36 cases). The studies reviewed suggested that TCD ultrasonography was the technique of choice in studies that are more recent. The median year of publication for studies of static autoregulation using ^{133}Xe or ^{85}Kr was 1985 (range 1970 – 1996) whilst for TCD ultrasonography the median was 1993 (1983 – 1997) [13].

The continuous measurement capability of TCD ultrasonography offers the potential to assess the dynamic component of cerebral autoregulation, which reflects the transient changes in cerebral blood flow following rapid changes in blood pressure, as well as the static component. The dynamic component of cerebral autoregulation corresponds to the transient response whilst the static component reflects the steady state response of the cerebral autoregulation with blood pressure as

an 'input' to the feedback mechanism. Aaslid *et al.* (1989) reported the results of dynamic autoregulation testing in normal volunteers using TCD ultrasonography. They examined the changes in blood flow velocity, as an index of relative changes in cerebral blood flow, in response to a transient drop in blood pressure induced by rapid deflation of bilateral thigh cuffs. The thigh cuffs were first inflated to supra-systolic blood pressure for two minutes [120]. The interest in the dynamics of cerebral autoregulation has been directly related to the availability of TCD ultrasonography due to its excellent temporal resolution. Thus, TCD ultrasonography has brought greater understanding to the physiology of cerebral autoregulation in humans and the study of dynamic autoregulation may become the method of choice for the clinical assessment of autoregulation (e.g. after stroke) [13].

1.5.3 Oscillations of Cerebral Blood Flow & Blood Pressure

Rhythmic oscillations are a common phenomenon in biological systems, occurring on a metabolic level as well as in highly complex regulatory mechanisms, such as those involved in glycolysis and neurohumoral systems. Slow, non-heart beat related rhythmic oscillations of intracranial (ventricular) pressure with a frequency of 0.5 to 2 cycles/min were first described by Lundberg (1960), and were termed 'B-waves' [121]. Although there have been many studies in this area, the pathophysiological understanding of the dynamics of B-waves and their interrelation with other systemic (e.g. blood pressure), cerebrovascular, or metabolic factors remains unclear. It has been suggested that B-waves are a result of fluctuation of one of, or a combination of, a number of factors such as Pco_2, respiratory efforts, central autonomic and vasomotor activity (brainstem), and blood pressure [122]. Magnæs (1976) was one of the first to postulate that B-waves were generated by changes in intracranial blood volume reflecting blood pressure waves and cerebral autoregulation [122]. Rosner *et al.* (1983) postulated that fluctuations of cerebral perfusion pressure evoked an autoregulatory response of the cerebral vessels, leading to fluctuations of intracranial volume and intracranial pressure [122]. Fujii *et al.* (1990) observed spontaneous rhythmic change in vascular calibre, also known as 'vasomotion', in cats. It was shown that vasomotion in vivo was common, of relatively large amplitude ($19 \pm 2\%$ of the mean), and was independent of vessel diameter [123]. These findings were confirmed by Auer *et al.* (1981) who also found rhythmic diameter oscillations of cat

pial vessels within a wide range of frequencies between 0.5 and 8 cycles/min at normal blood pressure. These oscillations could be influenced by changing the blood pressure (blood pressure increase provoked oscillations) and the vasomotor tone (vasodilatation increased the amplitude of oscillations) [124]. Auer *et al.* later (1983) observed rhythmic fluctuations of the pial vessel diameter simultaneously with B-waves and postulated that B-waves were a sum phenomenon of intracranial volume oscillations mediated by pial vessels [125]. Some studies have found an association between B-waves and bad prognosis in patients with raised intracranial pressure, whilst others have denied the prognostic significance of B-waves [126].

Cerebral blood flow velocity, as measured by TCD ultrasonography, also shows slow rhythmic oscillations. Lindegaard *et al.* (1987), using long-term monitoring with TCD ultrasonography, assessed the amplitude of spontaneous oscillations of cerebral blood flow velocity in the middle cerebral artery and found in different normal individuals maximum deviations between ± 15% and ± 35% of the mean [127]. In normal conditions, Giller *et al.* (1993) and Diehl *et al.* (1991) also found large oscillations of up to ± 30% from the mean cerebral blood flow velocity with low frequencies between 0.4 and 9 cycles/min [128, 129]. These large fluctuations of cerebral blood flow velocity tend to occur in three separate frequency ranges: one between 0.5 and 2 cycles/min, one around 6 cycles/min, and the other around 12 to 15 cycles/min [130].

Of these three different frequencies of fluctuations, the one that has been most reported is the very low frequency fluctuations between 0.5 and 2 cycles/min. Lundar *et al.* (1990) and Newell *et al.* (1992) showed that these fluctuations are synchronous with B-waves of intracranial pressure [131, 132]. They are, therefore, also known as 'B-wave equivalent' (BWE) [133]. Although highly correlated with intracranial pressure, poor correlation has been found between these fluctuations and systemic circulatory indices such as blood pressure [129]. Newell *et al.* (1992) performed continuous monitoring of blood flow velocity (middle cerebral artery), intracranial pressure, blood pressure, and end-tidal carbon dioxide concentration. They found that both velocity waves and B-waves occurred despite a constant carbon dioxide concentration in ventilated patients, and that they were not accompanied by fluctuations in the arterial blood pressure [131]. Mautner-Huppert *et al.* (1989) postulated that such fluctuations in blood flow velocity are caused by phasic dilatation

and constriction of the small regulating arterioles (vasomotion), which in turn produce fluctuations in cerebral blood volume, and are eventually reflected in the intracranial pressure [126]. Others have also suggested causal associations between slow fluctuations of blood flow velocity and cerebral haemoglobin oxygenation in the jugular bulb (Sjo_2), brain tissue oxygen concentration (Po_2), the cortical cytochrome-c oxidase and the NADH-redox state [122].

Slow fluctuations of blood flow velocity (middle cerebral artery) of around 6 cycles/min have been reported by Diehl *et al.* (1991) [129]. These fluctuations, also known as Mayer or M-waves, were found not to be synchronized with B-waves and were not correlated with respiration [134]. In addition, fluctuations in the high frequency range, around 12 to 15 cycles/min, have been detected and were noted to correlate with respiration [131]. They are, therefore, also known as respiratory or R-waves [134].

Blood pressure also demonstrates slow spontaneous oscillations. Steinmeier *et al.* (1996) observed, in patients with brain damage, different slow rhythmic blood pressure waves that could be clearly distinguished. They were classified as either Hering-Traube waves, which are attributed to and closely related to respiratory activation of the brainstem, or Mayer or M-waves, which are probably generated in the ventrolateral medullary surface [122]. In the rat model, Julien *et al.* (1995) found that the low-frequency oscillations of blood pressure were mostly secondary to rhythmic fluctuations in the vasomotor sympathetic tone. The high-frequency (respiratory) oscillations of blood pressure were associated with cardiac output of pure mechanical origin [135].

Lastly, the side-to-side and day-to-day variations of TCD derivatives (blood flow velocities, hemispheric blood flow velocity ratios and pulsatility indices) in 35 normal adult subjects were studied by Sorteberg *et al.* (1990). It was observed that blood flow velocity varied from side to side, with magnitude of variation of approximately 20% from the mean. There were, however, no significant variations from day to day [136].

1.5.4 Mechanisms of Cerebral Autoregulation

The mechanisms of cerebral autoregulation have been investigated for several decades. Three main hypotheses have been suggested: the myogenic, metabolic, and neurogenic hypotheses. Each hypothesis will be discussed individually.

The myogenic mechanism theory states that the smooth muscle of cerebral vessels is responsive to changes in transmural pressure; the small arteries and arterioles constrict or dilate in response to increases or decreases in the transmural pressure. In other words, as an intrinsic characteristic of cerebral blood vessels, there is vasoconstriction with hypertension and vasodilatation with hypotension. This is in accordance with the principle proposed by Bayliss in 1902 [137]. A rapid variation in intravascular pressure may modify the status of the actin and myosin filaments in the smooth muscle cells. However, the mechanism by which a force applied to vascular smooth muscle initiates an active response is poorly understood. It has been suggested that stretching arterial smooth muscle cells results in activation of membrane permeabilities to ions, such as calcium, and results in changes in membrane potentials [32].

The metabolic hypothesis states that changes in cerebral perfusion pressure leads to accumulation or clearance of vasoactive metabolites in the perivascular space. These metabolites, which may include carbon dioxide, hydrogen ion, adenosine, potassium, and calcium, act as mediators of the coupling between neuronal activity and cerebral blood flow. The exact role of these substances in the coupling between blood flow and metabolism remains to be elucidated. Moreover, changes in systemic metabolites may also greatly influence cerebral blood flow. Hypercapnia and hypoxia are two of the most powerful stimuli that can increase global cerebral blood flow. The metabolic theory of cerebral autoregulation suggests that a reduction in local blood flow results in the release and accumulation of vasoactive chemicals that produce vasodilatation of cerebral vessels. It has been proposed that accumulation of adenosine during hypotension and its increased clearance during hypertension could play a role in cerebral autoregulation [56]. Kontos *et al.* (1985) demonstrated that the dominant mechanism of autoregulation is a flow-dependent metabolic mechanism that depends on changes in parenchymal tissue oxygen tension, which in turn causes secondary changes in adenosine production [138, 32]. This metabolic theory is supported by the fact that focal cerebral ischaemic lesion may be able to influence blood flow in remote areas [see Section 1.2.6] [139].

The neurogenic hypothesis suggests that the autoregulatory adjustment of the calibre of resistance vessels is mediated by vascular innervation. This hypothesis assumes that there is a reflex arc by which changes in cerebral perfusion pressure control the diameter of resistance vessels via efferent extrinsic vasomotor nerves [56].

The extrinsic nerves originate mainly from the cranial ganglia, which provide sympathetic, parasympathetic and sensory input to the cerebral circulation by exerting vasoconstrictor influence on the larger cerebral arteries. Stimulation or denervation of these α-adrenergic perivascular sympathetic nerves may result in changes of cerebral blood volume, intracranial pressure and formation of cerebrospinal fluid [32]. This, in turn, may modify the autoregulatory function of the smaller arteries and arterioles further downstream [37]. The role of the parasympathetic system, which originates from the sphenopalatine and otic ganglia, is still unclear.

More recently, possibility has been raised that the cerebrovascular endothelial cells may contribute to cerebral autoregulation. There is evidence that cerebral autoregulation may be influenced by the release of endothelium-derived relaxing factor (EDRF) and contractile factor (EDCF) from the endothelium [32]. This hypothesis is supported by some studies which have demonstrated that disruption of the endothelium abolished the pressure-dependent myogenic depolarisation, action potential generation, and constriction of the cerebral blood vessel (cat middle cerebral artery) [140]. This is an exciting field because the endothelium may become a target for stroke therapy in the future.

It is not clear which of these mechanisms is (or are) predominantly responsible for cerebral autoregulation. However, the evidence thus far accumulated suggests that the cellular basis for cerebral autoregulation is inherent in the intrinsic property of vascular smooth muscle to react to changes in transmural pressure (and blood pressure). Neurogenic and metabolic factors may act to modulate this intrinsic vascular myogenic response and also influence the rapidity of the response, the slope of the pressure-flow relationship, and the range of pressures over which cerebral autoregulation is effective [56]. Wagner *et al.* (1985), however, showed that cerebral autoregulation was a function of cerebral perfusion pressure gradient (i.e. affected by changes in blood pressure, intracranial pressure and jugular venous pressure) and could not be accounted for predominantly by the myogenic mechanisms [141]. Indeed, there are important issues that remain unresolved and need further investigation. For example, it is possible that cerebral blood vessels may autoregulate by different mechanisms according to their location and size.

An important piece of research by Baumbach *et al.* (1985) showed that cerebral autoregulation was heterogeneous in three major ways: regional, segmental

and temporal [142]. The investigators found regional heterogeneity of the autoregulatory response during both acute reductions and increases in systemic arterial blood pressure. Changes in blood flow were less (i.e. cerebral autoregulation is more effective) in brainstem than in cerebrum during decreases and increases in cerebral perfusion pressure. Segmental heterogeneity of cerebral autoregulation suggests that large and small arteries and arterioles contribute in different ways to autoregulation. Determination of segmental cerebral vascular resistance indicated that, while small cerebral vessels (< 200 μm in diameter) made significant contribution to cerebral autoregulation at mean arterial blood pressures between 80 and 100 mmHg, the role of large cerebral arteries (> 200 μm) became increasingly essential to the autoregulatory response at pressures above 100 mmHg. It was also shown that the autoregulatory response was influenced by the rate of change in blood pressure, i.e. temporal heterogeneity of autoregulation. Sudden increases in blood pressure produced transient increases in cerebral blood flow, which were not observed under steady-state conditions. In addition, the blood-brain barrier was shown to be more susceptible to hypertensive disruption after rapid, as compared to step-wise, increases in blood pressure. It was therefore proposed that when investigating cerebral autoregulation, regional segmental, and temporal differences in the autoregulatory response should be taken into consideration [142].

1.5.5 Assessment of Cerebral Autoregulation

Cerebral autoregulation can be regarded, at least partially, to represent tight coupling between oxygen supply and demand of the brain. With constant metabolic demand, changes in cerebral perfusion pressure or blood pressure that would increase or decrease cerebral blood flow are compensated by adjustments in vasomotor tone to maintain a constant supply of oxygen and hence constant cerebral blood flow. As already mentioned, there are many factors, whether physiological or pharmacological, which can modulate the autoregulatory response. These multiple influences on cerebral blood flow generate the opportunity to study its autoregulatory mechanisms by disturbing different 'input' variables.

Assessment of cerebral autoregulation is an important adjunct to measurement of cerebral blood flow for diagnosis, monitoring and prognosis of cerebrovascular disease. It may be useful as part of the clinical management of conditions such as

severe head injury, subarachnoid haemorrhage, and severe chronic extracranial stenosis, which are known to impair cerebral autoregulation [see Section 1.3.2]. In these patients, greater control of the fluctuations in cerebral perfusion pressure and haemodynamic consequences of interventions may be desirable.

Research performed on human cerebral autoregulation has endeavoured to establish whether it is normal or impaired in various states. These studies usually employed the 'autoregulatory curve' as a model [see Figure 1 on Page 15] and considered cerebral autoregulation to be an 'all or nothing' phenomenon. Instead of viewing the assessment of cerebral autoregulation as an 'either or' examination, it is perhaps more appropriate to regard it as a series of stages dependent on the feedback gain of the multiple mechanisms involved. Cerebral autoregulation is also not a physical quantity with measurable properties, but rather a concept, derived from complex interactions between several variables [13]. The method of assessment of cerebral autoregulation is therefore determined by the models adopted to view the phenomenon. For example, the approaches used to examine static autoregulation differ greatly from those used to examine dynamic autoregulation.

In the past, most studies of cerebral autoregulation have looked at the relationship between cerebral blood flow and cerebral perfusion pressure or blood pressure. This static approach, which forms the basis for the 'autoregulatory curve', does not take into consideration the time course of changes in cerebral blood flow following changes in blood pressure. Static methods utilise steady-state values to test for changes in cerebral blood flow or blood flow velocity when blood pressure is changed significantly. Panerai (1998), in his exhaustive review of methods of assessing cerebral autoregulation in humans, found a great diversity regarding the essential methodological aspects of static autoregulation examination. In particular, there were significant variations in measurement techniques for cerebral perfusion pressure and cerebral blood flow, methods of altering blood pressure or cerebral perfusion pressure, research design, as well as analytic approach [13]. There is, therefore, no 'gold standard' for the assessment of static autoregulation.

The aforementioned mechanisms of cerebral autoregulation (myogenic, metabolic, and neurogenic) probably contribute in different ways to produce effective changes in cerebral vascular resistance in order to maintain a stable cerebral blood flow. Even the most rapid mechanism would require a specific and finite length of time to bring about haemodynamic changes. This means that when sudden changes of

blood pressure take place, it is expected that the return of cerebral blood flow to its original level will take a finite length of time to complete. Aaslid *et al.* (1989) first applied the term 'dynamic autoregulation' to describe transient changes in cerebral blood flow velocity following rapid changes of blood pressure. It was demonstrated that full restoration of TCD blood flow velocity to pre-test level was seen as early as 4.1 seconds after the step decrease in blood pressure [120]. In premature newborns, Panerai *et al.* (1995) also showed a similar temporal response; in most cases, the blood flow velocity returned to 'normal' within 10 to 15 seconds [143]. It is this speed of response that has limited the number of techniques that can be used to assess dynamic autoregulation. In particular, TCD ultrasonography is generally accepted as the most accessible, practical and valuable [see Section 1.5.2].

There are four main advantages of studying the dynamic component of autoregulation as the clinical assessment of cerebral autoregulation. Firstly, the test is not time-consuming and can be performed at the bedside. Secondly, it can be more reliably and consistently repeated. Thirdly, the short time interval that is required to carry out the test minimises the influences of other modulating factors such as Pco_2, mental activation, and haematocrit. These confounding factors are more relevant during static autoregulation tests when observations are made between much longer time intervals. Lastly, The quasi-quantification of dynamic responses is able to provide a more meaningful method of comparison between patients or groups of patients (e.g. acute vs. chronic stroke).

Cerebral autoregulation can be regarded as a feedback control system [144]. A model of input (blood pressure) and output (cerebral blood flow) is then delineated and variations in blood pressure can be considered as a disturbance to the system. To investigate the dynamics of cerebral autoregulation, it is helpful to observe the effect of clearly defined changes in cerebral perfusion pressure (or blood pressure) on cerebral blood flow (or blood flow velocity). Three useful models of pressure changes, by virtue of their mathematical simplicity, are stepwise changes, periodic oscillations, and spontaneous random fluctuations [145]. Studies that have used each model will be illustrated in turn.

Since Aaslid *et al.* (1989) first used the technique of sudden deflation of inflated thigh cuffs to induce a rapid stepwise drop in blood pressure ten years ago, it has become the most frequently reported technique of assessing dynamic autoregulation [120, 13]. Others, such as Newell *et al.* (1994), Strebel *et al.* (1995),

Tiecks *et al.* (1996) and White *et al.* (1997) have all used this approach in the past [146, 55, 147, 148]. The 'thigh cuffs' technique involves placing large (20cm) thigh cuffs around both thighs and the cuffs are inflated to 20 to 40 mmHg above systolic blood pressure, for at least two minutes. The rapid release of the thigh cuffs elicits an abrupt decrease in blood pressure approximately 200 msec later and the blood pressure remains low for four to five heart beats (about 5 to 7 seconds). The magnitude of blood pressure fall is in the region of 20%. By using TCD ultrasonography, Aaslid *et al.* (1989) found that the fall in blood pressure was associated with a decrease in blood flow velocity in the middle cerebral artery. Whilst the blood pressure remained low, the blood flow velocity returned to the pre-test level. After this phase, there was an overshoot in blood flow velocity while blood pressure returned to control. The competence of dynamic autoregulation was indicated by the rate of autoregulation, which was determined by the change in cerebral vascular resistance per second during the 2.5 seconds immediately after the step decrease in blood pressure. A highly significant inverse relation was found between rate of autoregulation and Pco_2. It concluded, "the response rate of cerebral autoregulation in awake normal humans is profoundly dependent on vascular tone" [120].

The 'thigh cuffs' technique has been widely used with useful results. There are, however, two important disadvantages of the technique. Firstly, the inflation of the cuffs can be painful and may produce sympathetic activation, which may interfere with the interpretation of result [13]. The fact that the procedure usually needs to be repeated several times, which suggests considerable variability of the procedure itself, may increase the overall discomfort induced by it. For instance, White *et al.* (1997) performed five cycles of inflation-deflation with a three minute rest interval between cycles [148]. Secondly, the stepwise drop in blood pressure is not always significant or consistent. White *et al.* (1997) and Junger *et al.* (1997) regarded a threshold of 10 mmHg as an acceptable decrease in blood pressure, whereas Tiecks *et al.* (1995) used a threshold of 15 mmHg and Newel *et al.* (1994) did not specify one [148, 42, 146, 149]. There is no universally agreed threshold over which the fall in blood pressure becomes significant. Little information is also available about the reproducibility of the blood pressure drop produced by the 'thigh cuffs' technique and the corresponding indices of dynamic autoregulation.

Another method of inducing significant changes of blood pressure is the Valsalva manoeuvre. Valsalva manoeuvre is defined as a forced expiration against closed glottis, with complex physiological consequences. In the study by Tiecks *et al.* (1996), subjects' mouths were connected to a pressure gauge and Valsalva manoeuvre was performed for 15 seconds by forceful expiration against the pressure gauge at a pressure of 30 mmHg [147]. There are four distinct phases of the Valsalva manoeuvre, identifiable by the characteristic changes in blood pressure, heart rate, cardiac output, and cerebral blood flow. Phase I is characterized by a rapid rise in blood pressure and reduction in heart rate after straining begins. Phase II is characterized by a fall in blood pressure (IIa) followed by tachycardia and partial recovery of blood pressure (IIb). After the release of straining, phase III is characterized by a brief drop in blood pressure and minor increase in heart rate. Lastly, phase IV is characterized by an overshoot of blood pressure above baseline followed by bradycardia [147, 13]. The sustained increase in intracranial pressure and transient decrease in blood pressure in the first seconds of the strain cause a fall in cerebral perfusion pressure. Typical changes in cerebral vascular resistance and blood flow velocity then occur, which can be measured and interpreted as cerebral autoregulatory response [150]. During dynamic autoregulation tests, the phases of greatest interest are II and IV, where there are often dramatic changes in blood flow velocity. Tiecks *et al* (1995) demonstrated that blood flow velocity decreased by 35% in phase IIa, then rose by 57% in phase IV, as compared with the baseline value [150]. Other investigators have evaluated head-up tilting and postural change, which are also capable of producing stepwise changes in blood pressure, as techniques in dynamic autoregulation tests [151]. In addition, Giller *et al.* (1991) investigated the feasibility of performing transient manual carotid artery compression in the neck, which was known to produce a brief hyperaemic response in the middle cerebral artery distribution, as a test of cerebral autoregulation. It was concluded that the technique, which used TCD ultrasonography, was useful and reliable [152].

Dawson *et al.* (1999) investigated the effects of different blood pressure stimuli. They examined the effects of dynamic 'pressor' stimuli, which were the cold pressor response (immersion of hand in cold water to induce somatic pain) and Valsalva manoeuvre, and 'depressor' stimuli, which were thigh cuff and isometric handgrip release. It was demonstrated that results (i.e. competence of cerebral autoregulation) varied according to the stimulus employed. It was concluded that

"these results cast doubts over the previously accepted normal values for dynamic autoregulatory competence, and indicate that, even in a healthy population, it varies according to the blood pressure stimulus used" [153]. In general, it should be the aim of the investigator to use a blood pressure stimulus that is non-invasive, acceptable to patients, and is able to produce significant blood pressure changes in the assessment of dynamic autoregulation.

Instead of generating stepwise changes in blood pressure, some investigators have studied the TCD blood flow velocity responses to slow induced oscillation in blood pressure. Birch *et al.* (1995) used cycles of 10 seconds of squatting followed by 10 seconds of standing to create periodic variations in blood pressure. It was demonstrated that when blood pressure was forced to oscillate with a period of 20 seconds, the middle cerebral artery blood flow velocity also has induced oscillations. The temporal relationship between the two oscillating parameters provided information about the competence of cerebral autoregulation [145]. This analytical technique will be discussed in detail in the next chapter. Aaslid *et al.* (1995) recognized that one of the disadvantages of stepwise drop in blood pressure was that a large drop in blood pressure may sometimes go below the lower limit of cerebral autoregulation, with theoretical harm to the cerebral blood flow. They investigated a technique of inducing oscillations in blood pressure by periodic inflation and deflation of thigh cuffs (15 seconds inflated above systolic blood pressure and 15 seconds deflated) in patients with head injury as well as normal controls. The technique was shown to "give a precise and physiologically realistic evaluation of autoregulatory response" [154]. Diehl *et al.* (1995) adopted cyclical slow breathing at a frequency of 6 breaths per minute as a stimulus to induce oscillations of blood pressure, with similar conclusions [144]. In most cases, with these techniques, patients need to be reasonably fit to perform periodic squatting and many patients cannot follow periodic slow breathing or find cyclical thigh cuff inflation uncomfortable [13]. In addition, forced breathing is known to provoke significant changes in arterial Pco_2, which can substantially influence the autoregulatory response [see Section 1.3.2] [145].

The aforementioned methods of assessment of the dynamic component of cerebral autoregulation in humans all have their advantages and disadvantages. Most of the problems associated with these methods derive from the particular techniques used to promote either stepwise changes or slow oscillations of blood pressure. These problems, which have been outlined according to each method, create difficulties in

standardization in the clinical setting. There is no single preferred method of assessment for patients with such diverse conditions as stroke, extracranial carotid stenosis, head injury, prematurity of the newborn, and various psychiatric diseases. In addition, patients differ in their ability to tolerate changes in blood pressure, postural changes, application of painful thigh cuffs, or performance of the Valsalva manoeuvre. For example, it will be difficult to expect an acute stroke patient to successfully perform a Valsalva manoeuvre especially if there are difficulties with communication and comprehension. Likewise, it is probably unreasonable to repeatedly perform the 'thigh cuffs' test on patients with severe head injury or premature newborns in the intensive care setting. This diversity of tolerance may produce significant additional complexity in analysis and comparison between patients or groups of patients. Furthermore, the stimulus to induce blood pressure changes should have minimal physiological effects on other systems such as sympathetic and mental activation, or parameters such as Pco_2 or pH. Disturbance of these factors may significantly influence test results.

It has therefore been suggested that observation of the spontaneous variation of cerebral blood flow velocity and blood pressure is the ideal method, because of its non-invasiveness and the avoidance of large blood pressure disturbances. This is particularly useful for patients in critical conditions, such as premature newborn and severe stroke. This method has been used in the examination of cardiac baroreceptor function. As already mentioned, blood pressure oscillates at a high frequency (Respiratory or R-waves) and low frequency (Mayer or M-waves). The respiratory activity is also accompanied by slow rhythmic fluctuations of the interval between heartbeats, that is, the R-R interval on the electrocardiogram. Malliani *et al.* (1991) confirmed the feasibility of using spontaneous variation of these parameters to provide information on the roles of sympathetic and parasympathetic activity in the control of circulatory oscillations [155]. Similarly, investigators have used spontaneous variations or oscillations of cerebral blood flow velocity and blood pressure for the assessment of dynamic autoregulation. Panerai *et al.* (1998) suggested that the use of undisturbed recordings of blood pressure and cerebral blood flow velocity might provide a safer technique for assessment of patients in whom a sudden drop of blood pressure is undesirable [156]. Steinmeier *et al.* (1996) found evidence that failure of cerebral autoregulation significantly modified the temporal relationship and correlation between cerebral blood flow velocity and blood pressure [122].

Tiecks *et al.* (1995) investigated whether static autoregulation correlated with dynamic autoregulation in patients with normal and impaired autoregulation. It was found that, in normal human subjects, measurement of dynamic autoregulation yielded similar results as static testing of intact and impaired autoregulation [149].

The subject of dynamic autoregulation testing is currently under intense research activity and the use of spontaneous variation of blood pressure and blood flow velocity may in the future become the clinical method of choice because of its many advantages. However, there is only limited experience with this technique and there exists essential gaps in knowledge that need to be bridged before it can be applied in routine clinical practice [13].

1.6 Summary

Stroke is an devastating disease with significant mortality and morbidity. Acute ischaemic stroke is four times more common than haemorrhagic stroke, and the most common clinical subtypes of stroke are those involving the anterior circulation, especially the middle cerebral artery. Anterior circulation strokes are also associated with the worst prognosis and highest rate of recurrence, as shown by the Oxfordshire Community Stroke Project (OCSP, 1990). In the past half a century, a great deal of research has been carried out on this important disease and, in particular, many investigators have studied the disturbances of cerebral haemodynamics after stroke. This progress has been greatly enhanced by the development of new imaging techniques such as PET scanning. These new techniques have made possible the in-depth exploration of many physiological aspects of ischaemic stroke, such as the vascular disturbances within the ischaemic penumbra. One of the most significant developments has been the increasing application of TCD ultrasonography to examine middle cerebral artery blood flow velocity in various cerebrovascular diseases. This technique has now become an indispensable tool in the assessment of cerebral autoregulation and cerebral vasomotor reactivity in the experimental setting. Moreover, clinical assessment of cerebral autoregulation is becoming more common for patients with conditions such as stroke (particularly haemorrhagic), head injury, and extracranial carotid stenosis, where autoregulation is often impaired and surgery may be a treatment option.

Evidence suggests that abnormalities in cerebral autoregulation and cerebral vasomotor reactivity are common after acute ischaemic stroke. This phenomenon is essential to the understanding of the pathophysiology of stroke itself. Theoretically, reducing systemic blood pressure in a patient with impaired cerebral autoregulation may passively reduce the cerebral blood flow, which may already be critically low within and around the ischaemic areas. Antihypertensive therapy, therefore, may exacerbate ischaemic damage or cause new 'watershed' infarcts within at-risk areas [75]. Furthermore, this phenomenon may have other important clinical implications, such as pharmacological manipulation of blood pressure after stroke, nursing care of acute stroke patients, and methods of mobilisation by physiotherapists. Evidence from randomised controlled trials is unfortunately lacking for many of these clinical aspects of acute stroke care.

In addition, there is limited knowledge on the recovery of cerebral autoregulation and cerebral vasomotor reactivity after acute ischaemic stroke. Only Agnoli *et al.* (1968) have compared the competence of cerebral autoregulation in stroke patients during the 'acute' (within 2 days) and 'chronic' (over 15 days) phases. They showed that impairment of cerebral autoregulation was more frequent in the acute than chronic group [83]. However, for this study, patients were not followed up from the acute to the chronic phase, but rather comparisons were made of different groups of individuals. A prospective, longitudinal study that examines the recovery of cerebral autoregulation during the acute, subacute, and chronic stages amongst the same stroke patients is needed. The study of the timing and nature of the recovery of cerebral autoregulation has important clinical implications. For instance, it may be 'safer' to manipulate blood pressure after stroke only when the impairment of cerebral autoregulation has 'resolved'.

The understanding of the physiological mechanisms as well as the static and dynamic components of cerebral autoregulation has evolved in the last 30 years through the development of new methods of assessment. Techniques involving TCD ultrasonography have become the methods of choice. Recently, the study of dynamic autoregulation has received much attention. Many investigators have tried to develop the 'ideal' method of assessment that is non-invasive, easy to perform at the bedside, cheap, safe, acceptable to patients, and able to yield useful results that are reliable and repeatable. Many of the methods that are described in the literature and in this thesis, particularly those that impose sudden and dramatic changes on the cardiovascular and cerebral circulation, fail to meet all these criteria. For this reason, it is likely that the observation of spontaneous fluctuations of cerebral blood flow velocity and blood pressure will be increasingly popular for the assessment of dynamic autoregulation.

The phenomenon of spontaneous variation of blood flow velocity after stroke needs further exploration. For example, there is evidence that, in patients with internal carotid artery stenosis, the spontaneous variations of cerebral blood flow in the affected and unaffected hemisphere are not synchronous [129]. After stroke, when cerebral autoregulation is often impaired, oscillations in cerebral blood flow velocity may also be asymmetrical and not synchronous. Results from our pilot study suggest that, in patients recovering from stroke, spontaneous variations of blood flow velocity in the left and right MCAs were not synchronous [157]. It is possible that there is asymmetry between the affected and unaffected hemispheres in relation to cerebral

autoregulation and vasoreactivity. This may have potential implications for future cerebrovascular research and methods of assessing cerebral haemodynamics.

Progress in research in cerebral autoregulation has also led to the development of less invasive techniques of inducing blood pressure oscillations. Techniques such as periodic inflation and deflation of thigh cuffs, although effective in inducing blood pressure oscillations, have been criticized as unacceptable to the patients. One technique that has recently been evaluated is isometric handgrip. Dawson *et al.* (1999) showed that isometric handgrip was a non-invasive technique that effectively increased blood pressure during static autoregulation testing [158]. Theoretically, it should be possible to stimulate periodic blood pressure oscillations by rhythmic handgrip. Although spontaneous blood pressure oscillations has been examined as a method of assessing cerebral autoregulation, to our knowledge, induced blood pressure oscillations by rhythmic handgrip has never been tested in stroke patients. We hypothesized that, after ischaemic stroke, the affected hemisphere may respond differently from the unaffected hemisphere to blood pressure oscillations because of impaired cerebral autoregulation. In the present study, we proposed that rhythmic handgrip may be a useful non-invasive technique for the assessment of cerebral autoregulation after stroke.

1.7 Objectives

In this study, we sought to assess the change in cerebral autoregulation after ischaemic stroke by using TCD ultrasonography to examine the relationship between oscillations of cerebral blood flow velocity and blood pressure. We were interested in both the spontaneous and induced oscillations by rhythmic handgrip. We performed this study to explore the following questions: -

1. Is there evidence of recovery in cerebral autoregulation during the first three months after acute ischaemic stroke?

2. Is there any difference in cerebral autoregulation between the affected and unaffected hemispheres after acute ischaemic stroke?

3. Does cerebral autoregulation behave as a high-pass filter model after acute ischaemic stroke?

4. How useful is the combination of TCD ultrasonography and rhythmic handgrip as a method of assessing cerebral autoregulation after stroke?

CHAPTER 2 METHODS

2.1 Introduction

The objectives of the present study have been clearly stated. In this study, we chose not to stimulate sudden and large stepwise changes in blood pressure by, for example applying the 'thigh cuffs' technique, head-up tilting, Valsalva manoeuvre, or inducing the cold pressor response. The main reason for avoiding these manoeuvres was that the stroke patients who were recruited in the study were often frail, immobile, dysphasic (with problems relating to comprehension and verbal expression), and in whom any induction of pain and discomfort should be prevented. As already discussed in Section 1.5.5, many of these manoeuvres can be associated with a degree of discomfort and inconsistency. Consequently, we opted to concentrate our investigation on the spontaneous fluctuations and slow induced oscillations of blood pressure.

The examination of spontaneous fluctuations necessitates continuous monitoring and recording of both blood flow velocity and arterial blood pressure. TCD ultrasonography was selected for the monitoring of cerebral blood flow velocity in both middle cerebral arteries [see Section 2.2.1], and the Finapres device for the monitoring of blood pressure [see Section 2.2.2]. We have also chosen to use rhythmic handgrip as the stimulus for blood pressure oscillations in this study [see Section 2.2.3]. The reasoning behind the choice of these techniques will be individually reviewed in this chapter. Furthermore, there are several physiological parameters that could have been examined but were omitted for reasons that will be discussed in detail in Section 2.2.5.

2.2 Apparatus, Techniques & Reasoning

2.2.1 Transcranial Doppler Ultrasonography

The principles, clinical applications, and many advantages of TCD ultrasonography have been clearly outlined in Section 1.5.2. The technique of vessel identification, appearances of normal blood flow signals, and derived indices are described here.

TCD ultrasonography can identify basal cerebral arteries according to the depth of insonation, the direction of blood flow, the velocity obtained, and the relationship with other identifiable arterial segments. The performance and interpretation of TCD ultrasonography therefore requires integrating all these elements to produce a mental picture of the vascular anatomy and flow in the cerebral circulation [106]. With some newer machines, it is possible to display a three-dimensional computerised 'mapping' of the arteries of the circle of Willis; for example the transcranial Doppler mapping (TCDM) system (Trans-scan, Eden Medical Electronics, WA, USA), which was developed by Aaslid *et al.* (1986) [116].

TCD examination can be performed on anyone who is able to remain stationary in the supine or semi-recumbent position for as long as the examination requires. No preparation is necessary and this hand-held technique can readily be performed at the bedside, in intensive care unit, during radiological (e.g. SPECT scan) or surgical procedures (e.g. carotid endarterectomy). The preferred position for the examiner is at the head of the bed to allow an assessment of each cerebral hemisphere and optimisation of scanning planes. The patient's head should face forward and the examiner adjusts the position of the transducer (i.e. probe) and angle of insonation to locate the target cerebral artery with the strongest possible Doppler signal.

Patient comfort is very important during TCD examination because it minimises head movements, which changes the artery-transducer angle, and avoids anxiety-induced respiratory changes, which causes hypercapnia or hypocapnia that may influence cerebral blood flow velocity [116].

TCD examinations are performed through the following four naturally occurring cranial windows (in addition to patent fontanelles and burr holes): transtemporal, transorbital, transforaminal (foramen magnum), and submandibular. Three transtemporal windows are available due to regions of thinning in the suprazygomatic portion of the temporal bone. The posterior window lies immediately anterior to the external auditory canal, the middle window lies 1.5 cm anterior to the

posterior window, and the anterior window lies 1.5 cm anterior to the middle window. The transtemporal windows are used to study the middle, anterior and posterior cerebral arteries, along with the anterior and posterior communicating arteries and the terminal portion of the internal carotid artery [95]. The other three transcranial windows are not relevant to this study and will not be described here.

After the water-based gel is applied to coat the transducer, the transtemporal windows are insonated to identify the site at which the strongest Doppler signal can be detected. By using a sample volume depth between 40 to 60 mm and an axial scan plane perpendicular to the skull, the examiner can usually detect strong flow toward the transducer, which indicates the position of the middle cerebral artery. This forward flow is shown on the TCD display screen as positive signal. Flow away from the transducer is indicated by negative signal [see Figure 4 on Page 38].

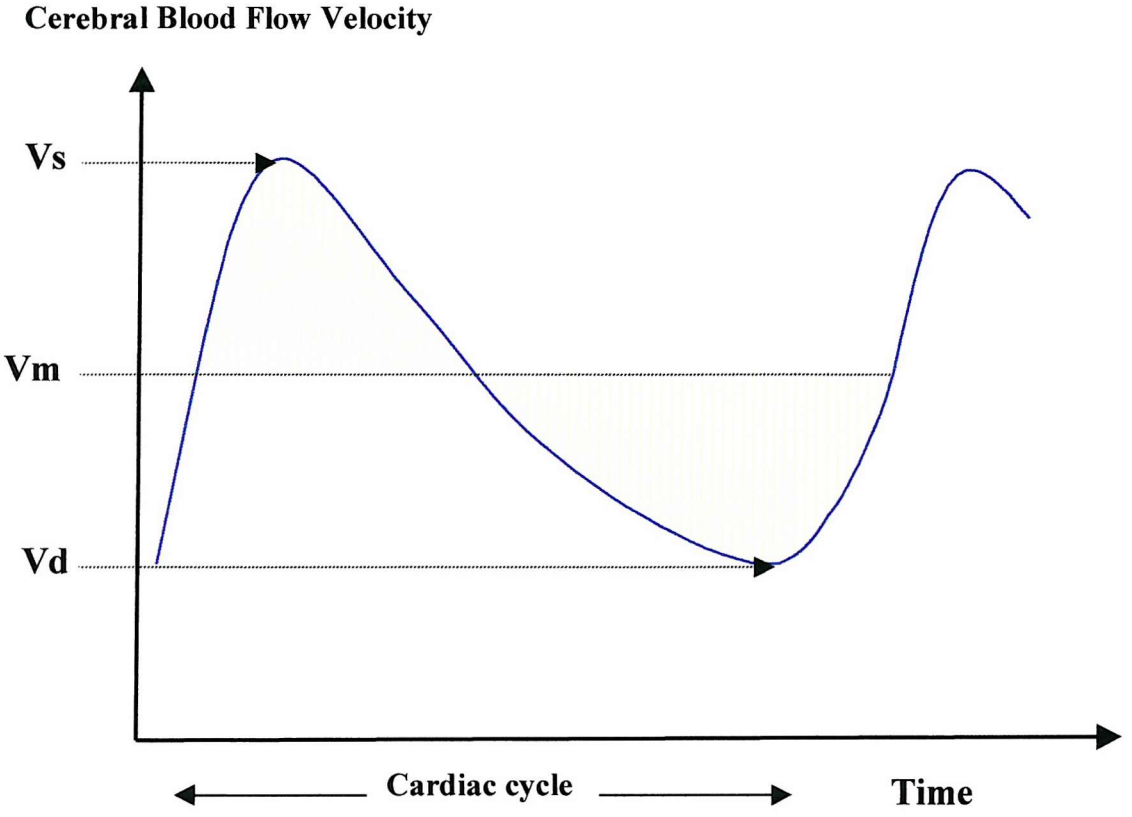
When reading the spectral display on the TCD screen, several indices appear as standard features. Peak systolic velocity (Vs) refers to the highest velocity during systole in the cardiac cycle. End-diastolic velocity (Vd) is the maximum velocity at the end of the diastole. Mean velocity (Vm) refers to the time-averaged maximum velocity over the entire cardiac cycle; this is also known as time averaged mean (TAM). Figure 5 (on Page 62) illustrates the derivation of these values. The peak velocities are readily derived from the velocity waveform and Vm is usually calculated automatically by the TCD system. Another TCD interpretation parameter used is the Gosling pulsatility index (PI). Gosling first described this index in an attempt to quantify Doppler waveforms in the evaluation of lower extremity arterial disease. The PI is calculated by the following formula [116]:

$$\mathbf{PI} = \frac{\mathbf{Vs - Vd}}{\mathbf{Vm}}$$

The PI is an indication of the resistance in the distal vasculature. This index is greatly influenced by many factors, including cardiac contractility, but in general will be higher with increased vascular resistance in the distal vessels, and lower with decreased distal vascular resistance.

There are other important physiological derivations of the monitored signal. Under conditions of laminar flow, which normally occurs in the MCA in the absence of turbulence or excessive arterial branching, the flow profile in the artery is parabolic. The maximum flow velocity (Vmax) occurs at the centre of the artery and is reflected as the maximum outline of the spectral tracing. The minimal flow velocity

Figure 5. This illustrates the method of determining the peak systolic (Vs), end-diastolic (Vd), and time-averaged mean (Vmean) cerebral blood flow velocity from the spectral outline TCD signal. This diagram has been adapted from Newell *et al.* (1992).



(Vmin) occurs near the wall of the artery. The instantaneous mean velocity (Vmean) reflects the average of all of the velocities taken from a particular cross-sectional sample of an artery. Vmean is therefore different from the aforementioned time averaged mean velocity (Vm), which is the maximum flow velocity (Vmax) averaged over the whole cardiac cycle. Experiments have shown that the Vmax, or the spectral outline, can be taken as an analog signal and used to reflect relative changes in velocity and blood flow during rapid blood pressure drops as well as during longer spontaneous oscillations of blood pressure and carbon dioxide [127, 159]. Vmax has been used as an index of relative changes in blood flow through the middle cerebral artery when monitoring, for example, during endarterectomy, cerebral vasomotor reactivity (e.g. with 5% carbon dioxide) testing, and cerebral autoregulation assessment that examined spontaneous variation of cerebral blood flow. Our previous work also showed that, in vasomotor reactivity studies that used acetazolamide, Vmax was more reliable and less prone to artefact than Vmean. However, the administration of acetazolamide was associated with changes in velocity profile within the blood vessel and affected the accuracy of results [160].

Laser-Doppler flow (LDF) measurement is another technique that enables continuous monitoring of cerebral blood flow. LDF uses the coherent properties of laser light to measure mean velocity of all the erythrocytes within an illuminated tissue volume of about 1 mm^3 [161]. It permits reliable, non-invasive recordings of the actual time course of cerebral blood flow. Florence *et al.* (1992) used LDF to examine the rapidity of cerebral autoregulation in rabbits [162], whilst Rosenblum *et al.* (1987) used this technique for intraoperative measurement of blood flow in patients with cerebral arterio-venous malformation [161]. The authors also remarked that the LDF flow value was an absolute physical measurement of microcirculatory flow, which could be calibrated in ml/g/min. Hudetz *et al.* (1992) found it to be a useful tool for the assessment of spontaneous variation of cerebral blood flow [163]. This technique was not available for the purpose of the present study. Many other investigators, such as Diehl *et al.* (1997) and Panerai *et al.* (1998), have successfully used normal TCD ultrasonography to assess cerebral autoregulation by examining spontaneous variations of blood pressure and blood flow velocity [134, 164].

For this study, a SciMed QVL 120 Doppler system (SciMed Ltd., Bristol, UK) was available. This digital Doppler ultrasound system has probe frequencies of 4 and 8 MHz, as well as a sensitive pulsed wave 2 MHz mode for transcranial applications.

Dual channel (e.g. using both probes simultaneously for left and right middle cerebral arteries) and dual depth (e.g. examining a middle cerebral artery at two different depths) are standard features [165]. In the pulsed wave mode at 2 MHz the same single crystal is used both as a transmitter and a receiver of ultrasound. The difference between transmitted and received frequency (the 'Doppler shift') is automatically calculated. The differential frequency also falls in the audio range and the signal is amplified and emitted through one of a pair of loud speakers (or headphones) depending on the direction of flow [see Figure 6 on Page 65]. Flow toward the transducer produced signals audible in the right headphone, and vice versa. The Doppler shift frequency is proportional to the velocity of the blood flow within the middle cerebral artery. The SciMed QVL 120 Doppler system uses a Fast Fourier Transform (FFT) algorithm to calculate all the velocities present in the returned signal from the moving blood (also known as 'spectral analysis'). It calculates the Doppler shift relative to the transmission frequency for every frequency received [see Figure 7 on Page 66]. The TCD then displays the true maximum (Vmax) and mean (Vmean) frequency signals as a full 16 level colour/grey scale spectrum ('sonogram') [165]. As compared to other conventional Doppler ultrasound systems that use a 'zero crossing technique' to measure blood velocity, the SciMed QVL 120 Doppler system is able to give a much more accurate picture of the spread of velocities present in the flow.

For the present study, the Vmax and Vmean of both middle cerebral arteries were continuously recorded and stored on a portable computer for off-line data analysis [see Section 2.2.4].

2.2.2 Finapres Device

Continuous monitoring of blood pressure can be achieved by two main methods: intra-arterial blood pressure monitoring and servo-plethysomo-manometry [166]. Although intra-arterial (most frequently intra-brachial) blood pressure monitoring is the 'gold standard' because it directly measures changes in arterial pressures within a large or medium sized artery, the procedure carries inherent risks that include trauma, haemorrhage, production of emboli, local and systemic infection, and pain [13].

Servo-plethysomo-manometry permits non-invasive and continuous beat-to-beat recording of peripheral arterial blood pressure pulse using a finger cuff [167]. The method is based on the principles of 'vascular unloading' and 'arterial volume clamp', which were first reported by Penaz in 1973 [168]. Others, such as Yamakoshi

Figure 6. The TCD probe is both a transmitter and a receiver of ultrasound. This diagram illustrates the way the Doppler shift (difference between the transmitted and received frequency) is amplified and emitted through a loudspeaker.

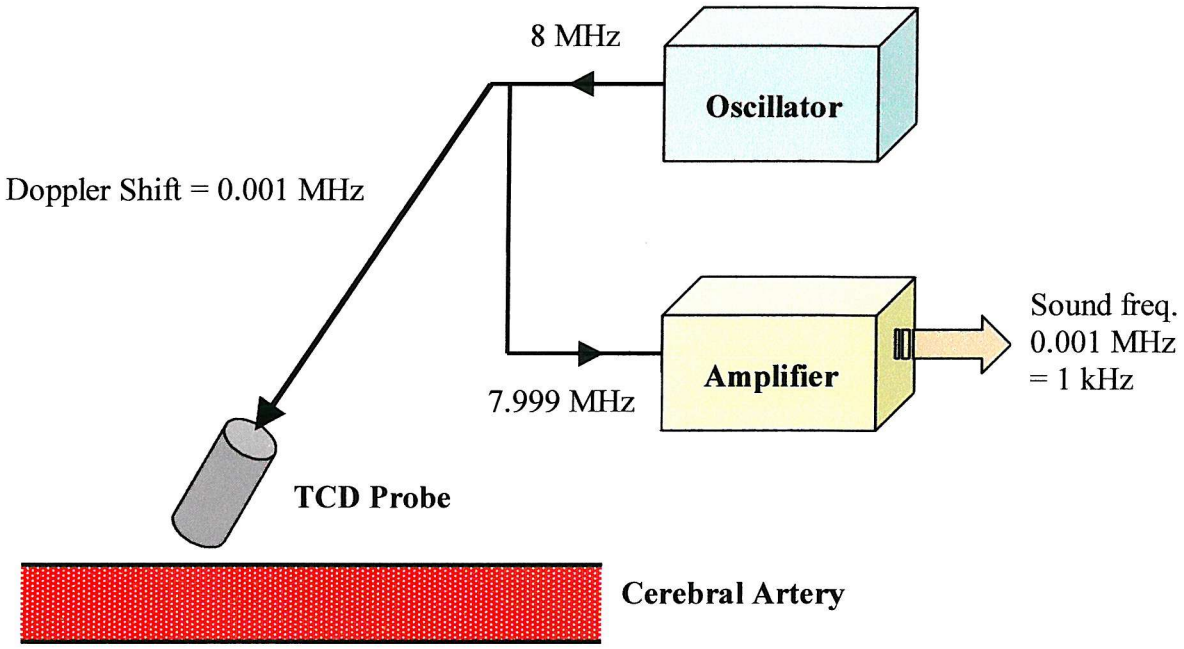
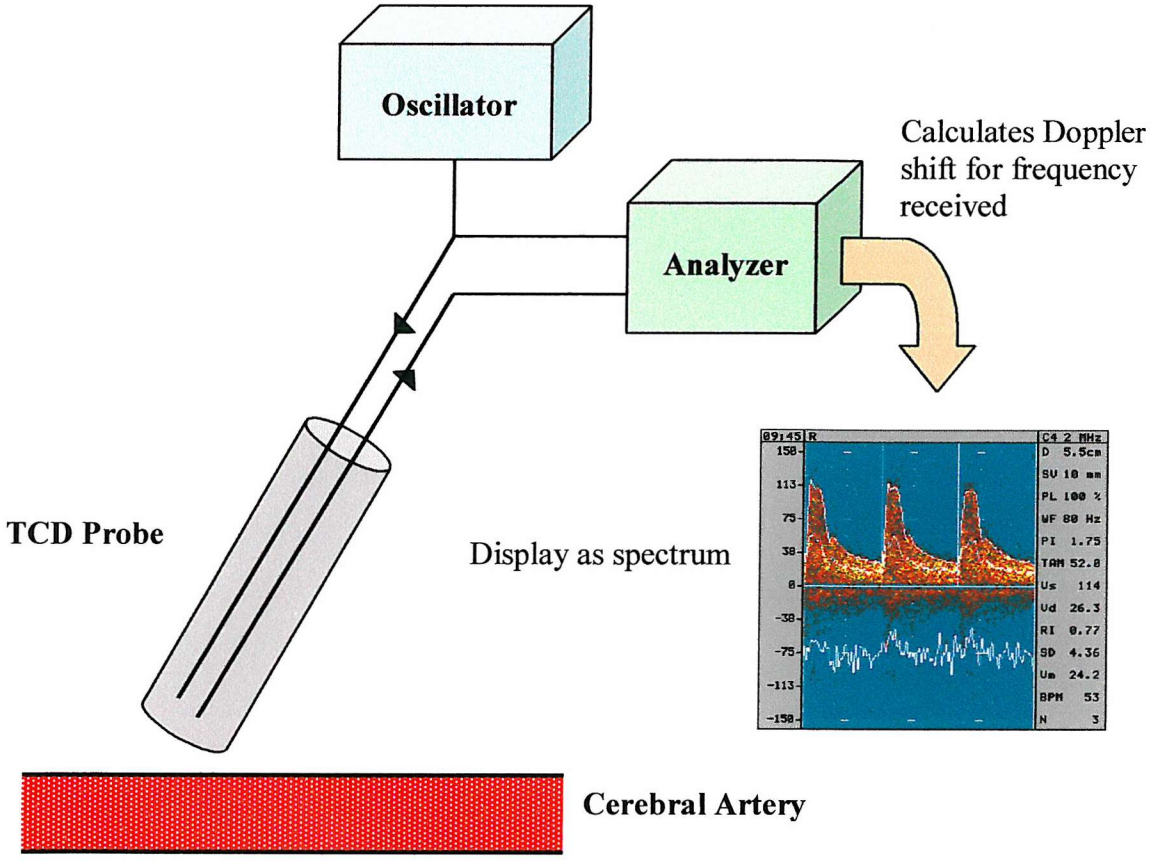


Figure 7. The TCD uses fast Fourier transformation to calculate all the velocities present in the returned signal from moving blood. It then calculates the Doppler shift relative to the transmission frequency for every frequency received and displays this as a colour spectrum on the monitor.



et al. (1980), have also used the Penaz method to develop a different blood pressure measurement device [168]. The development of a fully automated instrument was a long awaited step forward in the technique of blood pressure measurement. At present, the fully automated instrument, the Finapres device (Ohmeda Monitoring Systems, Englewood, CT, USA), exists in different forms, but the most commonly used commercial model is the Ohmeda 2300 Finapres device.

Finapres measurement of blood pressure has been shown to provide robust blood pressure information under most circumstances [167, 169] and is a reliable alternative to intra-arterial monitoring [168, 170, 171]. Imholz *et al.* (1988) demonstrated that the Finapres device reproduced intra-arterial blood pressure patterns faithfully [168]. Jellama *et al.* (1996) showed that Finapres device could be reliably used in clinical settings as a monitor of sudden changes in blood pressure, such as that induced by a 30 minutes head-up tilting [171]. Dawson *et al.* (1998) found a good agreement between the pulse interval derived from the Finapres device and electrocardiography, and concluded that the Finapres device could be used in the assessment of baroreceptor sensitivity or cerebral autoregulation [172]. Although the Finapres device does not enable valid estimates of absolute systolic, diastolic and mean arterial blood pressures, relative changes of blood pressure, as required for autoregulation testing, can be measured with satisfactory precision [173].

However, as expected physiologically, individual Finapres measurements are often different from intra-arterial measurements of blood pressure. The Finapres systolic blood pressure was shown by McAuley *et al.* (1997) to be consistently and significantly higher than the intra-brachial value; the difference between the systolic pressures measured by the two methods was also shown to increase significantly during the cold pressor response [167]. This phenomenon can be explained by the fact that the blood pressure waveform is modified on distal propagation by, for example, dispersion, reflection, and the state of arterial compliance. The resultant effects are amplification and narrowing of the wave, with an increased systolic, reduced diastolic and essentially unaltered mean blood pressure [167]. Omboni *et al.* (1993) demonstrated that standard deviations of diastolic blood pressure, mean arterial pressure, and pulse interval were similar when assessed by Finapres and intra-arterial monitoring, whereas standard deviation of systolic blood pressure was significantly overestimated by Finapres recordings [174]. In patients with peripheral vascular disease, Bos *et al.* (1992) found that Finapres measurements were not always equal to

intra-brachial measurements and that, during the Valsalva manoeuvre, the magnitude of the intra-arterial blood pressure response was sometimes over- or underestimated by Finapres device [170]. Furthermore, it has been suggested that assessment of autoregulation based on methods which induce large changes in blood pressure might also lead to changes in cardiac output which might then affect the pressure drop between the site of measurement and the large cerebral vessels [13].

The accuracy of Finapres blood pressure measurement may be influenced by various factors. For example, Jones *et al.* (1993) showed that systolic readings differed from intra-arterial values by more than the recommended standard when the Finapres finger cuff was applied too tightly or loosely; tight and loose applications often under- and over-read blood pressure values respectively [175]. The reliability and accuracy of the Finapres device is therefore influenced by even a small degree of finger cuff malapplication. Tanaka *et al.* (1993) examined the effect of finger temperature on Finapres measurements. It was found that Finapres systolic pressure was significantly affected by fingertip temperature, probably as a result of local vasoconstriction of arterio-venous shunts. The authors also suggested that finger warming may be a useful procedure to improve the reliability of Finapres readings [176]. Nevertheless, Hildebrandt *et al.* (1991) found that heating of the fingertip distal to the Finapres cuff produced a significant decrease in peripheral arterial pressure compared to the control recording made simultaneously from the other hand. The authors concluded that heat-induced vasodilatation may make Finapres recordings unrepresentative of systemic blood pressure [177]. Moreover, the Finapres cuff itself could induce vasoconstriction due to local venous occlusion, even when the appropriate cuff size is used.

Despite these findings, the Finapres device remains the method of choice for continuous monitoring of blood pressure, especially when the parameter of interest is relative changes rather than absolute values of blood pressure. Therefore, we chose to use a Finapres device and the available model was an Ohmeda 2300 Finapres device [see Photo 1].

2.2.3 Rhythmic Handgrip

Information about competence of cerebral autoregulation can be obtained by studying the temporal relationship and correlation between variations of blood pressure and cerebral blood flow velocity. As already mentioned in Section 1.5.5, three main forms

of blood pressure changes are: stepwise changes, slow induced oscillations, and spontaneous variation. All three forms can be used to study cerebral autoregulation but in this present study, spontaneous variation and slow oscillations of blood pressure were investigated.

Although blood pressure oscillations can be induced by many different physiological stimuli, such as periodic inflation and deflation of thigh cuffs, deep breathing, and repetitive squatting and standing. To our knowledge, there has yet been no study that has investigated rhythmic handgrip as a stimulus. Rhythmic handgrip is a simple, safe, and quick procedure, which can be performed at the bedside or on an outpatient basis, without the need for the subject to be particularly fit. It also has the advantage of being non-invasive and painless. The only requirement is to follow the commands of the operator to periodically handgrip with a specific force and hence a fully intact hearing and comprehension is preferable. Even patients with expressive dysphasia and hemiplegia can effectively perform rhythmic handgrip if they are able to follow verbal commands or prompting by gesture.

Handgrip is one of the many well-known measures that can effectively activate the sympathetic nervous system [178]. Other measures include the cold pressor response and cycling [178, 179]. It is for this reason that handgrip has been commonly used as a test of autonomic function, together with other tests such as the assessment of blood pressure changes during deep-breathing, lying-to-standing (or head-up tilting), and the Valsalva manoeuvre [180]. Vargas *et al.* (1980) also showed that non-invasive measurement of beat-to-beat variation of heart rate can also be used effectively to assess the autonomic function in the elderly population [181].

Static muscular exercise, such as sustained handgrip, produces a significant rise in arterial blood pressure, heart rate, cardiac output, and oxygen uptake [182, 183]. The response, which is reflex in nature, is thought to be initiated by stimuli from the exercising muscle. The blood pressure rise is mediated partly by a heart rate-dependent increase in cardiac output, and partly by peripheral vasoconstriction mediated via the α-adrenergic receptors of the peripheral autonomic nervous system [183]. There is also evidence that intracellular pH and diprotonated phosphate were linked to the reflex elevation of blood pressure during handgrip [184]. Any damage to the neurological pathways involved could lead to a diminished or absent cardiovascular response to sustained handgrip [182]. Furthermore, the magnitude of

cardiovascular response is determined by the percentage of the maximum voluntary contraction of a particular muscle group [182]. In general, handgrip of 20% to 30% of the maximum voluntary contraction can generate effective cardiovascular (i.e. blood pressure) response [185, 182, 179]. The reason why a larger muscle tension produces a greater response is not known. It has been suggested that the number and frequency of the impulse travelling in the reflex pathway may be increased either because a greater muscular bed pressure releases a stronger chemical stimulus to the muscle afferents, or because a muscle exerting greater tension has greater bulk and hence more muscle afferents are stimulated [182].

Khurana *et al.* (1996) found that, in normal individuals, isometric handgrip (at 30% maximum contraction for 5 minutes) produced a rise in diastolic blood pressure of 25.2 ± 1.3 mmHg (mean ± standard error). The authors concluded that isometric handgrip was a "specific, sensitive, reproducible, simple and non-invasive test of sympathetic function with relatively well-studied pathways" [186]. Jandik *et al.* (1985) showed that 3 minutes of isometric handgrip significantly increased heart rate (16.8 ± 10.7 beats/min) as well as systolic and diastolic blood pressure (25.5 ± 12 mmHg and 19.5 ± 12.8 mmHg respectively) [187]. Several investigators, such as Comi *et al.* (1986), have corroborated the reproducibility of sustained handgrip as a test of autonomic function [188, 189]. Interestingly, females have been shown to have a greater cardiovascular response to sustained handgrip [189].

Rhythmic handgrip is less widely used than isometric handgrip as a technique in the assessment of the cardiovascular system. Similar to isometric handgrip, rhythmic handgrip has also been shown to significantly increase heart rate and blood pressure in a periodic fashion [190, 179]. In the study by Pott *et al.* (1996), average heart rate increased from 74 ± 20 to 92 ± 21 beats/min and mean arterial blood pressure increased from 87 ± 7 to 105 ± 9 mmHg (mean ± standard deviation) during rhythmic handgrip at 1 Hz (i.e. 1 per second) [190]. Furthermore, during handgrip and other dynamic exercises, blood flow velocity (Vmean) in the middle cerebral artery increases in proportion to work rate [191]. It has been suggested that stimulation of the sympathetic nervous system and a resultant vasoconstriction of the middle cerebral artery might be the cause of the apparent increase in velocity (constriction of 0.2 to 0.4 mm could elevate Vmean by more than 20% at a constant flow). Pott *et al.* (1996), however, disproved this theory and found no evidence for a vasoconstrictive

influence of sympathetic nervous activity on the cerebral arteries during rhythmic handgrip [190]. Handgrip has also been shown to cause increased activation in the contralateral sensory area, the supplemental motor area, and the ipsilateral cerebellum [192]. This produces unequal increases in blood flow velocity on both sides. Ide *et al.* (1998) demonstrated that rhythmic handgrip increased heart rate, blood pressure, cardiac output and contralateral middle cerebral artery Vmean (13%), while the increment was smaller on the ipsilateral side (6%) [179]. This discrepancy has been confirmed by other investigators [190, 193], and indicates that Vmean is influenced by afferent input from the moving muscle [194]. In addition to the effects on the cerebrovascular and cardiovascular systems, rhythmic handgrip also increases minute ventilation and oxygen consumption in proportion to the intensity of rhythmic workload [195].

The exact physiology of rhythmic handgrip is yet to be fully elucidated. Rhythmic handgrip is not just a simple stimulus for periodic blood pressure changes, it also has interesting consequences on the cerebral hemispheres and circulation. For this reason, we have chosen to examine the effects of rhythmic handgrip on cerebral blood flow velocity and autoregulation. In this study, a sphygmomanometer was used to measure the maximum contraction for each subject and the percentage of maximum contraction of each handgrip (a handgrip dynamometer was not available). The speed of repetition of handgrip was determined as every 20 seconds (i.e. 20 seconds handgrip, followed by 20 seconds relaxed, and so on). This speed of repetition was chosen because the results from validation experiments showed that, in normal conditions, it could effectively generate oscillations of blood pressure [see Section 2.5.2]. In previous studies, subjects usually performed handgrip at 20 to 30% of maximum contraction; this regimen was therefore chosen for this study.

Stoll *et al.* (1998) compared bilateral handgrip to other tests of cerebral vasomotor reactivity in normal subjects and in patients with carotid artery disease. It was found that bilateral handgrip increased Vmean in the middle cerebral artery and served as a useful technique [196]. In the present study, rhythmic handgrip was compared to spontaneous variation as a potentially new non-invasive technique for the assessment of cerebral autoregulation after ischaemic stroke.

2.2.4 Other Equipment

For the purpose of averaging as part of the data analysis, it was necessary to ascertain the beginning (or a fixed point) of each cardiac cycle by performing continuous electrocardiography (ECG). A Datascope Multicare 3000 monitor (Datascope Corporation, NJ, USA) was available for this study [see Photo 2, Appendix 1, Chapter 7]. This was a flexible, user-configurable monitor with continuous ECG display. Three detachable ECG leads connected the monitor to the patient's chest and limbs. The signals from the Datascope Multicare 3000 monitor were transmitted to an isolated R-wave detector (serial number 89/002, frequency = 50 Hz), which was developed by the Medical Physics Department at Poole Hospital, Dorset [see Photo 3, Appendix 1, Chapter 7]. This enabled the detection of each R-wave of the ECG trace from the ECG monitor and a brief signal was automatically generated. Signals from the R-wave detector, as well as those from the TCD system and Finapres device, were continuously recorded and stored on a portable computer. The computer was a Hi-Grade Notino AS6000 laptop with a 233 MHz MMX Processor and 16 MB RAM. The protocol and validation studies for these instruments will be described in detail in Sections 2.4 and 2.5.

2.2.5 Omitted Physiological Parameters

In this study, the blood pressure, Vmean of both middle cerebral arteries, and the detection of each pulse were continuously and simultaneously recorded. There were, however, several physiological parameters that could have been examined during the course of the study but were omitted.

Firstly, Pco_2 was not monitored in this study. The effects of carbon dioxide on the cerebral circulation have been discussed in Section 1.3.2. Pco_2 is an important determinant factor of cerebral blood flow (and flow velocity), autoregulation and vasomotor reactivity in normal humans. High Pco_2 in the brain increases cerebral blood flow in areas with normal vasomotor reactivity [43]. End-tidal Pco_2 is most commonly monitored using an infrared gas analyser such as an in-line capnometer (Hewlett Packard, 47210A) [159, 145]. Giller *et al.* (1993) found that during changes of end-tidal Pco_2 (14 ± 6 mmHg), the mean diameter of large cerebral arteries changed by less than 4%, whereas the smaller arteries showed mean diameter changes as large as 29% [111]. During recordings of spontaneous variation of blood pressure,

however, Diehl *et al.* (1991) showed that carbon dioxide concentration was nearly constant during the recording period (variation $\leq \pm 5\%$) and did not correlate with the spontaneous blood flow velocity variations [129]. Other investigators have omitted the measurement of Pco_2 in their studies of cerebral autoregulation that used spontaneous variations of blood pressure and flow velocity [156, 130, 134, 164]. Although it may theoretically be useful to monitor end-tidal Pco_2 during the assessment of cerebral autoregulation, the exact relationship between Pco_2 and spontaneous variations of blood flow velocity is still unclear. For the purpose of this study, the appropriate end-tidal Pco_2 monitoring equipment was not available. The possible implications for the results will be explored in the 'Discussion' section.

Secondly, the measurement of Po_2 was not carried out. There is evidence that the dominant mechanism of cerebral autoregulation is a flow-dependent metabolic mechanism which depends on changes in tissue oxygen tension (Po_2), which in turn may cause secondary production of vasoactive substances such as adenosine [138]. Moreover, stroke patients are more likely to have abnormal arterial Po_2 and Pco_2, probably as a result of a combination of factors such as impairment of the medullary respiratory centre, reduced chest wall and diaphragmatic movement, and co-existing cardiopulmonary disease(s) [5]. Clarke *et al.* (1958) were the first to describe cyclical fluctuations of cat brain tissue Po_2 [122]. More recently, random and asynchronous brain tissue Po_2 waves were observed with a frequency of 6 to 12 cycles/min, but no correlation with respiration, heart rate, or blood pressure was found. Since then, these 'oxygen availability waves' have also been observed in humans. [122]. In animal studies, Steinmeier *et al.* (1996) found no correlation between blood pressure, intracranial pressure, blood flow velocity, and oxygen saturation in the jugular bulb (Sjo_2), which indicated the level of cerebral oxygenation [122]. Theoretically, although it may seem useful to perform continuous monitoring of Po_2 during the present study (e.g. by pulse oximetry measurements of arterial oxygen saturation from the finger), to our knowledge, few other studies that primarily examined cerebral autoregulation have simultaneously monitored Po_2.

Thirdly, although blood pressure was continuously monitored by the Finapres device, absolute blood pressure was not monitored during the study. As already discussed in Section 2.2.2, the Finapres is very reliable in assessing relative changes in arterial blood pressure, but not absolute blood pressure values. Some investigators,

such as Panerai *et al.* (1998), measured baseline blood pressure at rest using automated arm cuff [156]. This was performed mainly in studies that used sudden stepwise blood pressure changes (e.g. by the 'thigh cuff' technique) and the accuracy of the baseline blood pressure was therefore vital. Other investigators who used spontaneous variations of blood flow velocity and blood pressure to study cerebral autoregulation, such as Diehl *et al.* (1991, 1998), did not monitor absolute baseline blood pressure during the studies [129, 134]. Automated arm cuff was available for the present study, but from our experience, measurement of blood pressure using automated arm cuff induced discomfort or even pain while the cuff was inflated. Periodic arm cuff measurements may, therefore, affect cerebral blood flow velocity because of sympathetic nervous activation from the discomfort. Hence measurement of absolute blood pressure was not performed during each examination. However, baseline blood pressure was measured at each visit for subjects and controls; changes in baseline blood pressure over time could potentially account for any change in cerebral autoregulation.

Lastly, autonomic function was not assessed or used as a selection criterion. As already mentioned in Section 1.5.4, the 'neurogenic' mechanism of cerebral autoregulation states that the autoregulatory adjustments of resistance vessels are mediated by autonomic vascular innervation. The exact role of the cerebral nervi vasorum is, however, still unclear. It is possible that autonomic dysfunction, from whatever cause (e.g. multiple system atrophy), may be associated with impairment of cerebral autoregulation [197]. Previous reports on this subject have produced conflicting results and some investigators found well preserved cerebral autoregulation in patients with autonomic dysfunction [198]. Two main factors may have contributed to these differences. Firstly, differences in methodology may have been responsible. Secondly, the patients with autonomic dysfunction are a heterogeneous group, differing in their pathology and degree of degeneration. Symptoms suggesting low cerebral perfusion as a result of orthostatic hypotension are common in patients with autonomic dysfunction. Nevertheless, such patients have been observed to be able to tolerate, sometimes for years, blood pressure levels which would give normal subjects cerebral ischaemic symptoms [198]. It has not been clear whether cerebral blood flow fails to fall as low as it does in normal subjects with a comparably lowered blood pressure or whether the brain adapts in some other way to poor perfusion pressure and reduced cerebral blood flow. Thomas *et al.* (1980)

showed that cerebral autoregulation was preserved in patients with chronic autonomic dysfunction and the lower limit of autoregulation was reduced below that in normal subjects [198]. These findings help to explain the 'tolerance' of severe postural hypotension in some patients with autonomic dysfunction. Matta *et al.* (1996) also found that, in patients with sepsis syndrome and widespread secondary peripheral vasoparalysis, cerebral autoregulation and vasomotor reactivity remained intact [199].

Stroke itself is also associated with autonomic consequences. Barron *et al.* (1994) found that ischaemic stroke, especially in the right hemisphere, produced unbalanced cardiac autonomic activity favouring the sympathetic system (i.e. reduced parasympathetic activity), causing cardiac arrhythmias in some cases [200]. Giubilei *et al.* (1998) confirmed the finding of sympathetic predominance after ischaemic stroke and also found that "the flexible and dynamic properties of the autonomic nervous system were preserved" [201]. In the assessment of cerebral autoregulation, some investigators such as Kuo *et al.* (1997) excluded patients with possible autonomic dysfunction (e.g. those with diabetes mellitus), whereas others such as Diehl *et al.* (1998) did not [130, 134]. Although the association between autonomic dysfunction and cerebral autoregulation impairment cannot be ruled out, there is, however, no robust evidence to support a routine exclusion of patients with potential autonomic impairment from cerebral autoregulation tests. In this study, therefore, individual autonomic function was not tested.

2.3 Subjects & Controls

2.3.1 Selection Criteria

This was a prospective, controlled, and longitudinal study of cerebral autoregulation in patients with ischaemic stroke in the middle cerebral artery territory, during the acute, subacute, and chronic phases of recovery, using spontaneous and induced oscillations of cerebral blood flow velocity as measured by TCD ultrasonography.

Acute stroke patients ("subjects") were recruited from the Acute Stroke Unit, Royal Bournemouth Hospital. For convenience, controls were chosen from hospital patients admitted with and recovering from other minor systematic illness, but without a history of cerebrovascular disease [see Section 4.1.2]; most of them were discharged home the same day as the recording. CT brain scans had not been performed to exclude asymptomatic cerebral ischaemia in the controls. Retrospectively, it would have been more appropriate to recruit normal healthy volunteers as controls, but this was not possible for practical reasons.

Prior to recruitment into the study, the subjects must have fulfilled the following ten inclusion criteria:

1. The patient had clinical symptoms and signs of an acute stroke that has occurred within 7 days (with or without a previous history of stroke).
2. The acute stroke was confirmed to be ischaemic or non-haemorrhagic by CT or MRI brain scan (a formal radiologist report was needed).
3. The stroke affected all or part of the middle cerebral artery (MCA) territory, according to clinical signs and confirmed by CT or MRI brain scan.
4. The patient was able to lie still for at least 30 minutes without discomfort.
5. The patient was able to perform rhythmic handgrip for 5 minutes.
6. The patient had temporal windows through which TCD ultrasonography of both MCAs could be carried out consistently and without difficulty.
7. The patient lived in the local area to enable follow-up studies without excessive travelling.
8. The patient was able to communicate normally and understand verbal commands.
9. Signed informed consent was sought prior to any study procedure.
10. If the patient has given the consent but was unable to sign the consent form, for example due to paralysis of the dominant hand, the patient's relative could sign an 'assent of consent'.

The subjects must also *not* satisfy any of the following six exclusion criteria:

1. The patient suffered a very severe stroke or CT brain scan showed significant cerebral oedema. There would be a poor chance of long-term survival to allow for follow-up studies.
2. The patient was currently in, or had a history of, cardiac arrhythmia such as paroxysmal or sustained atrial fibrillation.
3. The patient had significant number of missed heartbeats, ectopic beats, or pauses (this was arbitrarily set as more than one every ten heartbeats).
4. The patient had respiratory difficulties, unusual breathing patterns, or other symptoms (such as frequent coughing, hiccuping, or tremor) that would make continuous TCD, ECG, and Finapres recording difficult.
5. The patient had significant peripheral vascular disease, or arthritis affecting the fingers, that would prevent accurate recording of continuous blood pressure using the Finapres device.
6. The patient had other serious concurrent medical condition(s), which would preclude him/her from follow-up studies.

Stroke affecting the MCA territory was chosen because it was most likely to be associated with perturbations of cerebral blood flow velocity and spontaneous variation of flow within the MCA. Haemorrhagic stroke was excluded because it often caused significant mass effect (by cerebral oedema) and arterial vasospasm including that of the MCA; this might in turn affect the cerebral haemodynamics in an unpredictable manner. In most cases, there was a better chance of obtaining good quality continuous bilateral TCD signals in those who had normal sinus rhythm, steady respiratory efforts and rhythm, MCAs that were easily insonated, and were able to remain stationary for the duration of the study. A steady respiratory rhythm would also prevent any large or irregular fluctuations of arterial Po_2 or Pco_2 (not monitored). We also checked that the recruited subjects and controls had a maximum chance of reliable continuous Finapres measurements, effective rhythmic handgrip, and returning for follow-up examinations.

The controls in this study also satisfied all of the selection criteria above except clauses 1 to 3, which are stroke-related. Individual written informed consent

was also obtained from every control before taking part in the study. It was our aim to recruit a similar number of controls who were age- and sex-matched.

2.3.2 Calculation of Sample Size

In general, calculation of the necessary sample size for a study first requires quantifying the objectives. It is also necessary to state the expected size of the changes that it is desired for the study to demonstrate [135]. With this information and allowing for circumstances such as loss to follow-up, it is then possible to estimate the probability of achieving statistical significance at, for example, 5% level. This probability is otherwise known as the 'power' of the study. For this study, quantification of the objectives was difficult for three main reasons.

Firstly, very few investigators have examined the use of spontaneous variation of cerebral blood flow velocity in the assessment of cerebral autoregulation. All the previous studies have been conducted amongst normal human adults and neonates, rats, and patients with carotid artery disease [130, 163, 164, 129, 156, 134]. Only two of these used normal controls. To our knowledge, there is no other study of this type that has ever examined patients after ischaemic stroke. Furthermore, no study has ever employed rhythmic handgrip in the assessment of cerebral autoregulation. For these reasons, there is very little evidence and original data for the purpose of power calculation in this study.

Secondly, there is a significant variation in the number of subjects and controls that were recruited into the studies mentioned above. Of those that studied patients with carotid artery stenosis, the number of patients varied from 8 [129] to 20 [156]. By contrast, Panerai *et al.* (1997) studied 83 neonates and Diehl *et al.* (1998) studied 50 normal volunteers [164, 134]. Other investigators who studied dynamic autoregulation in patients with carotid artery stenosis, for example Newell *et al.* (1994) and Diehl *et al.* (1995), included 7 and 20 subjects respectively; both studies had not included normal controls [146, 144]. Aaslid *et al.* (1996), who used periodic inflation and deflation of thigh cuffs to generate blood pressure oscillations to evaluate cerebral autoregulation, recruited 11 patients with head injury and 12 normal controls [154]. Due to the significant variation in methodology and subject selection criteria, previous studies have not provided a widely accepted minimum or standard number of individuals needed for the assessment of cerebral autoregulation; most

investigators have included between 7 and 20 subjects and a similar number of controls.

Thirdly, the present study was designed in part to evaluate the feasibility of using a new technique, i.e. spontaneous variation of cerebral blood flow velocity and rhythmic handgrip as a test of cerebral autoregulation. Therefore, this study may be regarded as a 'pilot' study and a basis upon which future investigations can be developed. Large numbers of subjects and controls are not usually justified in a pilot study setting.

For these reasons, calculation of the necessary sample size for this study was not straightforward and it was also felt that recruiting a large number of subjects and controls for this study would be difficult. After also taking into consideration the various practical limitations, for example the time available for data recording and analysis, we decided to aim to recruit ten subjects (to complete the full protocol including three separate examinations) and ten controls. This would have produced forty separate examinations, i.e. at least eighty 5-minute recordings with spontaneous variation of CBFV and forty 5-minute recordings with rhythmic handgrip.

2.4 Study Protocol

2.4.1 Screening Procedure for Subjects & Controls

The subjects and controls were selected according to the criteria outlined above. Before obtaining consent, each person was informally assessed for suitability to take part in the study. In particular, the medical notes were examined according to the selection criteria and all previous electrocardiograms (ECGs) were screened for cardiac arrhythmia such as atrial fibrillation. All potential subjects and controls then received full verbal and written explanation of the objectives and protocol of the research project. In addition, it was emphasised that participation was entirely voluntary and although the subjects or controls were encouraged to complete the whole study procedure, there was no obligation to do so and they were free to leave the study at any stage. Most importantly, they were reassured that participating in or abandoning the study would not influence their in-hospital management or total length of hospital stay. After any queries were answered and if consent was given, each was then asked to sign the 'consent' section of the information sheet. However, if the person gave verbal consent but was not able to sign the form, for example if the hemiparesis has affected the dominant hand, the person's relative could sign an 'assent of consent' form. The general practitioner of each person taking part in the study was also sent a letter explaining the purpose of this research project.

After consent was obtained, each subject and control underwent screening with TCD ultrasonography (SciMed QVL 120 Doppler system) to assess the ability to obtain reliable and consistent TCD signals of the blood velocities in both MCAs simultaneously. Acceptable TCD signals were those that were easily obtainable, had clear maximum velocity envelopes and normal waveform, and were steady even with minimal movements of the probe position. Those with signs suggestive of significant MCA stenosis, or with unacceptable TCD signals, were excluded from the study, whereas those with good TCD signals were invited to continue with the main part of the study protocol. A research room was available for this study and, whenever possible, the screening and recording procedures were performed in that room. The room, which contained a fully functional tilt table, was quiet and spacious, and the lighting, temperature, and position of the subject or control could also be adjusted. The protocol that applied to the subjects was slightly different from that of the controls. They will now be described individually.

2.4.2 Protocol for Subjects

Patients who suffered an acute ischaemic stroke in the MCA territory were recruited during the first seven days after stroke onset (the 'acute' phase). Subsequent follow-up examinations were conducted at six weeks (the 'subacute' phase) and at three months (the 'chronic' phase). These time intervals were chosen mainly because of the time available for the present study. However, it is also accepted practice to ensure that hypertensive stroke patients are on a regular antihypertensive treatment three months after stroke. Since there is yet no consensus of the optimal timing of starting antihypertensive treatment, six weeks (half way between zero and three months) and three months seemed appropriate for this study. Retrospectively, it would also have been informative and interesting to examine the cerebral autoregulation during the first two or three days of stroke, when the ischaemic penumbra is present and probably most vulnerable to changes in cerebral blood flow. Spontaneous hypertension [see Section 1.4.1] and sympathetic surge may also be potential confounding factors very soon after stroke. It was also difficult to assess the patients within due to practical reasons. Furthermore, we are planning a long-term follow up assessment of these patients at around one year.

Each subject was examined once during each of the three phases. These time intervals were followed as strictly as practically possible. Although the three different phases were arbitrarily defined, most experts accept that cerebral autoregulation may be impaired during the first week after stroke onset and recommend that blood pressure reduction may be 'safe' after around two weeks [5]. During each phase, the subject was investigated using the same protocol. In most of the cases, the subjects were still in hospital for the second examination (the subacute phase), but for the last examination (the chronic phase), many had already been discharged. Where necessary, transport was provided to take the patient to and from the hospital for follow-up studies.

Ten subjects (8 men and 2 women) were recruited for this study, eight of whom completed the examinations for all three phases. One subject was examined twice (during the acute and subacute phases) and one was examined only during the acute phase. A full report of the patient characteristics can be found in Section 4.1.

During each visit, the subject was positioned supine on the tilt table throughout the examination, with the head resting on one pillow. Every effort was made to ensure maximum comfort for the subject; this also prevented unnecessary

body movements or distress. TCD ultrasonography was performed and the MCA on each side was insonated through the temporal window and at the depth of the best signal (usually between 44 and 60 mm). The skin over the position of the window was marked during screening using a washable marker pen for easy identification. For continuous bilateral TCD recording, each probe was secured at the correct position using an elastic strap, which was gently fastened (with Velcro) around the head [see Photo 4 and 5, Appendix 1, Chapter 7]. It was ensured that the patient was as relaxed as possible before the study started to avoid any sympathetic influence on the spontaneous variations of blood pressure and cerebral blood flow velocity.

The subject was then attached to the ECG monitor (Datascope Multicare 3000) via three leads for continuous recording, and to the Finapres device (Ohmeda 2300) via a servo-controlled infrared finger cuff [see Photo 1 and 2, Appendix 1, Chapter 7]. The cuff was fixed to the middle finger (or whichever finger that produced the best Finapres blood pressure trace) of the paretic hand so that the non-paretic hand was reserved for rhythmic handgrip. For this study, only one finger cuff size (medium) was available and the same cuff was used for all the subjects and controls even though finger sizes differed between individuals.

Before applying the finger cuff, the hand was warmed by wrapping it in a towel together with two surgical gloves filled with warm water, one on top of the hand and another clasped in the palm of the hand. This was done to prevent vasoconstriction of arterio-venous shunts in the fingers, which could influence the accuracy of the recording [see Section 2.2.2]. We found that with cold fingers, the Finapres blood pressure values were generally lower, and the waveform was flatter, and there was often a slow downward drift toward lower values over time. After hand warming, however, the Finapres blood pressure values were consistently higher and the waveform was more distinctive and 'physiological' [see Section 2.5.1]. In this study, cold extremities were often found amongst the subjects, especially on the hemiparetic side, possibly as a result of secondary autonomic impairment and peripheral vasoparalysis after stroke.

Signals from the TCD system, Finapres device, and ECG monitor (via the R-wave detector) were downloaded instantaneously to the portable computer [see Photo 6, Appendix 1, Chapter 7]. Signals were transmitted via a 12-bit analog-to-digital converter (ADC-11) that was attached to the parallel port of the computer. A special software programme was written by the engineers of the Medical Physics Department

at Poole Hospital for the purpose of this study. This software enabled continuous recording and storage of data from all three sources. In all, six signals were recorded simultaneously as channels one to six; they were Vmax (left MCA), Vmax (right MCA), instantaneous Vm (of the monitored side), TCD power, pulse (detected R-wave), and instantaneous finger blood pressure, respectively. Data were sampled and recorded every 10 ms (i.e. 100 recordings every second), which has been shown to be well within the limits of capability of the portable computer [see Section 2.5.2]. In addition, the audio signals from one of the two TCD channels (left or right, not both) were continuously monitored and recorded using an ordinary audio cassette tape recorder (Sony TC-FX220) in order that signals could be played back for re-analysis at a later date if necessary.

Once the TCD probes were securely fixed in position, the ECG and Finapres devices connected, the computer ready to record, and the patient relaxed, the examination could commence [see Photo 7, Appendix 1, Chapter 7]. A typical examination comprised three sets of recordings, each lasting for five minutes. The first two recordings were performed with the subject lying still and relaxed and the third recording with rhythmic handgrip. Occasionally, when a recording contained irregularities of TCD signals, blood pressure, or pulse, extra whole 5-minute recordings had to be performed. If a problem arose near the beginning of a recording, for example if the subject sneezed and the TCD probes became displaced, then the recording was stopped and restarted. Whenever possible, the position of the TCD probes, Finapres finger cuff, and ECG leads were left undisturbed during each recording. Furthermore, the servo-adjust mechanism of the Finapres device was switched off during each recording to ensure that blood pressure was monitored without interruption.

For the first two recordings without rhythmic handgrip, the subject was encouraged to relax as much as possible (usually from at least 10 minutes before recording started), breath normally, and keep the eyes closed. The eyes were closed because there is some evidence that visual stimulation increases cerebral blood flow velocity [97]. The room was also darkened for a more relaxing ambience.

For the last recording with rhythmic handgrip, the subject was asked to grip the arm cuff of a sphygmomanometer, which was rolled up (deflated) into a size of a small tube for easy gripping, and inflated to a pressure of about 30 mmHg [see Photo 8, Appendix 1, Chapter 7]. The pressure of maximum contraction for each subject was

measured by gripping the cuff at full strength for 15 to 20 seconds; rhythmic contraction at 20 to 30% of this maximum was maintained during the recording to generate blood pressure oscillations. When the recording started, the subject was verbally instructed to alternately handgrip for 20 seconds and relax for 20 seconds (monitored by a stopwatch), for the full five minutes The strength of the rhythmic handgrip was adjustable during the recording by observing the sphygmomanometer pressure and asking the subject to grip more firmly or gently. During handgrip, regular respiration was encouraged to avoid deep breathing and breath holding.

Between each of the 5-minute recordings, the data recorded on the computer were checked for any obvious problems. In one or two occasions, the ADC-11 attachment became loose from the computer and data were recorded incorrectly and hence unusable; it was necessary to repeat the whole recording. In addition, individual 'spot' (not continuous) TCD recordings were performed for each side between the 5-minute recordings, so that hard copies were available for checking at a later date if necessary. At the end of all three recordings, the data were copied from the portable computer to 1.44 MB 3.5" floppy discs for backup. The size of the file for each recording was approximately 1.1 MB. This is a convenient size since each recording can be copied onto one floppy disc. These were then copied again onto the TCD computer for secondary backup. Each examination lasted approximately 45 minutes.

2.4.3 Protocol for Controls

The protocol for controls was similar to that for the subjects except that only one examination was required per control. In other words, three recordings were made; two of which were without, and one with, rhythmic handgrip. For these recordings, because there was no paretic or non-paretic side, the Finapres finger cuff was placed on the middle finger of the non-dominant (usually the left) hand, so that rhythmic handgrip could be performed using the dominant hand. In total, eleven controls (nine men) were recruited for the study. All eleven controls successfully completed the three recordings of each examination.

Retrospectively, it would have been more appropriate to examine the controls serially at the same intervals as the subjects. This will make the present study a true controlled study and allowed the evaluation of reproducibility of the technique used for assessing cerebral autoregulation. However, due to practical reasons and time constraints, that was not possible. Further studies are presently being planned.

2.5 Equipment & Technique Validation

2.5.1 Finapres Device

In the early part of the study, problems were encountered with inaccurate Finapres blood pressure traces in a few patients whose blood pressures were otherwise shown to remain normal and constant (checked with a sphygmomanometer). These traces were characterised by a typical slow downward drift toward low amplitude and featureless waveforms over approximately 10 minutes. Occasionally, the Finapres blood pressure eventually dropped to almost unrecordable values. This trend existed despite trying the cuff on different fingers or the opposite hand.

It was observed that the most common denominating feature in all these cases was the low temperature of the fingers. This observation was consistent with the findings of Tanaka *et al.* (1993) [176]. Hand warming, therefore, became a routine part of the preparatory procedure for each examination and it was observed that, with routine hand warming, problems with the Finapres trace became rarer.

2.5.2 Rhythmic Handgrip

For this study, the speed of repetition for rhythmic handgrip was determined by an earlier informal experiment, which was undertaken to evaluate various speeds. No comparison could be drawn with previous studies since none has ever used rhythmic handgrip to study cerebral autoregulation. The experiment was performed on ourselves and consisted of carrying out rhythmic handgrip at three different repetition speeds (squeezing and relaxing every 10, 20 and 30 seconds) whilst observing the changes in blood pressure as measured by the Finapres device. We found that rhythmic handgrip with a cycle period of 40 seconds (20 seconds on, 20 seconds off) produced satisfactory blood pressure oscillations. In addition, since the commands were simple to follow, it was generally felt that this speed was manageable even for patients with more severe stroke. Throughout the study, we regularly asked the subjects and controls for their opinions on the protocol and no problems were encountered with the rhythmic handgrip or the speed at which it was performed.

2.5.3 Speed of Data Acquisition

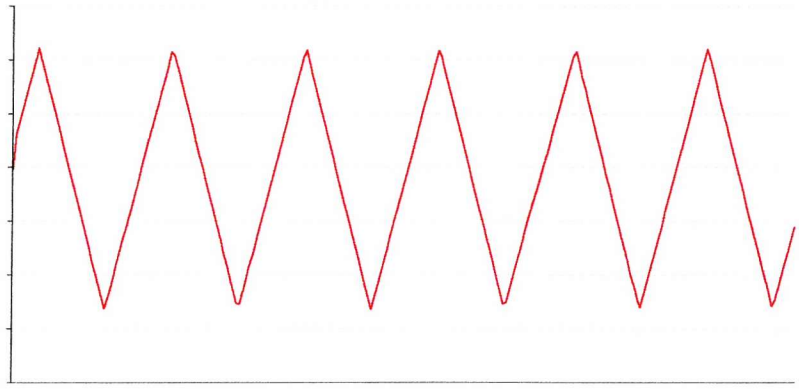
For this study, the faster the data acquisition, the more accurate the results would be. The speed of data acquisition usually depends on the speed of the computer processor. In general, good recordings of TCD and blood pressure signals would require a minimum sampling speed of 30 Hz (i.e. 33 ms^{-1}), whereas ECG recording needs a higher sampling speed of at least 200 Hz (i.e. 5 ms^{-1}). In order to ascertain the optimal sampling speed for this study, we used a signal generator to produce perfect triangular waves. The analog signals of these waves were sampled by the computer at different speeds and the results were compared. Previous studies by other investigators have used sampling speeds of between 5 and 20 ms^{-1} with good results [164, 146]. For this experiment, we chose to test sampling speeds of 5, 10, and 20 ms^{-1} using the portable computer that had a processor speed of 233 MHz. Our results showed that the computer was able to acquire data effectively and uniformly at all the speeds that were tested [see Figure 8A, 8B, and 8C on Page 87].

Although, theoretically, the results would be more accurate with faster data acquisition, it would also generate an enormous amount of data that may cause technical difficulties with data analysis. For example, sampling speed of 5 ms^{-1} would produce 200 data points per channel per second, of which there were six, i.e. 1200 individual data points per second. Some investigators used TCD systems that had the necessary data analysis software already installed so that blood pressure and cerebral blood flow velocity signals were acquired, displayed, stored, and analysed by the same system without the need to connect all the instruments to the computer. For example, Kuo *et al.* (1997) used a Multi-Dop X system (Multi-Dop X/TCD 7, DWL, Sipplingen, Germany) for the evaluation of cerebral autoregulation [130]. These systems could acquire data at high speed without the difficulties of dealing with unmanageable files.

In this study, we chose a sampling speed of 10 ms^{-1} because it was fast enough to for reliable TCD and blood pressure recordings without producing data files that were too large to manage. This speed, however, was not fast enough for continuous ECG recording. This problem was successfully overcome by using an R-wave detector, which only required a minimum sampling speed of approximately 30 ms^{-1}.

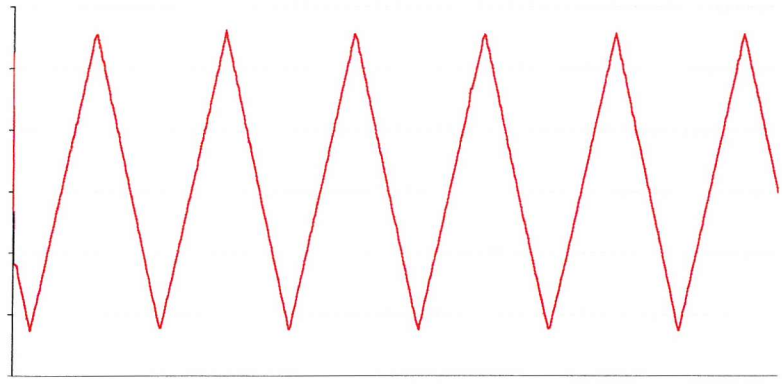
Figure 8. Validation of the speed of data acquisition required sampling triangular waves (from a signal generator) at different speeds and assessing the quality and uniformity of the sampled waves. The three graphs below represent sampling speeds as stated. All acquisition speeds appear satisfactory for the purpose of this study.

Fig 8A. At 20 ms^{-1}



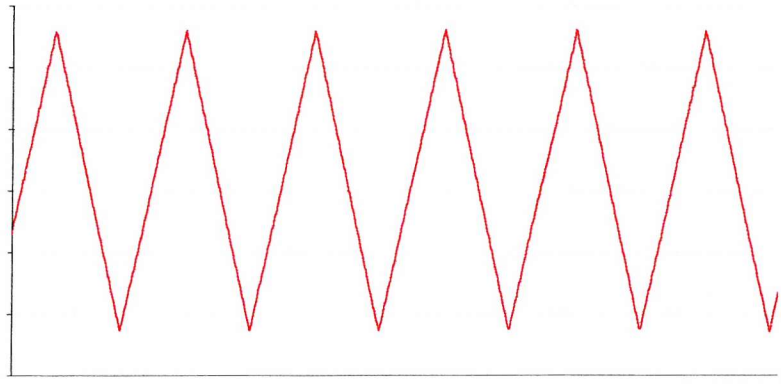
Time

Fig 8B. At 10 ms^{-1}



Time

Fig 8C. At 5 ms^{-1}



Time

2.6 Problems Encountered

Several problems were encountered during the study; they can be broadly classified into three categories: patient-related, equipment-related, and loss to follow up. The most significant problems and their methodological solutions are described in this section. Management of these problems and artefacts was important to reduce their potential influence on the accuracy and reliability of the results. The techniques of management will be described in detail in Chapter 3, 'Data Analysis'.

2.6.1 Patient-Related

We encountered four main patient related problems. They were breath holding, movements, cardiac arrhythmia, inconsistent Finapres recordings due to cold extremities and unusually small or large fingers.

As a selection criterion, every person who had a history of, or were currently suffering from, respiratory difficulties were initially excluded. However, four subjects and three controls displayed certain breathing patterns that became problematic during the recordings. Three subjects and two controls fell asleep during the recording and breathed deeply, snored, or yawned. Conversely, two subjects unintentionally held their breath whilst performing rhythmic handgrip. These breathing patterns may have caused fluctuations in Po_2 and end-tidal Pco_2, which may in turn have affected cerebral blood flow. Any unusual breathing patterns were recorded and the person was verbally encouraged to relax and breathe regularly and not too deeply. Retrospectively, it would have been useful to record the actual respiratory rate during each recording for a more precise assessment of the regularity and frequency of respiration. Occasionally, the TCD probes were displaced by coughing or sneezing. When this occurred near the beginning of the recording, the recording was stopped and restarted.

Recordings were sometimes interrupted by movements of the body, such as turning of the head, attempts to sit up, and stretching of limbs to relieve cramps or discomfort. This problem affected the recordings of two subjects and three controls. Body movements were occasionally triggered by sudden distraction, such as when verbal prompting was given to encourage regular breathing. Furthermore, in two controls, contractions of the facial muscles during breathing or swallowing of the saliva caused small movements at the temporo-mandibular region, which in turn

affected the angle of insonation by the TCD probes. This was usually reflected as periodic fluctuations of the TCD power in harmony with respiratory activity. Wherever appropriate, verbal prompting was given to avoid significant disturbance of the recording, but in most cases, it was necessary to terminate the recording and start again because the TCD probes had been displaced.

In two subjects and three controls, the heartbeats were not constantly in sinus rhythm. There were episodes of minor paroxysmal cardiac arrhythmia, in particular atrial or ventricular ectopic beats, missed beats, and short bursts of supraventricular or ventricular ectopic beats. During the study, no subject or control suffered life-threatening paroxysmal arrhythmia, such as ventricular tachycardia or atrial fibrillation, that required immediate cessation of the examination. Where there was arrhythmia of significant duration, at least one extra recording was performed to increase the amount of data for analysis.

Lastly, as already mentioned in Section 2.4.2, only a medium sized Finapres finger cuff was available for the study. In two subjects and one control, however, the middle finger (recommended) was either too small or large for a perfect fit. Other fingers, such as the thumb or little finger, were sometimes used to obtain the best blood pressure trace. Moreover, in three subjects, despite warming of the hands and using the appropriate finger size, the blood pressure trace in some recordings were still affected by the downward trend as described earlier. During a few recordings, however, these traces spontaneously improved without any readjustment to the cuff. Although there was no apparent reason for this phenomenon, it nevertheless highlights the problems that can be caused by the highly sensitive Finapres device.

2.6.2 Equipment-Related

During the study, there were three main problems related to the equipment. Firstly, in one of the early examinations, the signals from the TCD reached saturation when they were recorded onto the computer. This was easily overcome by reducing the size of signals into the computer by the use of resistors. Secondly, in five examinations, unpredictable short and sharp electrical 'spikes' were found to distort some of the signals. Thirdly, the R-wave detector was occasionally malfunctioning, resulting in the failure of some heartbeats to trigger a pulsed signal, or the generation of extra signals without corresponding heartbeats. The management of the last two problems will be described in detail in Section 3.2.

2.6.3 Loss during Follow Up

In total, two subjects were lost during follow up. One of the subjects was admitted with a mild stroke. The first examination was carried out successfully. After hospital discharge, he refused to return to hospital for the follow up examinations. Another subject was lost during follow up due to recurrence of stroke between the second and third examination. In addition, one control had a CT brain scan one week after the study and it showed signs of a previous ischaemic stroke within the territory of the anterior cerebral artery. The data of that control were excluded from the data analysis.

CHAPTER 3 DATA ANALYSIS

3.1 Introduction

3.1.1 Analytical Techniques for Assessing Cerebral Autoregulation

In the last 20 years, many different approaches have been used to assess static and dynamic cerebral autoregulation. The reason for this diversity is that cerebral autoregulation is a derived concept rather than a directly measurable quantitative phenomenon. The degree of competence or impairment, therefore, is even more difficult to define. The interpretation of data varies between investigators and there is no universally agreed 'gold standard' of data analysis. Often the chosen analytical technique also depends on other factors such as the number of subjects and controls, method of data collection, and available resources.

The assessment of *static* autoregulation aims to evaluate the overall efficiency of the autoregulatory action, i.e. the change in cerebral vascular resistance (CVR) in response to the manipulation of arterial blood pressure (BP). The linear regression method has been used to determine the static relationship between cerebral blood flow (CBF) and BP or cerebral perfusion pressure (CPP). If the regression slope is approximately zero, it can be assumed that the linear regression coincides with the plateau region of a normal 'autoregulation curve' (see Figure 1 on Page 15). If the slope is significantly positive, this can represent the linear portion of the curve below its lower, or above its upper, limit of autoregulation. However, the discrimination between a normal and abnormal autoregulation remains unclear. Values between 0.5 and 3 to 4 %/mmHg have been proposed as thresholds for an impaired autoregulation [13]. In a study by Panerai *et al.* (1998), recordings were classified as showing impaired autoregulation if the regression had a significant slope greater than 1.5 %/mmHg.

Other investigators have used the correlation coefficient (r) as a measure of autoregulation, the higher the correlation coefficient (r nearer to 1), the more severe the autoregulation impairment (pressure-passive relationship). The threshold of impairment, generally around $r = 0.5$ to 0.6, varies between investigators [13, 202]. Others, such as Tiecks *et al.* (1995), assessed static autoregulation by calculating an index of static autoregulation (sCA) as a ratio of the percentage change of CVR to that of BP, that is:

$$\textbf{sCA index} = (\%\Delta\ \textbf{CVR}) / (\%\Delta\ \textbf{BP}) \times \textbf{100\%}$$

Thus, a change in CVR that would fully compensate for the drop in BP would yield a sCA index of 100%, and no change in CVR would yield a sCA index of 0% [149]. A sCA index of between 85 and 100% has been shown to correlate well with intact cerebral autoregulation [13].

The assessment of *dynamic* autoregulation has traditionally also required the manipulation of BP. Recent observations of the frequency response of cerebral blood flow velocity (CBFV) to BP have demonstrated that human cerebral autoregulation behaves as a 'high-pass filter' model. With slow frequency variations in BP, effects on CBF variation are dampened by reflex changes in cerebral arteriole vessel diameters. Conversely, with higher frequency of BP variations, the effects are not counteracted and therefore pass directly to variations in CBF [203].

When it is considered as a mathematical model, BP can be regarded as an input function (the disturbance of the feedback system), and CBF or CBFV as an output function. The regulator of the system is the cerebral resistance vessels. The high-pass filter model of cerebral autoregulation suggests that very slow oscillations in BP as input function are almost totally excluded from the output function, whereas fast input oscillations are completely transmitted to the output function [134].

Cerebral autoregulation also exhibits frequency-dependent dynamic characteristics. The high-pass filter system influences both the timing (phase) and amplitude (gain) of the output, and the resultant change of timing (phase angle shift) and gain is dependent on the frequency of the input into the system [134].

In the context of cerebral autoregulation, gain can be thought of as an indicator of what magnitude of change in CBFV is caused by a unit change in BP. With an intact autoregulation, changes in arterial diameter attempts to minimise the effects of BP on CBF; a smaller gain would therefore indicate more effective autoregulation. Conversely, an increased gain would indicate reduced effectiveness (i.e. more BP variation is transmitted to CBFV) [203].

The phase describes the shift in degrees required to align the input signal (BP) with the output signal (CBFV) at a specific frequency. Since all signals are presumed to be sine wave functions, the maximum possible phase angle shifts are $\pm\ 180^0$ (or 0 to 360^0). When the two signals are in perfect synchronization, the phase angle is 0^0. In

general, the phase angle shift is large with intact autoregulation, whereas it approaches 0^0 with absent autoregulation [203].

According to the high-pass filter model, higher frequencies of BP oscillations would result in greater amplitudes of CBF oscillations (increased gain) and reduced time lag between BP and CBF oscillations (reduced phase angle) [204].

With this high-pass filter model of cerebral autoregulation, variations in BP should be transmitted to CBFV according to the following equation:

$$\mathbf{CBFV}\ (f) = \mathbf{BP}\ (f) \bullet \mathbf{HP}\ (f)$$

where CBFV (f) and BP (f) are frequency domain expressions of time courses for CBFV and BP, respectively, at a given frequency, f. HP (f) represents the gain and phase shift angle of the high-pass filter at this frequency [144].

The examination of this dynamic relationship between the input and output signals at individual frequencies is known as cross-spectral transfer function analysis, or 'cross-spectral analysis'. This consists of a combination of spectral analysis and transfer function analysis. Transfer function analysis in the frequency domain offers the capacity to describe the temporal and spatial relation between two physiological signals [130].

The first part of the analysis is spectral analysis, which generally uses fast Fourier transformation (FFT) to construct a spectrum. FFT transforms the mean CBFV and mean arterial BP time series into a series of sine wave [see Section 3.3.2]. The second part of the analysis is transfer function analysis, which examines the amplitude and phase of BP and CBFV oscillations at each frequency [see Section 3.4]. The comparison of the relative amplitudes and phases enables the calculation of gain and phase angle shift at each frequency. These mathematical indices then provide an indication of the competence of cerebral autoregulation [203].

Cross-spectral transfer function analysis has previously been used to study respiratory sinus arrhythmia, the arterial baroreflex, and renal autoregulation [203, 205]. It has also recently been used by various investigators such as Diehl *et al.* (1991), Tiecks *et al.* (1995), and Blaber *et al.* (1997), for the assessment of dynamic autoregulation in humans [129, 149, 203]. The transfer function not only examines the relative gain and phase angle shifts over a range of frequencies, it also provides an estimate of reliability of the relationship between the two signals, called coherence function. The coherence index expresses the fraction of output that can be explained by the input at each frequency [13]. The coherence index is, therefore, similar to the

correlation coefficient of linear regression, with a value between 0 and 1. At a given frequency, the coherence index is 1 if there is perfect correlation between the two signals, and 0 if there is no correlation. In general, a coherence index ≥ 0.5 was considered to indicate significant consistency in the recordings [206, 207].

In the past, different investigators have used the coherence index for different purposes. Giller *et al.* (1997) assessed the coherence index as a direct indication of cerebral autoregulation in eight patients with subarachnoid haemorrhage and six normal controls. It was found that five of the eight patients had coherence greater than 0.6, whereas four of the six normal controls had coherence less than 0.6 [202].

Other investigators, such as Diehl *et al.* (1998), Kuo *et al.* (1997), and Blaber *et al.* (1997), however, had different interpretations of the use of coherence function [134, 130, 203]. Diehl *et al.* (1998) considered coherence as the correlation between the oscillations of two signals with respect to phase angle shifts. In this study, the phase angle shift and gain were only calculated in cases with a coherence index of $\geq$ 0.4, because "the determination of mean phase angle shifts and gains between two variables is not appropriate if there is no or little coherence" [134]. Kuo *et al.* (1998) used the coherence function to provide an assessment of the linear relation at each frequency and of the statistical reliability of the transfer function [130]. Blaber *et al.* (1997) also stated that the coherence function did not have direct physiological significance but could be used as a statistical measure of the reliability of the transfer function and of the linearity of the input/output relation [203]. In general, the coherence function alone probably cannot be used to directly describe the competence of cerebral autoregulation. However, together with gain and phase angle shift, it may provide additional information on the relationship between CBFV and BP oscillations, as assessed by transfer function analysis.

Diehl *et al.* (1998) assessed the phase angle shifts and gains of fifty normal subjects. Spontaneous variations of BP and CBFV were recorded and cross-spectral transfer function analysis was performed to investigate the relationships between BP and CBFV waves in the high frequency (Respiratory waves, 12 cycles/min) and mid-frequency (Mayer waves, 6 cycles/min) regions [see Section 1.5.5]. It was shown that, as expected with a high-pass filter model, the phase angle for M-waves was larger and gain was smaller than was the case for R-waves. In other words, cerebral autoregulation was less effective in the higher frequency range of BP oscillation

[134]. This finding was confirmed by Zhang *et al.* (1998) who examined the gain, phase, and coherence index of ten healthy subjects. They found that gain increased substantially with increasing frequency from 0.07 to 0.20 Hz (i.e. 4 to 12 cycles/min) in association with a gradual decrease in phase. The coherence index was greater than 0.5 in the frequency range of 0.07 to 0.30 Hz (i.e. 4 – 18 cycles/min) and less than 0.5 at < 0.07 Hz [204].

In this study, the investigators also assessed the 'impulse response function' (IRF). The impulse response function was derived as the inverse Fourier transformation of the transfer function estimated under steady-state conditions to predict the change in CBFV when the cerebrovascular system was driven by a transient change in mean arterial pressure. The predicted changes in CBFV were then compared with the measured changes by evaluation of the mean value and standard deviation of residuals between each data point of the two signals 10 seconds before and 20 seconds after the thigh cuffs deflation. The null hypothesis supposed that the mean value of residuals was zero. If spontaneous variation in CBFV were caused by changes in BP and the dynamic relationship between the two variables could be modelled by the transfer function, then the changes in CBFV predicted by the impulse response function should precisely match the measured changes in CBFV during acute hypotension [204]. In neonates, Panerai *et al.* (1998) also found that impulse responses of CBFV for BP were significantly different between the group with normal autoregulation and another group with abnormal autoregulation [164].

3.1.2 Objectives of Data Analysis

The aim of data analysis was to perform cross-spectral transfer function analysis to evaluate the competence of cerebral autoregulation in stroke patients at various stages of recovery. We hypothesized that there would be a difference between the affected and unaffected hemispheres, as compared to controls, and that there would be an improvement in autoregulation during the first three months after stroke.

For the part of the study that examined the spontaneous oscillations of BP and CBFV, the gain, phase angle shift, and coherence function were calculated. We called this the 'steady-state transfer function analysis'. For the part that involved rhythmic handgrip (RHG) as a stimulus of BP oscillations, only the gain and phase angle shift were calculated. We called this the 'rhythmic handgrip transfer function analysis'.

Furthermore, the steady-state transfer function analysis also examined the phase angle shift, gain, and coherence for three frequency ranges of BP oscillations (high, low, and very low). These frequency ranges corresponded to the three frequency ranges of CBFV oscillations (B-wave equivalent, M-wave, and R-wave). This was performed in order to test the existence of a high-pass filter model of cerebral autoregulation, which may behave differently after acute ischaemic stroke.

The results were then compared between the different groups: affected vs. unaffected hemisphere; subject vs. control; visits 1, 2 and 3; and the three frequency ranges. A result was defined as statistically significant if $P < 0.05$.

In order to perform cross-spectral transfer function analysis on the recorded data, it was necessary to ensure that the data were as reliable and easy to analyse as possible. The artefacts that were present amongst the data were found and managed individually. This 'cleaning up' process will be discussed in detail in the next section.

3.2 Management of Data Artefacts

In this section, the management strategies of data artefacts are discussed. These were all done off-line using programmes specially written by Dr Mike Lunt (senior medical physicist at Royal Bournemouth Hospital). These programmes took the format of macros, which were applied to the raw data within the Microsoft Excel package. The aim of this 'cleaning up' process was to reduce as much as possible the inconsistency of the data caused by the artefacts, and increase uniformity and hence the reliability of the final results. The main artefacts that required the use of these programmes were those related to R-wave detection and spurious electrical spikes, as previously described in Section 2.6.3.

3.2.1 R-wave Artefacts

In this study, the R-wave detector was used to produce a pulsed signal (the 'R-wave pulse') for every R-wave that was detected. Under normal conditions, R-wave pulses should be evenly spaced, giving moderately consistent beat-to-beat intervals. They should be of the same duration so that the start of the pulse was also consistent. The R-wave detector, however, produced various artefacts that could be grouped into the following three main categories:

1. 'Missing' R-wave pulses.
2. 'Spurious' R-wave pulses.
3. R-wave pulses that were too long or short (hence inaccurate beat-to-beat interval).

The first category refers to the missing R-wave pulses. This occurred when a normal heartbeat was, for unknown reason, not recognised. An R-wave pulse was, therefore, not produced. The second category refers to the spurious R-wave pulses. This occurred when a normal heartbeat was recognised but more than one R-wave pulse was produced. These extra pulses are referred to as 'spurious'. Two computer programmes were written for the management of these two artefacts.

The first programme (Ctrl b) identified beat-to-beat intervals (i.e. time between consecutive R-wave pulses) that were more than 2.5 to 3 standard deviations from the mean for the full 5-minute recording. These irregular beat-to-beat intervals were identified on a printout of the data and each one was characterised as one of three types. Firstly, a correct interval caused by an irregular heart rate (i.e. ectopic

beat); secondly, a long interval caused by a missing R-wave pulse; and lastly, a short interval caused by a spurious pulse.

A spurious R-wave pulse was removed manually by simply replacing the abnormal data with a zero baseline. For missing R-wave pulses, a second programme (Ctrl r) was used to insert a pulse where there should have been one. This programme took a typical heartbeat from the 5-minute recording and recognised the typical relative position of the R-wave pulse to the rise in CBFV and BP. This was used as a template to identify the 'correct' position of the missing R-wave pulse by calculating the best goodness of fit. A new R-wave pulse was then inserted at the typical position.

In this study, data values of the ectopic beats were not manipulated or processed. Although some investigators believe that ectopic beats can alter the integrity of the data and their analysis, the effects of these beats on the relationship between oscillations of BP and CBFV remain to be elucidated. Moreover, the presence of ectopic beats was not a reason to classify a recording as of poor quality [see Section 3.3.3]. In the future, we plan to develop more sophisticated methods of extracting ectopic beats and investigate whether and how ectopic beats influence the final results.

Where the R-wave pulses were too long or short, three programmes were written to correct them. A normal R-wave pulse lasted 50 to 60 ms, which corresponded to 5 to 6 sampling points at 10 ms intervals. Occasionally, the R-wave pulse lasted for longer (e.g. 80 to 100 ms), or shorter (e.g. 20 to 30 ms) than normal. The beat-to-beat interval was, therefore, unreliable and could be inaccurate. The first programme (Ctrl i) identified heartbeats with R-wave pulses that were too long or short (i.e. not 50 to 60 ms), and used the (Ctrl r) programme above to find the 'correct' position of the R-wave pulse that corresponded to the 'true' start of beat. The next programme (Ctrl m) then corrected the start of the R-wave pulse. The last programme (Ctrl n) made all R-wave pulses the same length, at 50 to 60 ms.

3.2.2 Electrical Spikes

In this study, large aberrant electrical spikes were found in five examinations. There was no predictable pattern of occurrence and, as already mentioned in Section 2.6.3, the cause of these spikes was unknown. These spikes affected different channels, most notably Vmax (left and right), BP, and R-wave pulses, at different times. It was

necessary to remove these spikes because of their effects on the calculation of beat averages. Each spike usually only lasted 10 ms (i.e. one sampling point).

Two programmes were specially written for the identification of possible spikes. One programme (Ctrl s) identified spikes affecting R-wave pulses and another (Ctrl f) identified spikes affecting Vmax (left and right) and BP. Each spike was removed manually or automatically, and the value was replaced with an averaged value derived from the two points before and after it (i.e. linear interpolation).

Electrical spikes were not unique to the present study. Narrow spikes in the Vmca signal were also detected in a study by Panerai *et al.* (1995). These spikes were also removed by linear interpolation and no explanation was given for their occurrence.

After processing the raw data with the above programmes, they could then be used in the next stage of data analysis, spectral analysis.

3.2.3 Potential Blood Pressure Drift

It is possible that the BP might have slowly drifted during the 5-minute recordings. This was managed using three different methods.

Firstly, prior to FFT, any systematic drift in the BP data was adjusted by normalisation (an automatic process of the software). Secondly, during rhythmic handgrip transfer function analysis, the BP waveforms of seven 40-second cycles were treated as independent segments and averaged [see Section 3.5]. Hence, any drift in BP would have been eliminated. Lastly, during steady-state transfer function analysis, different FFT spectrums for BP were also averaged to eliminate any drift in BP over time [see Section 3.4]. This was the same method used in the studies by Giller *et al.* (1997), and Zhang *et al.* (1998) [202, 204].

3.3 Spectral Analysis

The two main steps of spectral analysis were firstly, the calculation of beat-to-beat and time sequences, and secondly, the fast Fourier transformation (FFT). These steps are necessary prior to the steady-state transfer function analysis. The rhythmic handgrip transfer function analysis has a different protocol and will be described in Section 3.5. In this section, CBFV in the middle cerebral artery, as measured by TCD ultrasonography, is referred to as 'Vmca'. 'R-waves' refer to respiratory waves, i.e. oscillations of BP or Vmca in the high frequency range, and not the R-waves of ECG signals.

3.3.1 Calculation of Beat-to-Beat & Time Sequences

During the recording, the computer sampled every 10 ms, which gave 100 samples per second, (i.e. 6000 per minute, 30,000 per 5-minute recording). Since a normal heart rate under steady condition was about 80 per minute, on average about 75 data points were sampled for every heartbeat. For steady-state transfer function analysis, the variables of interest were Vmca (left and right sides) and BP.

In order to perform spectral analysis, it was first necessary to obtain beat-to-beat averages of Vmca and BP. Mean values of Vmca and BP were calculated for each cardiac cycle of the 5-minute recording. The resultant beat-to-beat sequence of mean Vmca and BP values were unequally spaced. This sequence was linearly interpolated and resampled with a time interval of 250 ms in order to produce a uniformly spaced time sequence. The start and end of the beat-to-beat sequence were extrapolated with the same time interval. This new time sequence of mean Vmca and BP values was then ready for the process of FFT.

3.3.2 Fast Fourier Transformation

FFT is a method of classical spectral analysis that has many applications, both in medical as well as electronic engineering fields. Spectral analysis assumes that any time-domain recording, such as mean BP and Vmca, can be regarded as a summation of sinusoidal oscillations of various frequencies. The FFT algorithm transforms time-domain signals into a spectrum in the frequency-domain. The spectrum takes the form of a histogram whose x-axis corresponds to frequency intervals, and y-axis to

amplitude of oscillations, such that the estimated spectral power in a frequency range is equal to the sum of amplitude squared within the corresponding frequency range.

For the FFT algorithm, each 5-minute recording was divided into subintervals of equal length, each lasting for 2 minutes and overlapping by 50%. Only 4 minutes and 40 seconds of each recording was used in order that direct comparisons can be made with the results of rhythmic handgrip transfer function analysis [see Section 3.5]. Each 5-minute recording was divided into 3 subintervals. Since two 5-minute steady-state recordings were performed per visit, there were in total 6 subintervals per visit. This protocol of interval subdivision is similar to the ones used by Giller *et al.* (1997) and Zhang *et al.* (1998) [202, 204]. Given that for coherence function, the statistical error decreases as the number of subinterval increases, there was a trade-off between having fewer but longer subintervals (higher spectral resolution, lower statistical reliability) and more but shorter subintervals (lower spectral resolution, higher statistical reliability). For example, Kuo *et al.* (1998) used 29 subintervals of 1 minute duration (15-minute recording), each overlapping by 50% [130]. The duration of 2 minutes was chosen for this study because we were particularly interested in the spectral analysis of the 'very low' frequency range, which corresponded to oscillations of Vmca at 0.5 to 2 cycles/min, that is, 'B-wave equivalent'. An overlap of 50% was chosen in this study because Carter *et al.* (1973) have shown that, in order to increase the number of subintervals in coherence calculation, a overlap of up to 50% was effective [208].

Computation of the spectrum was performed for BP and Vmca on each 2-minute subinterval using the FFT after a Hanning window was applied to each subinterval. Given that the beat-to-beat sequence of BP and Vmca have been resampled with a time interval of 250 ms to produce signals with a uniform time axis, each 2-minute subinterval therefore consisted of 480 points each 250 ms apart. The frequency intervals (x-axis) of the FFT spectrum ranged from 1 cycle in 2 minutes (0.0083 Hz) to 240 cycles in 2 minutes (2 Hz). These corresponded to cycle periods of 120 seconds to 0.5 second.

At the end of spectral analysis, for each visit, 6 pairs of FFT spectrums for BP and Vmca were produced for steady-state recordings (without RHG). Comparisons of the BP and Vmca FFT spectrums using transfer function analysis yielded coherence function, gain, and phase angle shifts.

3.3.3 Quality of the Recordings

In the study, the recordings were classified to either good or poor quality. This was a qualitative and descriptive exercise, and the method has not been validated in other studies. The purpose of qualifying the recordings was so that only the good quality recordings were selected for the final data analysis.

For this purpose, each 5-minute recording was divided into ten 30-second segments. Segments with large number of artefacts, mainly paroxysmal arrhythmia and temporary loss of TCD or Finapres signals, were classified as poor quality. As already mentioned in Section 3.2.1, we did not regard the presence of ectopic heart beats as a reason to classify a recording as poor quality. The total number of good quality segments was taken as the quality score out of ten. In this study, a score of ≥ 8 was arbitrarily chosen as the threshold for being good quality.

This process was performed prior to data analysis so that there could be no chance of selection bias. It was also performed in a blinded fashion. The person who judged the quality (Dr Mike Lunt) was neither aware of whether the recording was from a subject or control, under steady state or with RHG, nor at which visit the examination was undertaken.

The elimination of some of the poorer recordings meant that there were fewer, but better quality, available data for analysis. A summary of the quality of each recording in the study can be found in Section 4.2.

In this study, we found the majority of the recordings were of good quality with very few artefacts. However, a small proportion of recordings were affected by large numbers of artefacts that were not amenable to management by manipulation as described in Section 3.2.

3.4 Steady-State Transfer Function Analysis

3.4.1 Phase Angle Shift & Gain

The phase angle shift and gain were calculated from the steady-state transfer function analysis. The transfer function, $H(f)$, between the two signals can be defined as:

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

where $S_{xx}(f)$ is the autospectrum of changes in BP and $S_{xy}(f)$ is the cross-spectrum between the two signals. The transfer function magnitude $|H(f)|$, which is gain, and phase spectrum $|\Phi(f)|$ can be derived from the real part $H_R(f)$ and the imaginary part $H_I(f)$ of the complex transfer function as:

$$\textbf{Gain} = |\mathbf{H}(f)| = \{[\mathbf{H_R}(f)]^2 + [\mathbf{H_I}(f)]^2\}^{1/2}$$

$$\textbf{Phase Angle Shift} = \Phi(f) = \textbf{arctan}\ [\mathbf{H_I}(f) / \mathbf{H_R}(f)]$$

The transfer magnitude (gain) and phase reflect the relative amplitude and time relationship between the changes in BP and Vmca at different frequencies within a specified frequency range [204]. In some studies, such as Blaber *et al.* (1997), the gain was calculated as 20 log $|H(f)|$ to give values in decibels (dB), In general, a gain of 1 indicates that the output varies by the same fraction of the mean value as the input [203]. A gain value of less than 1 indicates that the output varies less than the input, and a value of greater than 1 indicates that the output varies greater than the input. The definitions of positive and negative phases, however, differ between authors. According to Diehl *et al.* (1995), Panerai *et al.* (1998), and Birch *et al.* (1994), a positive phase is when the output leads input, whereas according to Blaber *et al.* (1997), it is the opposite [144, 164, 145, 203]. In the present study, the former definition was used.

As already discussed in Section 1.5.3, the fluctuations in Vmca, like BP, can be divided into 3 components at specific frequency ranges (B-wave equivalent, M-wave, and R-wave). In this study, therefore, the frequency ranges of particular interest were also designated as high frequency (HF, 0.15 to 0.4 Hz), low frequency (LF, 0.04 to 0.15 Hz), and very low frequency (VLF, 0.016 to 0.04 Hz), according to the protocol used by Kuo *et al.* (1997) [130]. The HF component corresponded to the R-wave (9 to 24 cycles/min), the LF component to the M-wave (2.5 to 9 cycles/min), and the VLF component to the B-wave equivalent (1 to 2.5 cycles/min). Phase angle shift and gain were analysed for each of the three frequency ranges.

In our study, the gain was calculated as the ratio of the percentage change in amplitude of Vmca oscillation to that of BP oscillation, at each frequency point. The gain of each frequency range was calculated by averaging the gain value (at that frequency range) from each of the six subintervals for that visit. Then the gain values of different subjects were averaged to obtain a 'group' mean gain. The same process for the subsequent visits yielded a mean gain value for each frequency range at each visit.

The phase angle shift was calculated from the separate averages of the sine and cosine components of each of the FFT spectrums. Similar to gain, the phase angle shift of each frequency range was calculated by averaging the value (at each frequency range) from each of the six subintervals for that visit. Then the phase angle shifts of different subjects were averaged to obtain a 'group' mean phase angle shift. The same process for the subsequent visits yielded a mean phase angle shift for each frequency range at each visit.

The same analysis was also performed for the recordings of the controls, except that each control was only examined once.

3.4.2 Coherence Index

Coherence function is a quantitative measure of similarity between signals in the frequency-domain. It is similar to correlation coefficient, normalised to yield values between 0 (signals totally uncorrelated) and 1 (signals identical) [208]. Coherence takes into account the change in signal content over time. For example, frequency components may wax and wane in amplitude and the relative phase between components in the two channels may alter with time. Noise may occur sporadically or continuously in one or both channels, and new frequency components may arrive by additive process. The coherence function provides an analytical tool by which all of these changes can be monitored and quantified in the frequency domain [208]. As already mentioned, in the past, the coherence function has been interpreted in different ways by different investigators [see Section 3.1.1].

Coherence function was used by Diehl *et al.* (1998) in the analysis of cerebral autoregulation [134]. The form of coherence function (COH) analysis was called magnitude squared coherence (MSC), the formula of which is shown below [134]:

$$\mathbf{COH} = \left(\frac{\sum_{i=l}^{u} a_i b_i \cos(\alpha_i - \beta_i)}{\sum_{i=l}^{u} a_i b_i} \right)^2 + \left(\frac{\sum_{i=l}^{u} a_i b_i \sin(\alpha_i - \beta_i)}{\sum_{i=l}^{u} a_i b_i} \right)^2$$

The terms a_i and b_i represent the amplitudes and α_i and β_i the phases of the oscillations in Vmca and BP, respectively. l and u are the lower and upper limits of the frequency band (e.g. high and low frequency bands). The coherence index provides a measure of the consistency between the frequency points within a frequency band. In the study by Giller *et al.* (1997), however, the coherence was assessed as the consistency across the subintervals at each frequency and the coherence indices averaged over the frequency points to provide a "mean coherence" [202]. Similarly, Zhang *et al.* (1998) averaged the coherence indices over the frequency points within a frequency band, and over the subjects, giving a "group-averaged coherence function" [204].

In the present study, coherence function was only assessed for the recordings without RHG. In the study by Giller *et al.* (1997), the frequencies that were included for coherence assessment ranged from 1.5 to 11.3 cycles/min (i.e. cycle period between 90 and 5.3 seconds). In order to produce comparable results, we used a similar range of frequencies (cycle period between 120 and 5.3 seconds) for the analysis of coherence. In this study, coherence between Vmca and BP oscillations was calculated according to the aforementioned formula for magnitude squared coherence.

Furthermore, similar to the analysis of phase angle shift and gain, coherence index was also calculated for each of the three frequency ranges as described in Section 3.4.1. The coherence index of each frequency range was calculated by averaging the value (at each frequency range) from each of the six subintervals for that visit. Then the coherence indices of different subjects were averaged to obtain a 'group' mean coherence. The same process for the subsequent visits yielded a mean coherence for each frequency range at each visit.

We hypothesized that, if the high-pass filter model of cerebral autoregulation existed, coherence indices of the three frequency ranges would be significantly different with the highest coherence in the high frequency range. Moreover, phase and

gain would also demonstrate similar trends with decreasing phase and increasing gain at higher frequencies. We also compared the results of visits 1, 2, and 3, and the affected and unaffected hemispheres, in order to examine for any trend of recovery over the first three months of stroke recovery.

3.5 Rhythmic Handgrip Transfer Function Analysis

For this part of data analysis, a special programme that was written by Dr A Birch (Department of Medical Physics and Medical Engineering, Southampton General Hospital) was used. This programme has been tested in cerebral autoregulation studies that involved periodic squatting and standing, with useful results [145].

The data of Vmca (left and right sides) and BP, after the management of data artefacts, were analysed using the programme. An additional channel, which contained information on the start and end of each cycle of RHG, was also created. RHG was performed with a cycle period of 40 seconds (20 seconds on, 20 seconds off). Within each 5-minute recording, there were seven complete 40-second cycles, making up a total of 4 minutes and 40 seconds (the last 20 seconds of recording was discarded).

The seven complete cycles were averaged (separate Vmca and BP waveforms) in order to reduce the relative amplitude of the cardiac pulsations. The average waveform was then processed by means of FFT using the first 4 harmonics (single-frequency sinusoidal oscillations), i.e. 1, 2, 3, or 4 cycles per 40 seconds (cycle period of RHG). The first harmonic (i.e. 1 cycle per 40 seconds, or 0.025 Hz) was also known as the 'fundamental'. From the FFT, only the fundamental components of the Vmca and BP were used to extract the phase angle shift and gain. The amplitude of oscillation at the fundamental frequency was assessed for Vmca and BP, and expressed as a percentage difference from the mean. The gain was then calculated as the ratio of the percentage change in amplitude of Vmca oscillation to that of BP. The phase angle shift was calculated as the angle difference (in radians or degrees) between the peaks of Vmca and BP oscillations at the 0.025 Hz component. The 'power' was calculated as the percentage of the average waveform that was made up by oscillations at the fundamental frequency.

Phase angle shift and gain were calculated for each recording and visit. As for steady-state transfer function analysis, a positive phase was defined as when the Vmca oscillations (output) led BP oscillations (input), and a gain greater than one was when Vmca oscillations varied more than BP oscillations. The same programme was used for the RHG recordings of subjects and controls. We compared the results of the three visits in order to examine for any trend suggesting a recovery of cerebral autoregulation.

3.6 Statistical Analysis

In this study, statistical analyses and interpretation were advised and performed by a statistician consultant (Dr Peter Thomas, Bournemouth University). The aim of statistical analysis was to investigate whether cerebral autoregulation in the affected hemisphere was impaired after stroke, and whether there was significant change over the first three months. The results of the affected hemisphere were compared to those of the unaffected hemisphere and controls. Recovery of cerebral autoregulation would be reflected by significant changes in the mean phase angle shift and gain over the three visits. The association of phase, gain, and coherence with frequency of BP oscillations was also explored.

For the steady-state and rhythmic handgrip transfer function analyses, the mean phase angle shifts and gains of the affected and unaffected hemispheres were compared between the three visits. Repeated measures analysis of variance (ANOVA), linear regression analysis, and two-tailed *t*-test were performed for these purposes. There were two chosen within-subject variables for the repeated measures ANOVA. The first one was 'visit', which was a polynomial variable (i.e. 1, 2, or 3). The second was 'hemisphere', which was a simple stratified variable (i.e. affected or unaffected). The repeated measure ANOVA examined for any possible dynamic interaction between hemispheres and visits.

For steady-state transfer function analysis, repeated measures ANOVA were also performed to examine for any dynamic interaction between frequency ranges (very low, low, and high), visits, and hemispheres. Linear regression analysis and two-tailed *t*-test were also used to compare the means and to examine for the presence of any significant trend, whether linear or 'sphericity-assumed'. The α-level was set at $P = 0.05$.

Recorded data for phase angle shift, gain and coherence (for each hemisphere, visit, frequency range, with and without rhythmic handgrip, and for subjects and controls) were tested for their distribution using Shapiro-Wilk test (SPSS 9.0), which is suitable for 50 or fewer observations. In total, 67 out of a total of 77 (87%) data sets were found to be normally distributed. This therefore justifies the use of ANOVA for data analysis, which assumes data to be of an approximately normal distribution [see Appendix 2, Chapter 7, for a full report of the results of Shapiro-Wilk test].

CHAPTER 4 RESULTS

4.1 Characteristics of Subjects & Controls

4.1.1 Subjects

In this study, we examined ten subjects who displayed the following characteristics:

NAME	SEX	AGE	SIDE OF INFARCT	OCSP CLASS†	TIME TO VISIT 1‡	HX CVA¥	HX ↑BP	↓BP Rx¶	HX DM§
B L*	M	67	Right	TACI	3	No	Yes	Yes	Yes
A O	M	71	Right	PACI	5	1998	No	No	No
Q W	F	86	Right	PACI	2	No	Yes	Yes	No
J M*	M	69	Left	PACI	6	No	No	No	Yes
G D*	M	85	Left	TACI	2	No	Yes	Yes	No
H O*	M	66	Right	PACI	2	No	No	No	No
C M	M	48	Right	PACI	7	1997	No	No	No
O G*	F	82	Left	PACI	3	No	No	No	No
B P*	M	71	Left	PACI	6	1998	No	No	No
R C	F	80	Left	TACI	5	No	Yes	No	No

† Oxford Community Stroke Project clinical classification for stroke: TACS, PACS, LACS, and POCS

‡ Time delay from stroke onset to first examination (visit 1) in days

¥ Previous history of ischaemic or haemorrhagic stroke, or transient ischaemic attack; year of event

¶ Currently taking antihypertensive treatment § Current or previous history of diabetes mellitus

The mean age of all ten subjects was 72.5 years with a standard deviation (SD) of 11.4 years and standard error of the mean (SEM) was 3.6 years. Of the ten subjects, six had good quality (score ≥ 8/10) TCD recordings on both sides and on all three visits (subjects with *); recordings of these subjects were included in the final analysis. Of the six subjects who had good recordings, the mean age was 73.3 years (SD=8.1yr., SEM=3.3 yr.). The mean time delay to first visit was 4.1 days. Four subjects had a history of hypertension and three were on antihypertensive treatment. None had carotid stenosis of >50% by carotid Doppler ultrasound (European Carotid Stenosis Trial criteria). A summary of the subjects' baseline blood pressure and cerebral blood flow velocity (MCA) can be found in Appendix 3, Chapter 7.

4.1.2 Controls

In this study, we examined eleven controls with the following characteristics:

NAME	SEX	AGE	DIAGNOSIS†	TIME TO VISIT 1‡	HX ↑BP	↓BP Rx¶	HX DM§
E F*	M	65	Chest infection	21	No	No	No
C L	M	71	Anaemia	3	No	No	No
G W*	M	60	Dizziness ? cause	1	No	No	No
R A*	M	48	Chest pain ? cause	1	No	No	No
E H*	F	80	Chest pain ? cause	1	Yes	Yes	No
J B	M	70	Chest infection	4	No	No	No
K A*	F	79	Dizziness ? cause	1	Yes	Yes	No
J D	M	86	Anaemia	9	No	No	No
E T*	M	68	Chest pain ? cause	1	No	No	No
H T*	M	83	Pleural effusion	2	No	No	No
A M	M	68	Deep vein thrombosis	1	No	No	No

† Main diagnosis on admission

‡ Time delay from admission to examination in days

¶ Currently taking antihypertensive treatment

§ Current or previous history of diabetes mellitus

The mean age of the eleven controls was 70.7 years with a SD of 11.0 years and SEM was 3.3 years. Of the eleven controls, seven had good quality (score ≥ 8/10) TCD recordings on both sides (controls with * above); recordings of these controls were included in the final analysis. Of the seven controls who had good recordings, the mean age was 69 years with a SD of 12.6 years and SEM of 4.8 years. The mean time delay from admission to examination was 4 days. No control had a history of stroke, transient attacks, or diabetes mellitus. Two controls had a history of hypertension and were on antihypertensive treatment. A summary of the controls' baseline blood pressure and cerebral blood flow velocity (MCA) can be found in Appendix 4, Chapter 7.

4.2 Quality of Recordings

4.2.1 Subjects

Each recording was scored out of 10 according to the overall quality, as outlined in Section 3.3.3. The following table shows the quality of each recording for the six subjects who were included in the analysis, at each visit. In the following table, each cell contains the quality of all the recordings for each examination; for example, 'Visit 2, with RHG'. 'L' and 'R' denote the side of TCD monitoring, and the number is the score out of 10. At any particular visit, the quality of the recording on each side may have been different.

NAME	VISIT 1		VISIT 2		VISIT 3	
	No RHG	RHG	No RHG	RHG	No RHG	RHG
B L*	L5 R5 GOOD ×2	GOOD	GOOD ×2	GOOD	GOOD ×2	GOOD
J M*	GOOD ×2	GOOD	GOOD ×3	GOOD	GOOD ×2	GOOD
G D*	L9 R7 GOOD ×2	GOOD	GOOD ×2	GOOD	GOOD ×2	GOOD
H O*	GOOD ×2	L8 R8	GOOD L10 R9	GOOD	L10 R7 L10 R9 GOOD	GOOD
O G*	L9 R8 L9 R10	L8 R3 L9 R9	GOOD ×2	GOOD	GOOD ×2	GOOD
B P*	GOOD ×3	L8 R8	GOOD ×3	L10 R8 L8 R8	GOOD ×2	GOOD

The following table refers to the quality of each recording of the four subjects who were excluded from the analysis.

NAME	VISIT 1		VISIT 2		VISIT 3	
	NO RHG	RHG	NO RHG	RHG	NO RHG	RHG
A O	GOOD ×2	L7 R10	GOOD ×2	GOOD	GOOD ×2	GOOD
Q W	GOOD ×2	GOOD	GOOD ×2	L2 R1	×	×
C M	GOOD ×2	GOOD	×	×	×	×
R C	L10 R6 L10 R8 L10 R7	L9 R0	L9 R6 L10 R4	L3 R7	GOOD ×2	GOOD

4.2.2 Controls

The following table refers to the quality of each recording of the seven controls who were included in the analysis. Each control underwent one visit.

NAME	NO RHG	RHG
E F*	GOOD ×2	GOOD
G W*	L8 R10, L6 R7 GOOD	L9 R9
R A*	GOOD ×2	GOOD
E H*	GOOD ×2	GOOD
K A*	GOOD ×2	GOOD
E T*	GOOD ×2	GOOD
H T*	GOOD L10 R9	GOOD

The following table refers to the quality of each recording of the four controls who were excluded from the analysis.

NAME	NO RHG	RHG
C L	L8 R9 L8 R9	L4 R9
J B	L7 R7 L10 R2	L7 R0
A M	GOOD ×2	L2 R10 L3 R10
J D	L9 R9 GOOD ×2	L10 R0

4.3 Steady-State Transfer Function Analysis

For this part of the analysis, phase angle shift, gain, and coherence were calculated for each subject for the affected and unaffected hemispheres, for each visit, and for each of the three frequency ranges: very low (VL), low (L), and high (H).

4.3.1 Phase Angle Shift

The following table shows the results for subjects, with the mean phase angle shift, SD, and SEM, for each hemisphere, visit, and frequency range. The numbers refer to phase angle shifts in degrees^0 (all phase angle shifts were positive).

VISIT	HEMISPHERE	FREQ.	MEAN	SD	SEM
1	AFFECTED	VL	16.51	30.27	11.44
		L	17.67	20.69	7.82
		H	7.28	11.05	4.17
	UNAFFECTED	VL	9.30	30.72	11.61
		L	33.08	9.66	3.65
		H	10.91	9.87	3.73
2	AFFECTED	VL	2.24	30.00	11.34
		L	28.48	15.93	6.02
		H	7.10	7.40	2.80
	UNAFFECTED	VL	15.06	28.67	10.84
		L	30.91	18.52	7.00
		H	10.42	5.86	1.92
3	AFFECTED	VL	14.40	24.63	9.31
		L	35.61	14.39	5.44
		H	8.28	9.23	3.49
	UNAFFECTED	VL	20.57	35.94	13.59
		L	34.69	12.76	4.82
		H	10.47	10.89	4.12

A recovery in cerebral autoregulation would be indicated by a positive increase in the mean phase angle shift (towards $+180^0$) over visits, for any given frequency range. Repeat measure ANOVA was performed to examine for any dynamic interaction between hemispheres, visits, and frequency ranges. This analysis examined, for example, whether the difference in mean phase angle shift between the affected and unaffected hemispheres changed significantly with subsequent visits and/or different frequency ranges.

The results showed no significant dynamic interaction ('sphericity-assumed') between hemispheres, visits, and frequency ranges (P = 0.08).

However, there was significant overall interaction between the mean phase angle shifts of the three frequency ranges when the values of the three visits and both hemispheres were combined (P = 0.002). When the mean phase angle shifts of the three visits and all frequency ranges were combined, no significant overall difference between the two hemispheres was shown (P = 0.34). In addition, when the mean phase angle shifts of both hemispheres and all frequency ranges were combined, no significant overall interaction between the visits was shown (P = 0.68).

Furthermore, we showed a significant quadratic (non-linear) trend of changing phase angle shift from very low, to low, to high frequency ranges (P = 0.004) [see Figures 9A and 9B]. The result was also significant when a linear trend for visits was combined with a quadratic trend for frequency ranges (P = 0.01) [see Figures 9C and 9D].

The following table shows the results of the mean phase angle shifts for the controls at different frequency ranges. A two-tailed heteroscedactic *t*-test (unequal variances) was performed between the mean phase angle shifts of affected hemispheres (visit 1) and controls (where the greatest difference was expected) for each frequency range. The P value for each *t*-test is shown in the table. A *t*-test was also performed between the mean phase angle shifts in the very low and high frequency ranges, but no significant difference was found (P = 0.33).

CONTROL	MEAN	SD	SEM	*t*-TEST (P)
VL	19.99	26.39	8.35	0.81
L	29.22	26.15	8.27	0.33
H	11.14	7.98	2.52	0.45

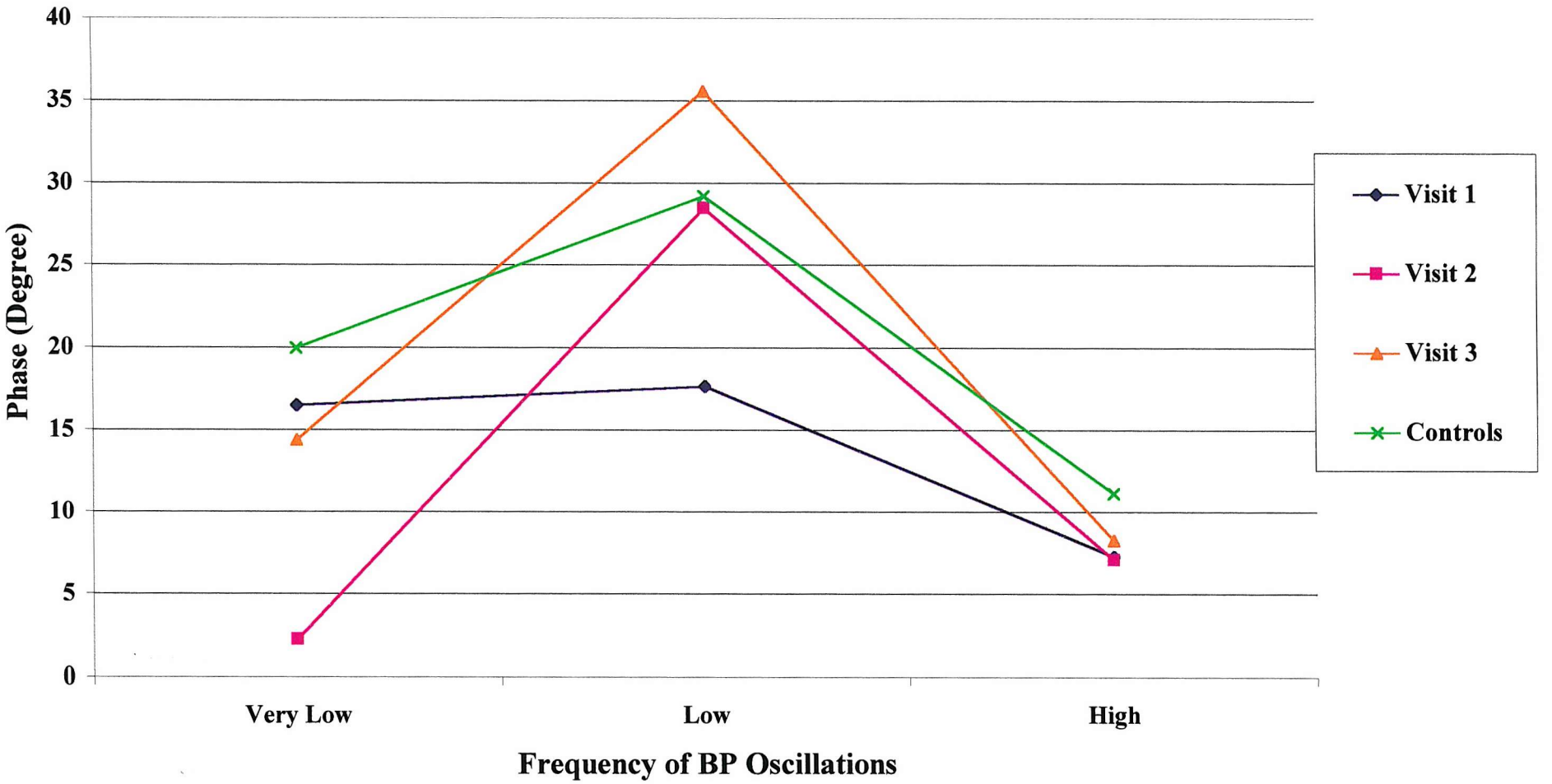


Figure 9A. Steady-state phase in the *affected* hemisphere for visits 1 to 3 and for controls. It shows a consistent trend of changing phase with increasing frequency of BP oscillations.

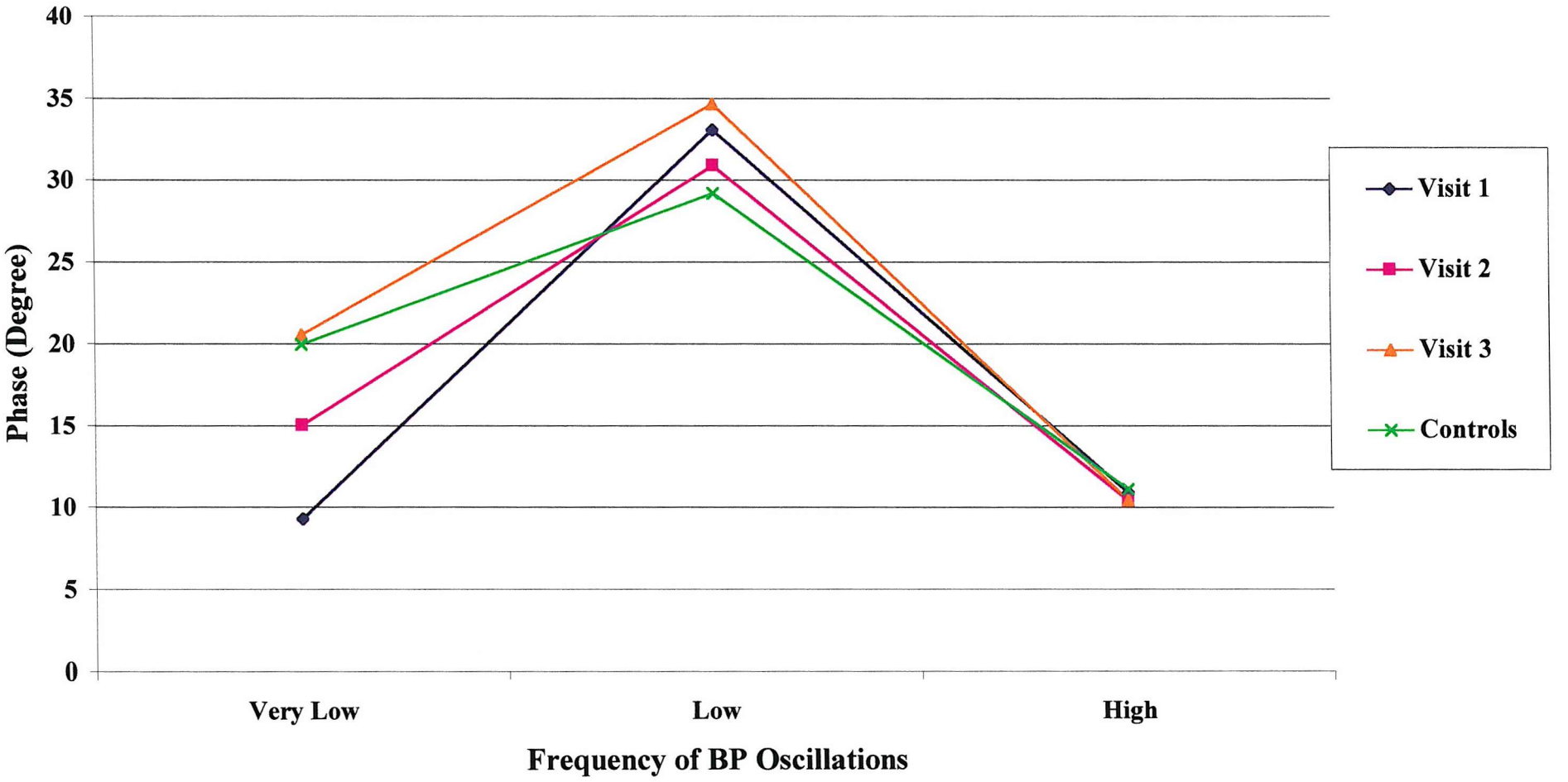


Figure 9B. Steady-state phase in the *unaffected* hemisphere for visits 1 to 3 and controls. It shows a consistent trend for changing phase with increasing frequency of BP oscillations.

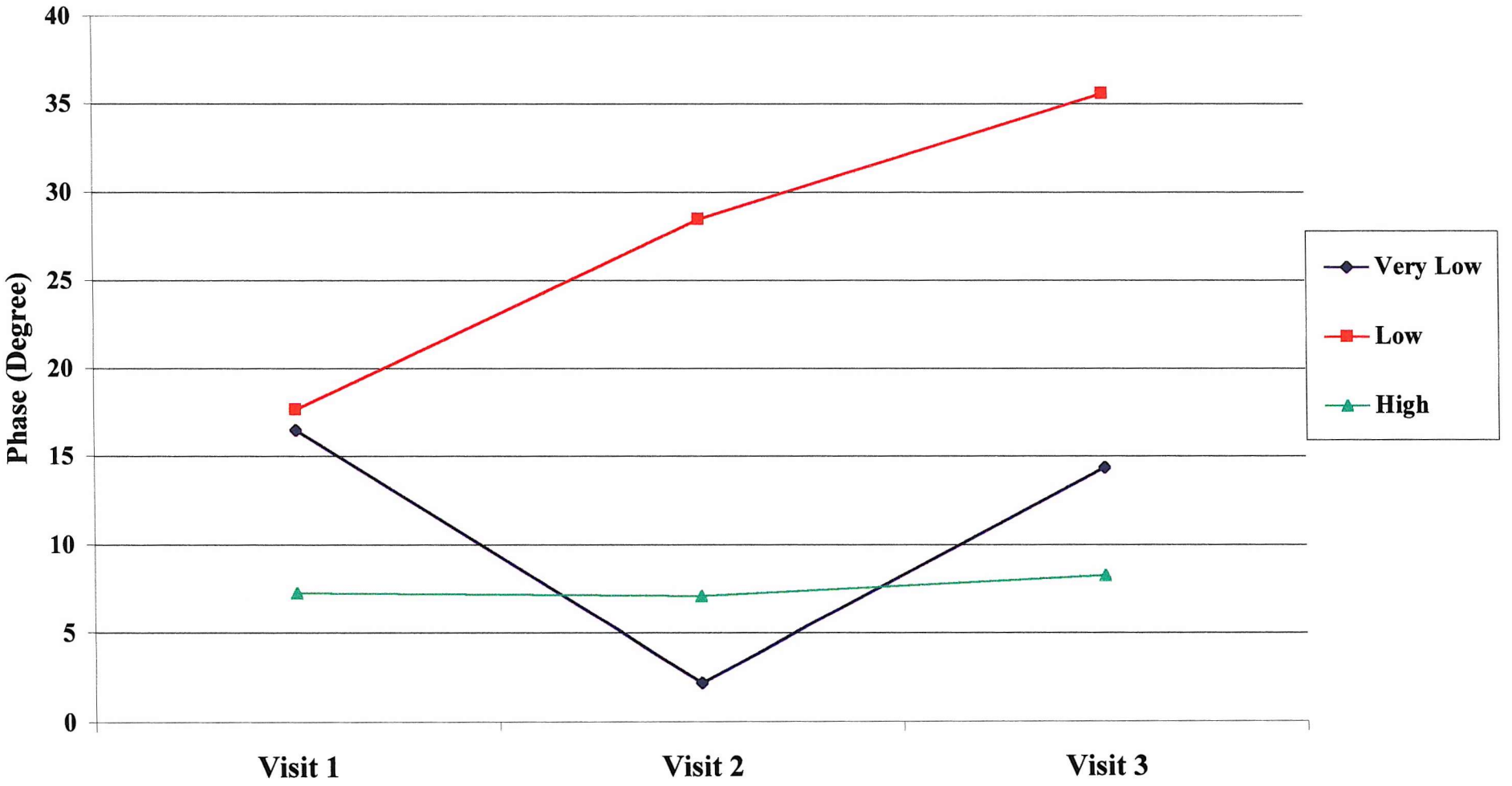


Figure 9C. Steady-state phase in the *affected* hemisphere for different visits & frequency ranges.

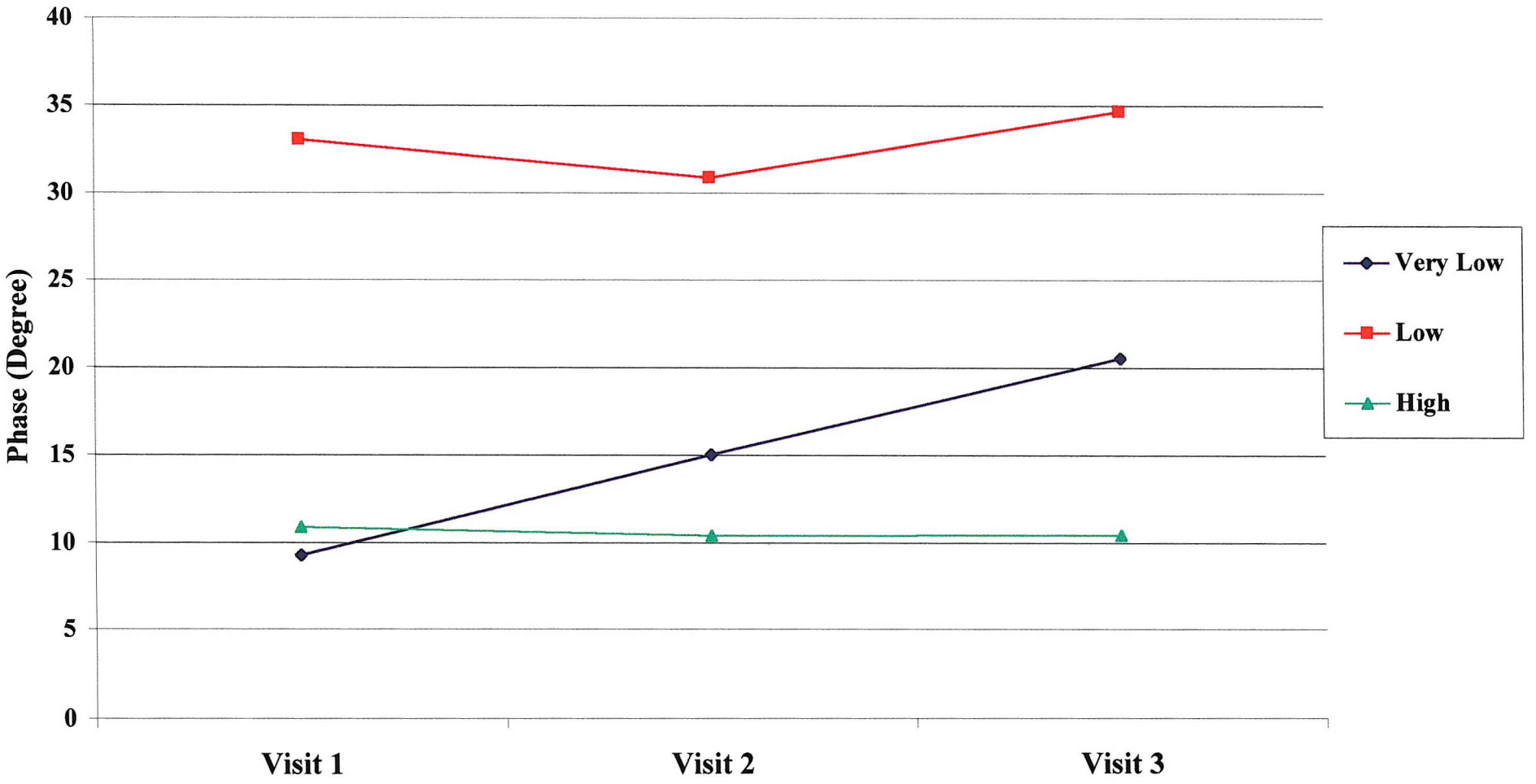


Figure 9D. Steady-state phase in the *unaffected* hemisphere for different visits and frequency ranges.

4.3.2 Gain

The following table shows the results for subjects, with the mean gain, SD, and SEM, for each hemisphere, visit, and frequency range. The numbers refer to gain with no specific units since they represent ratios (some authors use cm/sec/mmHg as units).

VISIT	HEMISPHERE	FREQ.	MEAN	SD	SEM
1	AFFECTED	VL	1.67	1.53	0.58
		L	2.10	0.76	0.29
		H	3.53	2.62	0.99
	UNAFFECTED	VL	1.04	0.18	0.07
		L	1.62	0.44	0.16
		H	2.57	0.86	0.33
2	AFFECTED	VL	1.41	0.40	0.15
		L	2.25	0.55	0.21
		H	3.86	1.92	0.73
	UNAFFECTED	VL	1.51	0.64	0.24
		L	2.18	0.66	0.25
		H	3.12	1.21	0.46
3	AFFECTED	VL	1.71	0.64	0.24
		L	2.40	0.58	0.22
		H	2.97	0.38	0.15
	UNAFFECTED	VL	1.61	0.54	0.20
		L	2.59	0.91	0.34
		H	3.15	0.90	0.34

A recovery in cerebral autoregulation would be indicated by a decrease in the mean gain (towards 0) over visits, for a given frequency range.

Repeat measure ANOVA showed that there was no significant dynamic interaction ('sphericity-assumed') between hemispheres, visits, and frequency ranges ($P = 0.33$).

However, significant overall interaction between the mean gains of the three frequency ranges was shown when the values of the three visits and both hemispheres

were combined ($P < 0.0001$). When the mean gains of the three visits and all frequency ranges were combined, no significant overall difference between the two hemispheres was shown ($P = 0.25$). In addition, when the mean gains of both hemispheres and all frequency ranges were combined, no significant overall interaction between the visits was shown ($P = 0.66$).

Furthermore, using regression analysis, we showed a significant linear trend of increasing gain from very low, to low, to high frequency ranges ($P = 0.002$) [see Figures 10A and 10B]. Two-tailed *t*-test that was performed on the mean gains of very low and high frequency ranges showed that the mean difference was 1.71 (95% CI = 0.61 to 2.80, $P = 0.007$). The result was also significant when a linear trend for visits was combined with a linear trend for frequency ranges ($P = 0.02$) [see Figures 10C and 10D].

The following table shows the results of the mean gains for the controls at different frequency ranges. A two-tailed heteroscedactic *t*-test was performed between the mean gains of affected hemispheres (visit 1) and controls (where the greatest difference was expected) for each frequency range. A *t*-test was also performed between the mean gains in the very low and high frequency ranges but no significant difference was found ($P = 0.32$).

CONTROL	MEAN	SD	SEM	*t*-TEST (P)
VL	2.53	1.53	0.48	0.28
L	2.62	1.99	0.63	0.47
H	3.66	3.05	0.97	0.93

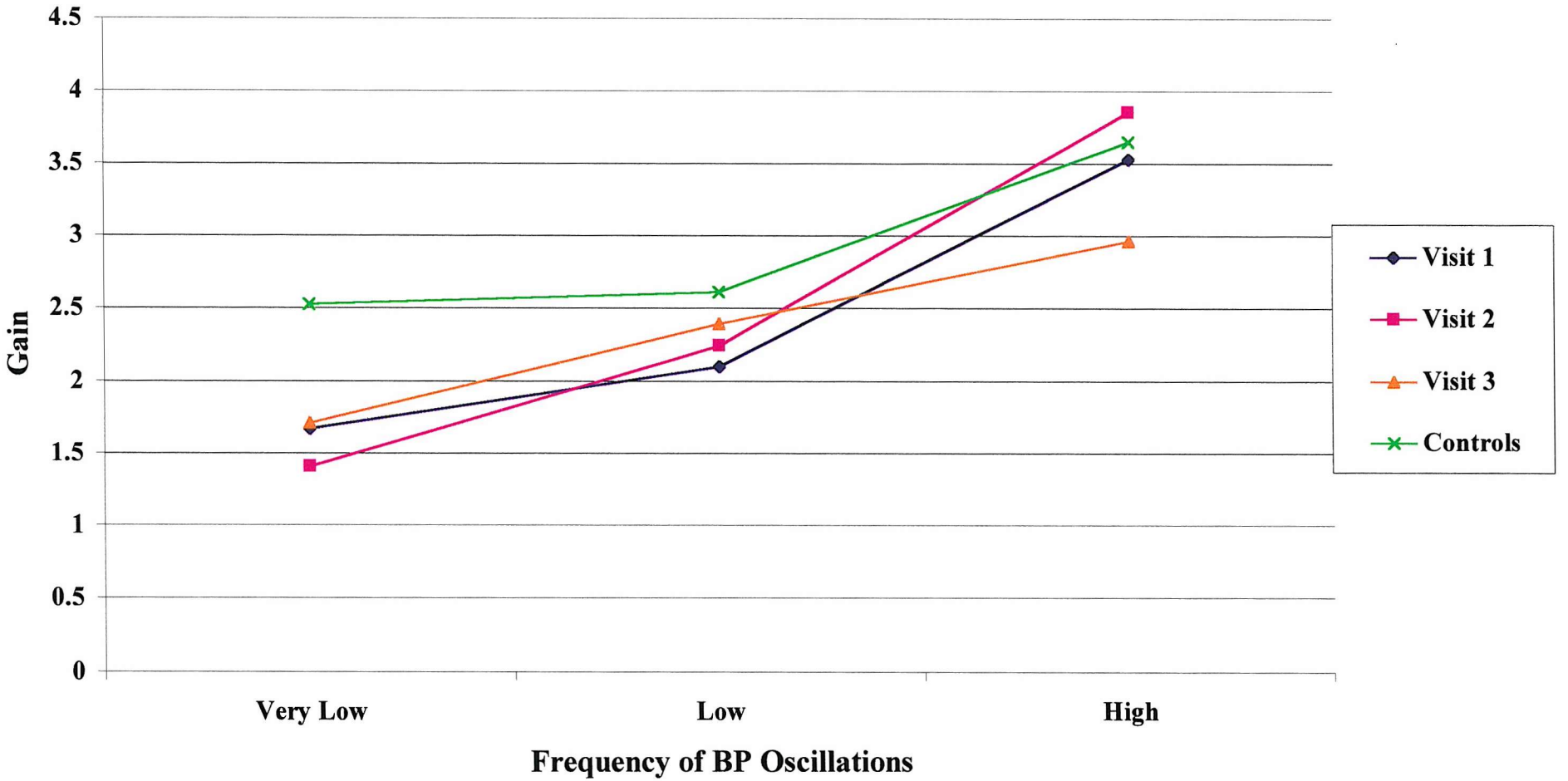


Figure 10A. Steady-state gain in the *affected* hemisphere for visits 1 to 3 and for controls. It shows a consistent trend of increasing gain with increasing frequency of BP oscillations.

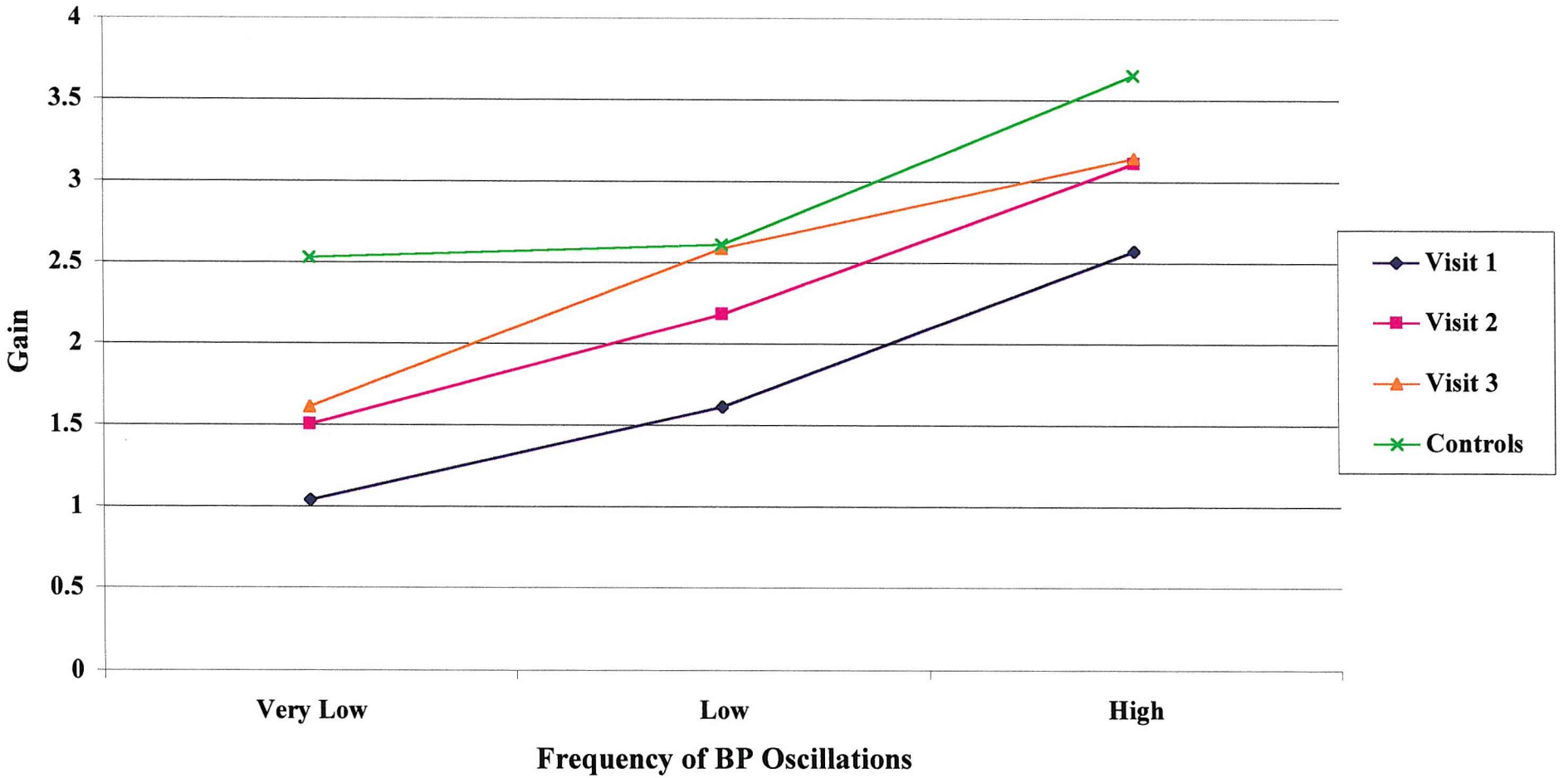


Figure 10B. Steady-state gain in the *unaffected* hemisphere for visits 1 to 3 and for controls. It shows a consistent trend of increasing gain with increasing frequency of BP oscillations.

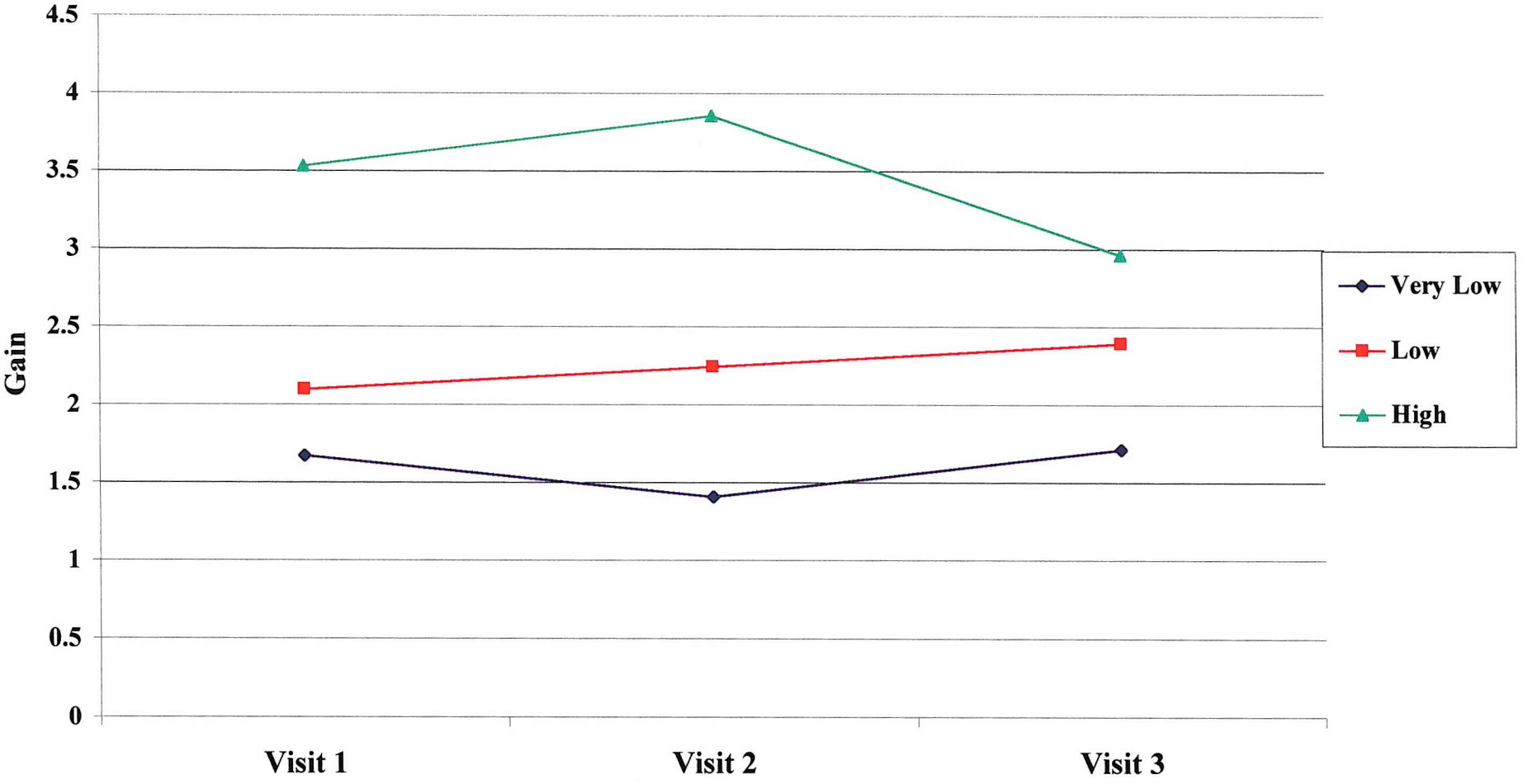


Figure 10C. Steady-state gain in the *affected* hemisphere for different visits & frequency ranges

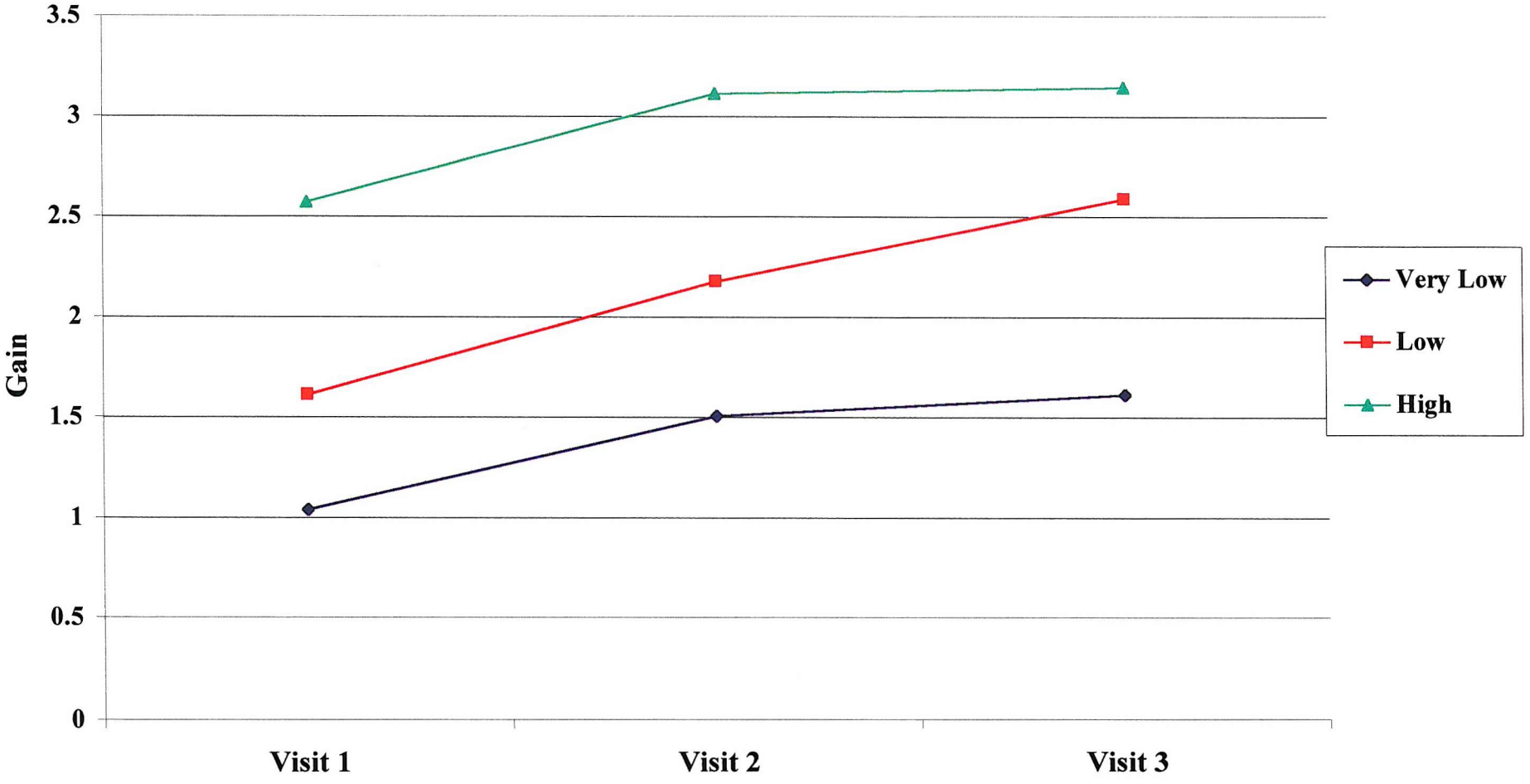


Figure 10D. Steady-state gain in the *unaffected* hemisphere for different visits & frequency ranges.

4.3.3 Coherence Index

The following table shows the results for subjects, with the mean coherence index, SD, and SEM, for each hemisphere, visit, and frequency range. The numbers refer to coherence index that has no specific units.

VISIT	HEMISPHERE	FREQ.	MEAN	SD	SEM
1	AFFECTED	VL	0.42	0.16	0.06
		L	0.61	0.19	0.07
		H	0.69	0.16	0.06
	UNAFFECTED	VL	0.52	0.16	0.06
		L	0.69	0.16	0.06
		H	0.73	0.12	0.05
2	AFFECTED	VL	0.49	0.18	0.07
		L	0.58	0.18	0.07
		H	0.67	0.22	0.08
	UNAFFECTED	VL	0.42	0.13	0.05
		L	0.66	0.11	0.04
		H	0.74	0.20	0.08
3	AFFECTED	VL	0.55	0.25	0.10
		L	0.66	0.21	0.08
		H	0.73	0.15	0.06
	UNAFFECTED	VL	0.60	0.20	0.08
		L	0.66	0.22	0.08
		H	0.72	0.18	0.07

Repeat measure ANOVA showed that there was no significant dynamic interaction ('sphericity-assumed') between hemispheres, visits, and frequency ranges ($P = 0.19$).

However, significant overall interaction between the mean coherence indices of the three frequency ranges was shown when the values of the three visits and both hemispheres were combined ($P = 0.003$). When the mean coherence indices of the three visits and all frequency ranges were combined, no significant overall difference

between the two hemispheres was shown (P = 0.38). In addition, when the mean coherence indices of both hemispheres and all frequency ranges were combined, no significant overall interaction between the visits was shown (P = 0.53).

Using regression analysis, we showed a significant linear trend of increasing coherence from very low, to low, to high frequency ranges (P = 0.003) [see Figures 11A and 11B], but no significant trend with visits [see Figures 11C and 11D]. Two-tailed *t*-test performed on the mean coherence indices of very low and high frequency ranges showed that the difference was 0.21 (95% CI = 0.06 to 0.36, P = 0.01).

The following table shows the results of the mean coherence indices for the controls at different frequency ranges. A two-tailed heteroscedactic *t*-test was performed between the mean coherence indices of affected hemispheres (visit 1) and controls for each frequency range. A *t*-test was also performed between the mean coherence indices in the very low and high frequency ranges. No significant difference was found (P = 0.06).

CONTROL	MEAN	SD	SEM	*t*-TEST (P)
VL	0.59	0.19	0.06	0.08
L	0.61	0.20	0.06	0.98
H	0.76	0.19	0.06	0.44

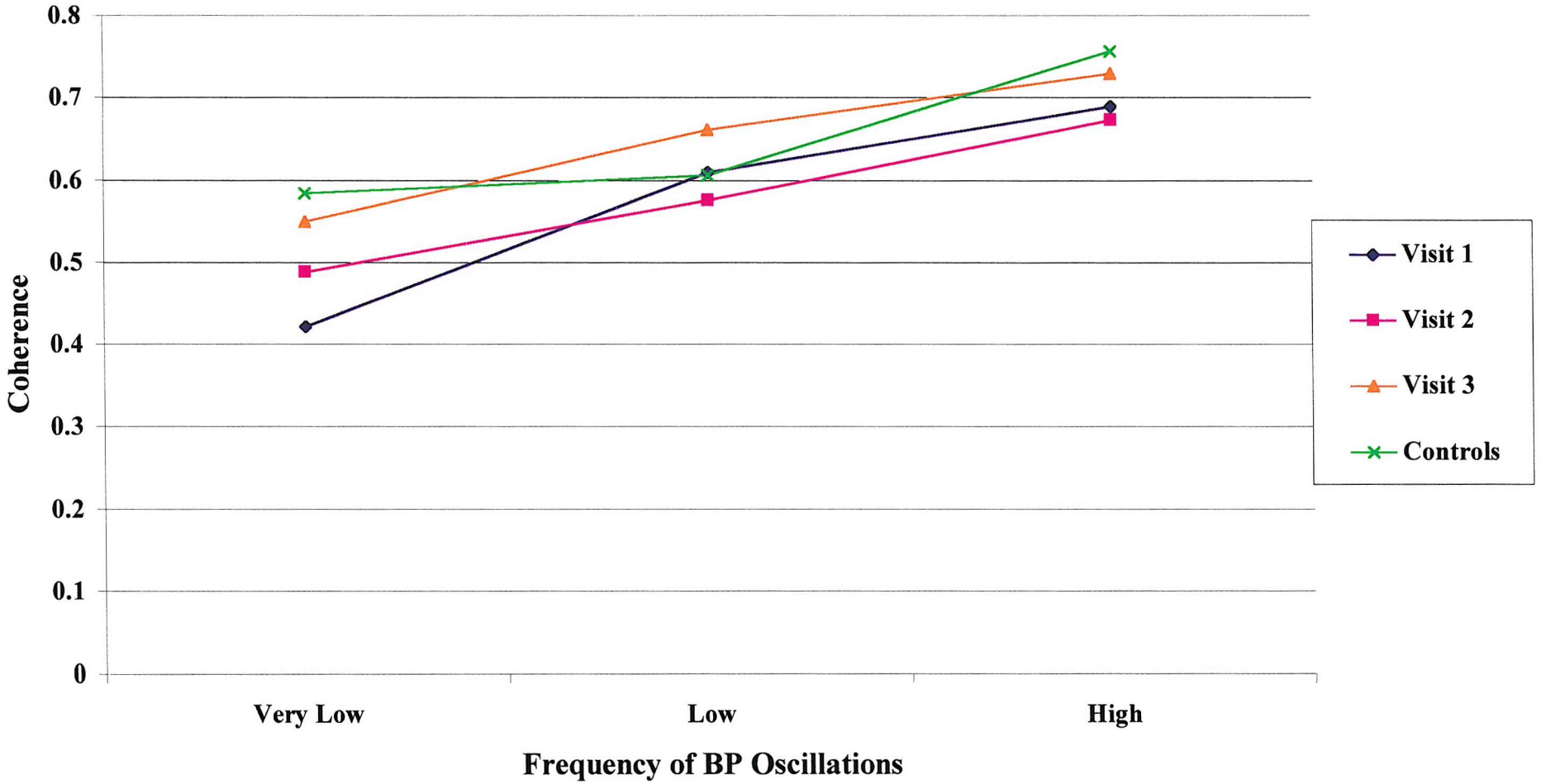


Figure 11A. Steady-state coherence in the *affected* hemisphere for visits 1 to 3 and for controls. It shows a consistent trend of increasing coherence with increasing frequency of BP oscillations.

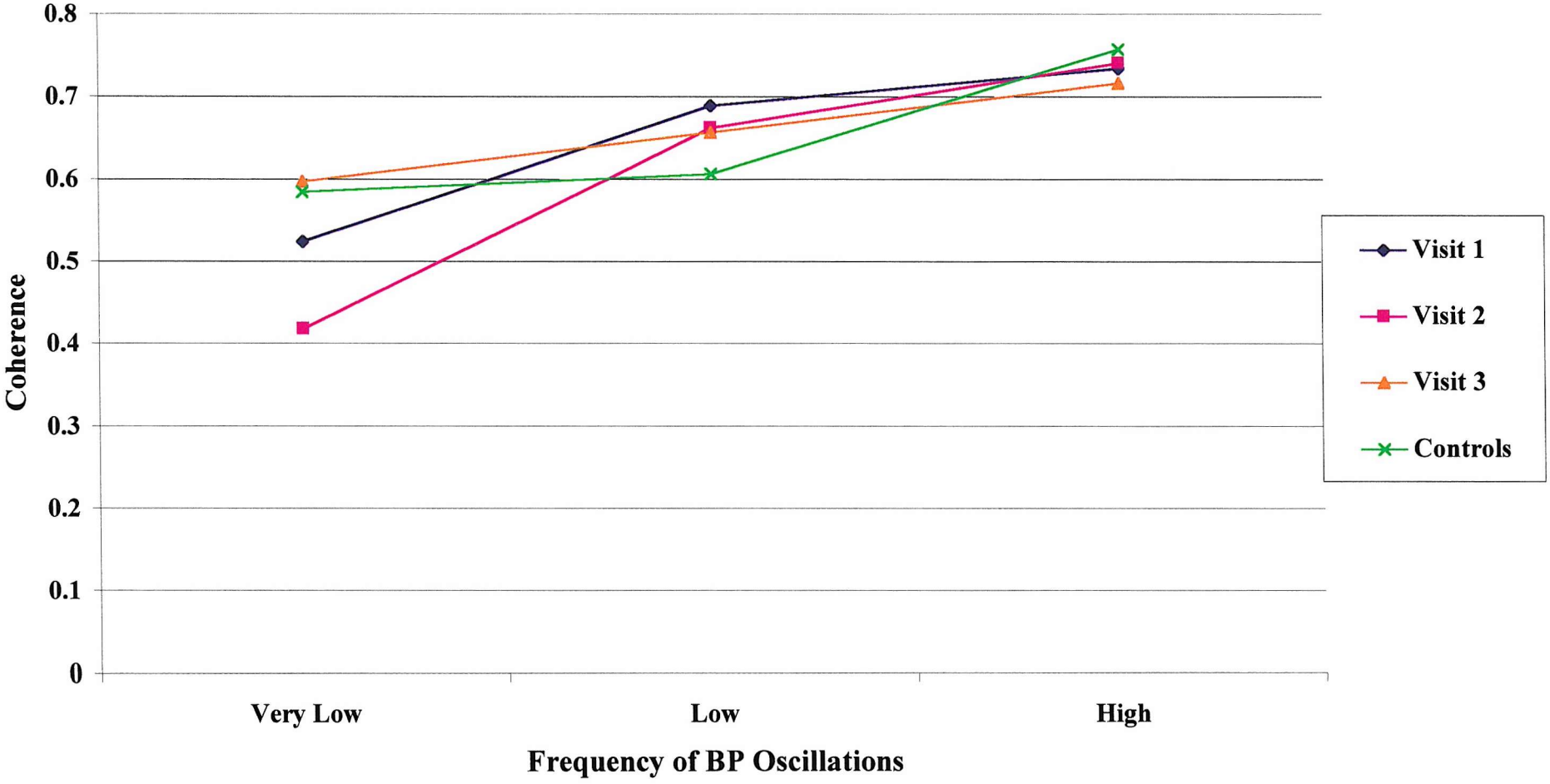


Figure 11B. Steady-state coherence in the *unaffected* hemisphere for visits 1 to 3 and for controls. It shows a consistent trend of increasing coherence with increasing frequency of BP oscillations.

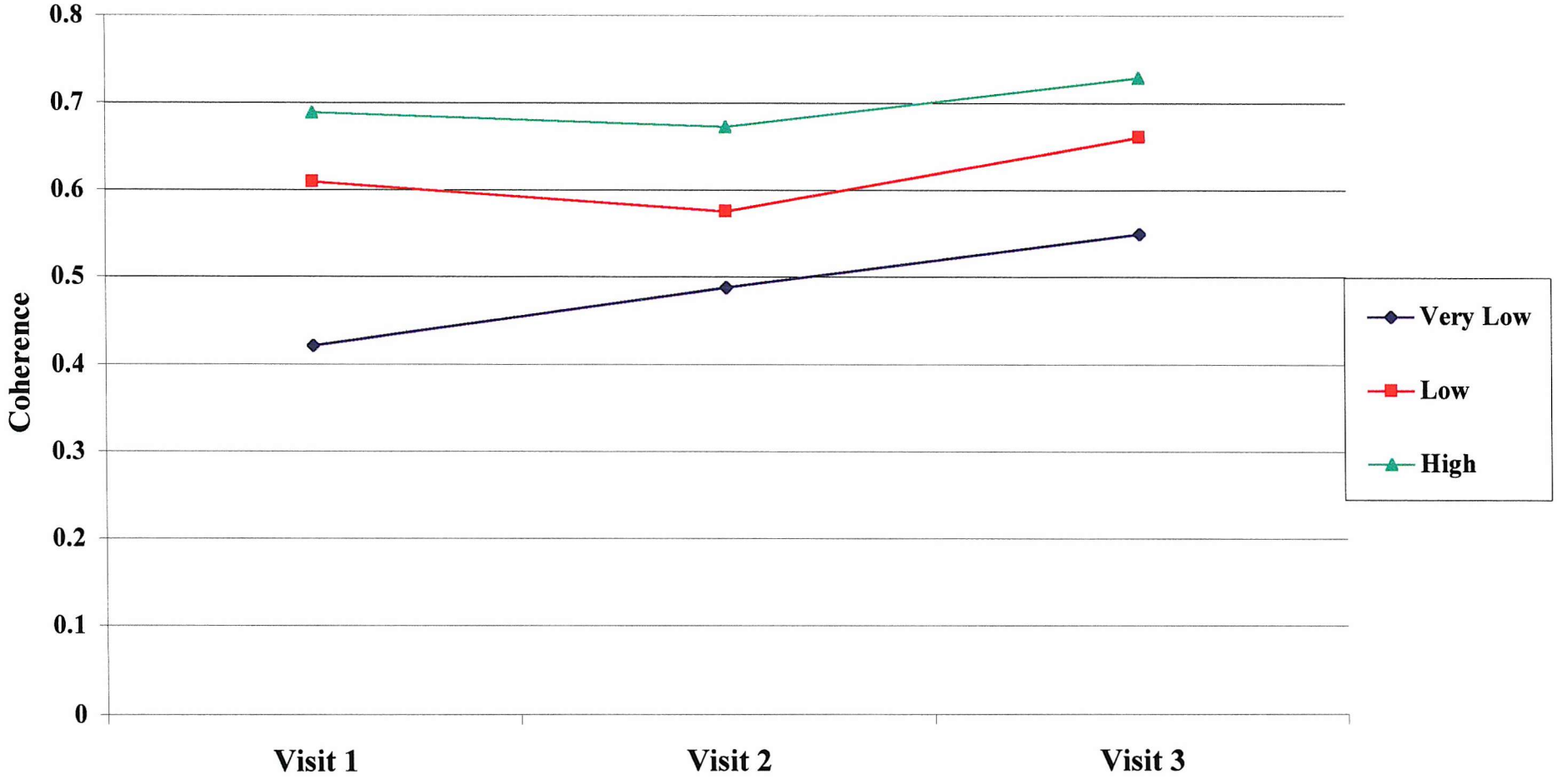


Figure 11C. Steady-state coherence in the *affected* hemisphere for different visits & frequency ranges

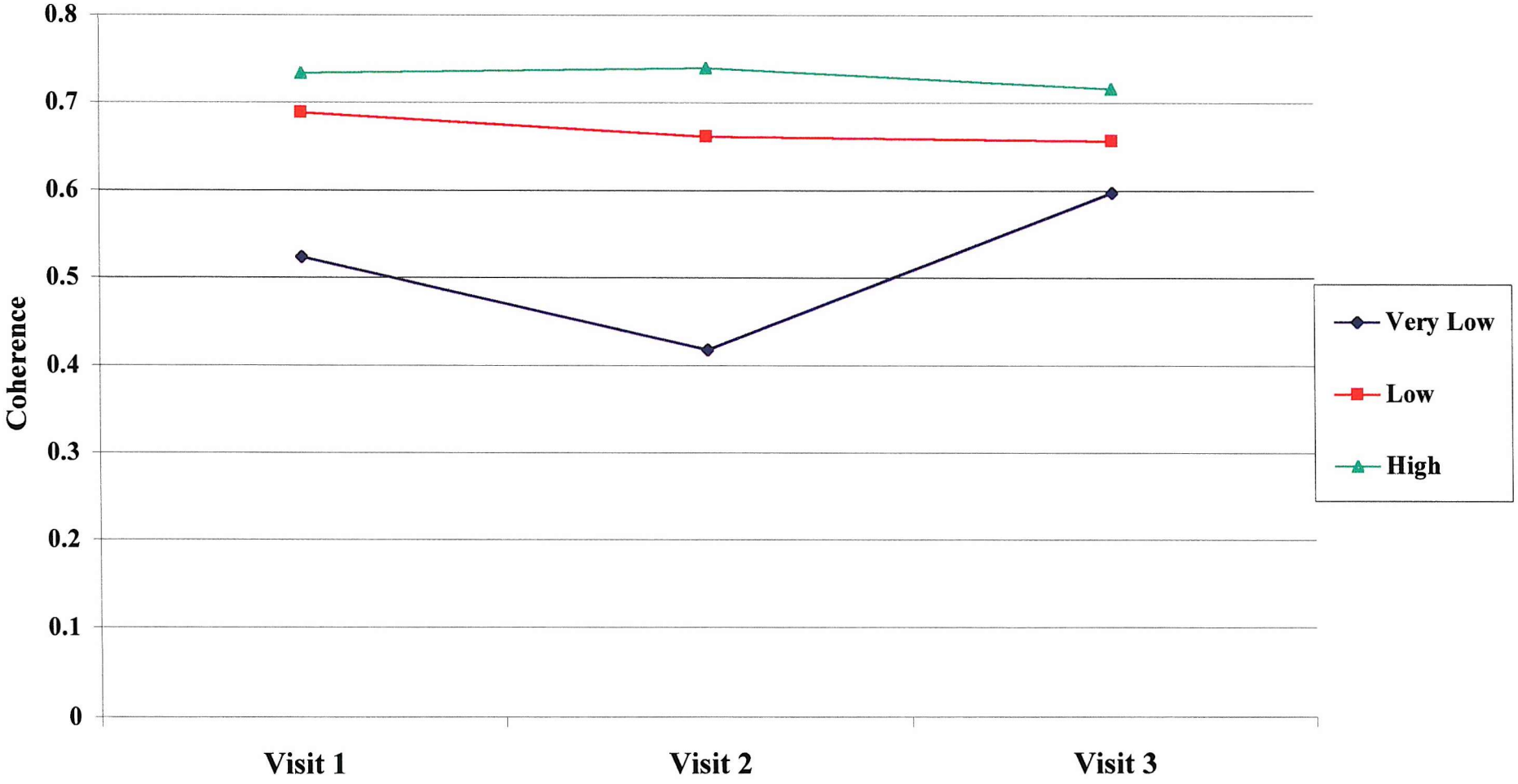


Figure 11D. Steady-state coherence in the *unaffected* hemisphere for different visits & frequency ranges

4.4 Rhythmic Handgrip Transfer Function Analysis

For this part of the analysis, phase angle shift and gain were calculated for each subject for the affected and unaffected hemispheres, and for each visit. See Figure 12.

Rhythmic handgrip produced an average change in blood pressure of 5% in the subjects. A full report of percentage change in blood pressure induced by rhythmic handgrip, as well as the 'power' of the blood pressure oscillations, that is the percentage of the average blood pressure waveform that was made up by oscillations at the fundamental frequency, can be found in Appendix 5, Chapter 7. The closer the power is to 100%, the closer the blood pressure waveform resembles a sine waveform at the frequency of the rhythmic handgrip (i.e. at 0.025 Hz). The average power of blood pressure oscillations was 82% in the subjects.

4.4.1 Phase Angle Shift

The following table shows the results for subjects, with the mean phase angle shift, SD, and SEM, for each hemisphere and visit. The numbers refer to phase angle shifts in degrees^0. See Figure 12.

VISIT	HEMISPHERE	MEAN	SD	SEM
1	AFFECTED	-26.50	55.30	22.58
	UNAFFECTED	+2.67	33.80	13.80
2	AFFECTED	-10.25	35.28	14.40
	UNAFFECTED	+14.17	37.65	15.37
3	AFFECTED	+33.83	35.81	14.62
	UNAFFECTED	+26.67	32.47	13.26

Again, repeat measure ANOVA was performed to examine for any dynamic interaction between hemispheres and visits. A recovery in cerebral autoregulation in the affected hemisphere would be implied if the difference in mean phase angle shift between the affected and unaffected hemispheres became smaller with subsequent visits.

The results showed that there was no significant interaction ('sphericity-assumed') between hemispheres and visits ($P = 0.42$). In other words, any difference

in the mean phase angle shift between the affected and unaffected hemispheres did not significantly change over visits.

The mean phase angle shifts of the three visits were then combined to examine for any significant overall difference between the affected and unaffected hemispheres. Two-tailed *t*-test showed that the overall mean phase angle shift in the affected hemisphere was not significantly different from the unaffected hemisphere (mean difference = -15.5^0, 95% confidence limits = -49.5^0 to $+18.5^0$, P = 0.30).

The mean phase angle shifts of the affected and unaffected hemispheres were then combined to examine for any significant overall interaction between visits. The result was not significant (P = 0.07). Figure 12 shows that there are similar trends of increasing phase angle shift with subsequent visits in both the affected and unaffected hemispheres.

Using regression analysis, we showed a significant linear trend of increasing phase angle shift with subsequent visits (P = 0.04) when the mean phase angle shifts of the affected and unaffected hemispheres were combined. Two-tailed *t*-test that was performed on the mean phase angle shifts of visit 1 and visit 3 showed that the mean difference was -42.2^0 (95% CI = -80.1^0 to -4.2^0, P = 0.04).

The mean phase angle shifts for controls were $+3.9^0$ (SD = 40.2^0, SEM = 15.2^0) in the right hemisphere and -2.9^0 (SD = 47.4^0, SEM = 17.9^0) in the left hemisphere. Two-tailed *t*-test was performed on the mean phase angle shifts between the affected hemispheres and controls (averaged over both hemispheres) at visit 1 (where the largest difference was expected). The result was non-significant (P = 0.54).

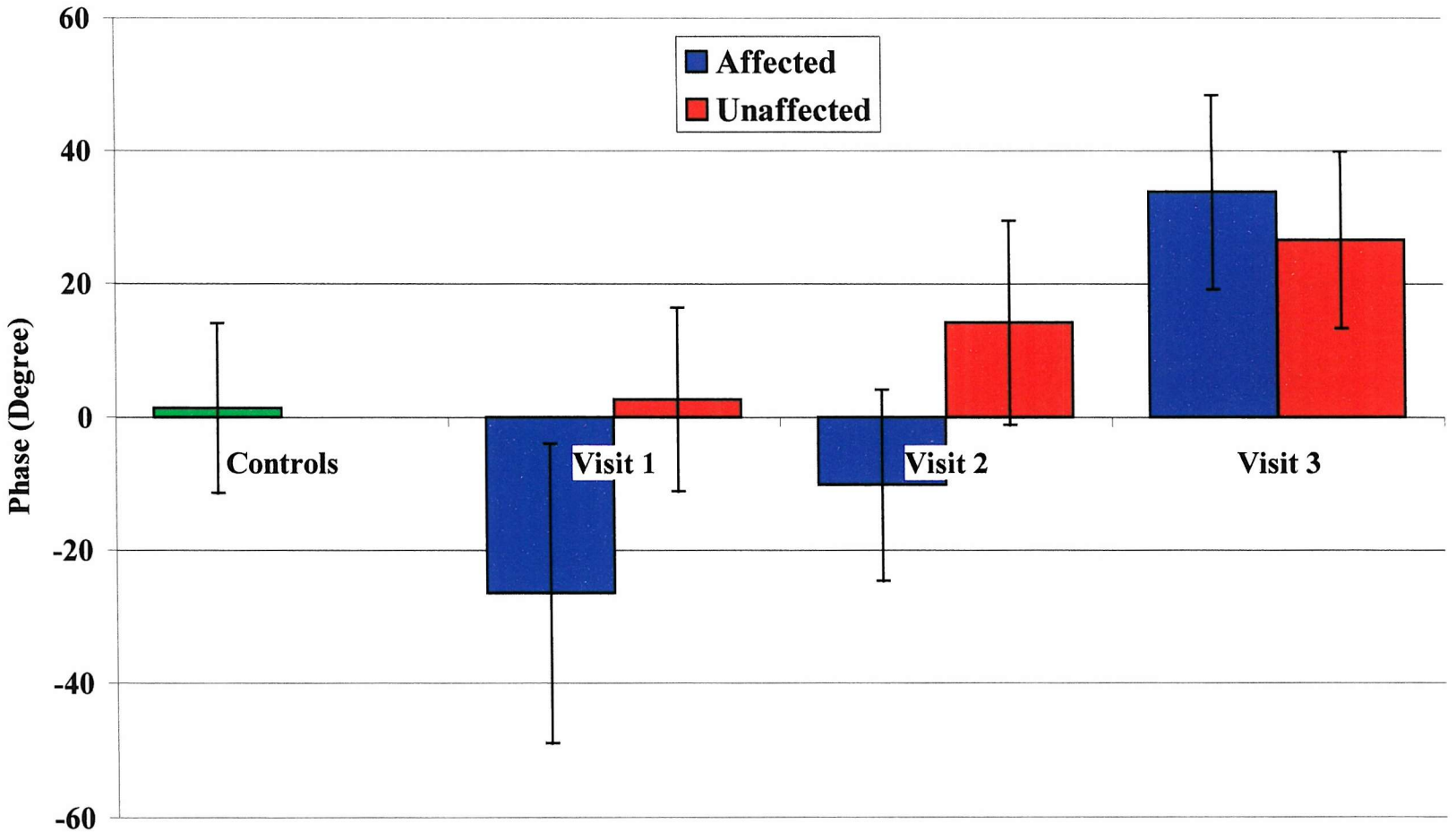


Figure 12. RHG transfer function analysis: phase for visits 1 to 3 and for controls (+ 1 SEM). It shows a linear trend of increasing phase with visits in both hemispheres (P = 0.04).

4.4.2 Gain

The following table shows the results for subjects, with the mean gain, SD, and SEM, for each hemisphere and visit. See Figure 13.

For the controls, rhythmic handgrip produced an average change in blood pressure of 6%, and the average power of the blood pressure oscillations was 78%. There was no significant difference in the average change in blood pressure, or in the power of the blood pressure oscillations, between subjects and controls (P = 0.74 and 0.69, respectively). A full report of percentage change in blood pressure induced by rhythmic handgrip and the power of the blood pressure oscillations can be found in Appendix 6, Chapter 7.

VISIT	HEMISPHERE	MEAN	SD	SEM
1	AFFECTED	1.43	1.20	0.49
	UNAFFECTED	0.73	0.42	0.17
2	AFFECTED	0.94	0.51	0.21
	UNAFFECTED	0.59	0.32	0.13
3	AFFECTED	0.81	0.62	0.26
	UNAFFECTED	0.58	0.21	0.08

A recovery in cerebral autoregulation in the affected hemisphere would be implied if the difference in mean gain between the affected and unaffected hemispheres became smaller with subsequent visits.

Repeat measure ANOVA showed that there was no significant interaction ('sphericity-assumed') between hemispheres and visits (P = 0.47). In other words, any difference in the mean gain between the affected and unaffected hemispheres did not significantly change over visits.

The mean gains of the three visits were then combined to examine for any significant overall difference between the affected and unaffected hemispheres. Two-tailed *t*-test showed that the overall mean gain in the affected hemisphere was not significantly different from that in the unaffected hemisphere (mean difference = 0.43, 95% CI = -0.02 to +0.88, P = 0.06).

The mean gains of the affected and unaffected hemispheres were then combined to examine for any significant overall interaction between visits. The result

was not significant (P = 0.35). Figure 13 shows that there are similar trends of decreasing gain with subsequent visits in both the affected and unaffected hemispheres.

Linear regression analysis demonstrated a non-significant linear trend of decreasing gain with subsequent visits (P = 0.24) when the mean gains of the affected and unaffected hemispheres were combined. Two-tailed *t*-test on the mean gains between visit 1 and visit 3 showed the mean difference was 0.39 (95% CI = -0.35 to +1.13, P = 0.24).

The mean gains for controls were 0.84 (SD = 0.38, SEM = 0.14) in the right hemisphere and 1.00 (SD = 0.38, SEM = 0.14) in the left. Two-tailed *t*-test was performed on the mean gains between the affected hemisphere and controls (averaged over both hemispheres) at visit 1. No significant difference was demonstrated (P = 0.63).

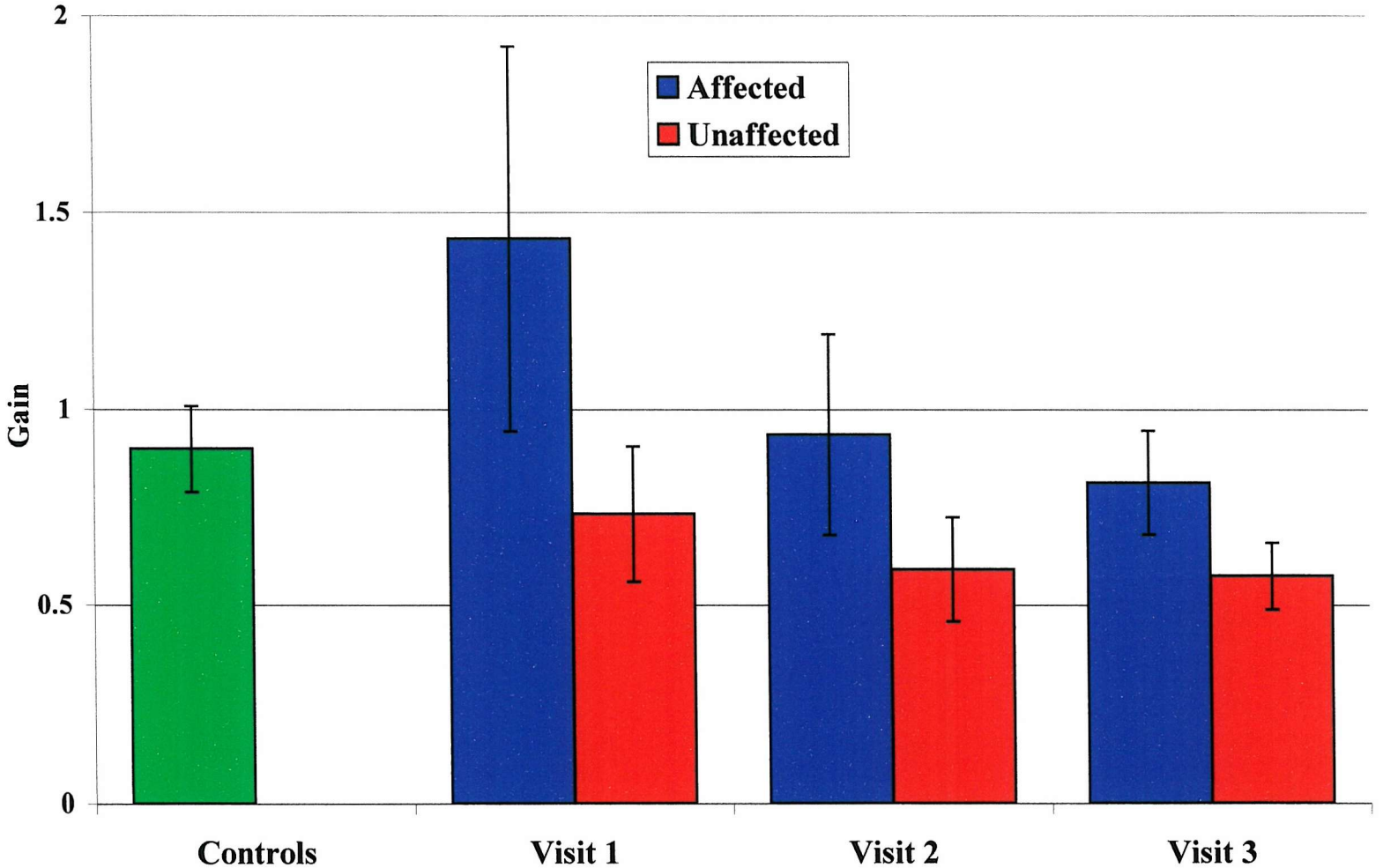


Figure 13. RHG transfer function analysis: gain for visits 1 to 3 and for controls (+ 1 SEM). It shows a linear trend of decreasing gain with visits in both hemispheres (P = 0.24).

4.5 Summary

In this study, the following important findings were observed from the steady-state transfer function analysis in both the subjects and controls:

1. The frequency range of spontaneous BP oscillations significantly influenced the phase angle shift. Phase angle shift *increased* from very low to low frequency and then *decreased* from low to high frequency. In subjects, this trend was also significant when combined with a linear trend of increasing phase angle shift with subsequent visits.
2. The frequency range of spontaneous BP oscillations significantly influenced the gain, such that gain increased with increasing frequency. In subjects, this trend was also significant when combined with a linear trend of increasing gain with subsequent visits.
3. The frequency range of spontaneous BP oscillations significantly influenced the coherence, such that coherence increased with increasing frequency.

The following important findings were observed from the rhythmic handgrip transfer function analysis in the subjects:

1. There was a significant linear trend of increasing phase angle shift with subsequent visits. This trend was present in both the affected and unaffected hemispheres.
2. There was a non-significant linear trend of decreasing gain with subsequent visits. Again, this trend was present in both the affected and unaffected hemisphere.
3. There was no significant difference in phase angle shifts and gains between the affected and unaffected hemispheres.

CHAPTER 5 DISCUSSION

5.1 Interpretation of Results

5.1.1 Recovery of Cerebral Autoregulation

Using rhythmic handgrip as a technique in the assessment of cerebral autoregulation, this study demonstrates a significant trend of increasing phase angle shift (P = 0.04), and a non-significant trend of decreasing gain, with subsequent visits up to three months after acute ischaemic stroke. This finding suggests a steady change in cerebral autoregulation throughout the first three months after stroke. Both the observed trends were linear in nature, suggesting that cerebral autoregulation may begin to recover within seven days after acute ischaemic stroke, and that this recovery may not yet reach a plateau at three months but may continue to occur beyond this time.

There was also a similar but non-significant trend in the unaffected hemisphere alone (P = 0.24) and we showed no significant difference between the affected and unaffected hemispheres (P = 0.06). These observations suggest that impaired cerebral autoregulation after acute ischaemic stroke may also involve the unaffected hemisphere. Hence, cerebral haemodynamic disturbances after stroke may not only be a local phenomenon in and around the area of ischaemia, but may be global in nature. Other investigators such as Høedt-Ramussen and Skinhøj (1964) have observed a similar phenomenon in man [26]. Moreover, no significant difference between the results for subjects and controls was found, possibly as a result of low statistical power.

Interpretation of the results from the steady-state transfer function analysis is more difficult. We found no significant trend of either increasing phase angle shift or decreasing gain with visits. However, for phase angle shift and gain, the results were significant when the trends of frequency ranges were combined with the trends of visits. The implications of these results are not clear, but it suggests that, after ischaemic stroke, the changes in phase angle shift and gain (hence cerebral autoregulation) may be under the influence of complex interactions between frequency ranges and visits. Furthermore, the trends that were observed were not all linear in nature. For phase angle shift, the results were significant when a linear trend for visits was combined with a quadratic trend for frequency ranges (P = 0.01), whereas for gain, the results were significant when a linear trend for visits was

combined with a linear trend for frequency ranges (P = 0.02). It is possible that phase angle shift and gain reflect different aspects of cerebral autoregulation. In other words, phase angle shift and gain may behave in different ways to reflect changes in cerebral autoregulation at different time intervals after stroke, when frequency ranges are also considered.

From the steady-state transfer function analysis, the non-significant trend of gain appears to indicate an *increasing* gain with subsequent visits, especially in the unaffected hemisphere (see Figure 10C and 10D). If this observation is interpreted alone, it suggests *worsening* of cerebral autoregulation during the first three months after stroke. This is unlikely to be the case in light of the contrasting results that were obtained from the rhythmic handgrip transfer function analysis. This finding may be a consequence of inadequate statistical power, for example, due to the small number of subjects who were recruited in this study and recordings that were included for data analysis.

The results of coherence can be interpreted in several ways. In this study, coherence is regarded as a test of linearity providing a measure of the consistency between the frequency points within a frequency band. As already mentioned in Section 3.4.1, coherence has also been interpreted by some investigators, such as Giller (1990), as a direct indication of cerebral autoregulation [217]. Zhang *et al.* (1998) stated that "a low coherence (< 0.5) may be produced by a number of processes: 1) extraneous noise is present in the measurements; 2) the system relating output to input (i.e. cerebral autoregulation) is non-linear; 3) the output is due to more than one input; 4) there is no relationship between input and output" [204]. In the present study, we found a significant trend of increasing coherence with increasing frequency of blood pressure oscillations (P = 0.003). Our results show that all the coherence indices in the low and high frequency ranges were greater than 0.6, and the majority in the very low frequency range were greater than 0.5. This observation suggests that the frequency points within a frequency band are consistent, and the consistency increases with frequency. However, no significant association was found in relation to visits or hemisphere, or between subjects and controls. The high coherence indices that were observed suggest that the variations in blood pressure and Vmca were similar to a high degree. This may indicate that blood pressure variation produces the Vmca variation, or that both are affected by the same unmeasured input. However, no interpretation of cause and effect can be made from these results since

cross-spectral transfer function analysis assumes an open loop between input and output, where in many cases there are both feed-forward and feedback mechanisms involved [203]. Furthermore, according to the aforementioned interpretations by Zhang *et al.*, the high coherence in our results may also indicate: 1) absence of extraneous noise in the measurements; 2) the system relating output to input is linear; 3) the output is due mainly to the input. Lastly, according to the interpretation by Giller *et al.* (1990), since perfect autoregulation can be indicated by a coherence index of around zero, we speculate that the relatively high coherence indices that were obtained in the control group may indicate that cerebral autoregulation in some controls was impaired. However, it may also simply have been the result of inadequate statistical power, for example, due to the small number of recordings that were included in the data analysis.

As already mentioned in Section 1.4.2, only two investigators, Mori *et al.* (1993) and Agnoli *et al.* (1968), have ever attempted to examine the impairment of cerebral autoregulation at different time intervals after stroke, with very different results and conclusions [65, 83]. Moreover, these studies had significant methodological differences, which make the process of precise comparison of results difficult. Other investigators such as Giller *et al.* (1997) have observed signs of abnormal cerebral autoregulation in patients following subarachnoid haemorrhage [129]. Diehl *et al.* (1991) examined spontaneous oscillations of CBFV in patients with severe internal carotid artery stenosis and found significant asymmetry between the patient and control groups [202]. Hu *et al.* (1999) found that, using transfer function analysis at different frequency ranges, patients with high-grade carotid stenosis had significantly lower phase angle shift in the low frequency range. They concluded that transfer function analysis of spontaneous fluctuations of CBFV and blood pressure could be used to identify patients with impaired cerebral autoregulation [218]. To our knowledge, our study is the first to be performed in a longitudinal fashion, examining the same subjects at various pre-defined time intervals after acute ischaemic stroke. Strict but universal selection criteria were employed for the subjects as well as controls, in an attempt to minimize diversity and hence statistical inaccuracy. Our study is also the first to demonstrate a steady change in phase angle shift and gain over the first three months after acute ischaemic stroke, as well as the first to use rhythmic handgrip as a technique of stimulating blood pressure oscillations in the assessment of cerebral autoregulation.

In both parts of the data analysis, comparison between the results of subjects and controls failed to yield any significant results. Furthermore, there were no significant differences between the results of the various frequency ranges in the control group. Similar to the explanation for the high coherence indices that were found in the controls, these findings may also indicate that cerebral autoregulation in some controls may have been impaired, for example, due to unrecognized and asymptomatic neurological diseases. They may also reflect a lack of statistical power and the heterogeneity within the group, as well as within the general population, in relation to cerebral autoregulation and haemodynamics. In retrospect, it would have been more appropriate to recruit normal healthy volunteers as controls since impaired cerebral autoregulation would have been less likely.

5.1.2 High-Pass Filter Model of Cerebral Autoregulation

Precise comparison of our results with those of other studies is difficult because none of the methods involving TCD ultrasonography represents a 'gold standard' for comparison. However, the work of Zhang *et al.* (1998) and Kuo *et al.* (1997) can be used for the closest comparison to our study because of the similar methodology and findings [204, 130].

With the results from the steady-state transfer function analysis, we demonstrated significant associations between the frequency of blood pressure oscillations and phase angle shift ($P = 0.002$), gain ($P < 0.0001$), and coherence ($P = 0.003$). Gain and coherence increased with increasing frequency from very low to high frequency range. This was in association with an initial increase in phase angle shift from very low to low frequency range, followed by a significant drop from low to high frequency range. These findings suggest that dynamic cerebral autoregulation is dependent on the frequency of blood pressure oscillations and that autoregulation is less effective at the higher frequency ranges. Our results, therefore, confirm that the behaviour of cerebral autoregulation is consistent with a high-pass filter model, and that this model remains applicable after acute ischaemic stroke and throughout the first three months of recovery. Moreover, the trends that exist in the controls closely follow those in both the affected and unaffected hemispheres in the subjects [see Figures 9 to 11]. This observation suggests that the high-pass filter model applies to all the observed groups, and the frequency-dependence behaviour of dynamic cerebral autoregulation may be a basic phenomenon that is not affected by ischaemic stroke.

Spontaneous fluctuations in blood pressure had long been noted by cerebral and cardiovascular physiologists but had been difficult to study until the recent introduction of cross-spectral transfer function analysis [155]. It is now generally accepted that blood pressure fluctuations can be classified in various frequency components as described in Section 1.5.3. These different frequency fluctuations are also believed to be associated with different underlying mechanisms. The high frequency component originates from fluctuations in cardiac output induced by respiratory movement, and the low frequency component from fluctuations in sympathetic vasomotor control by the central nervous system [130]. The mechanism underlying the very low frequency component is less clear, but it was thought to be generated by thermoregulation or hormonal system [155].

Most of the previous studies that have examined spontaneous fluctuations in blood pressure and CBFV have mainly dealt with fluctuations within the frequency range of 0.5 to 2 cycles/min (0.008 to0.033 Hz). This is probably because of the possible relationship with B-waves of the intracranial pressure, as described in Section 1.5.3 [133]. Diehl *et al.* (1995) and Newell *et al.* (1992) are two of the few studied that have examined fluctuations at higher frequency ranges, with even fewer discussing the possible underlying mechanisms [144, 131].

In the present study, we used the same frequency ranges as defined in the study by Kuo *et al.* (1998): 0.016 to 0.04 Hz as very low frequency (VLF), 0.04 to 0.15 Hz as low frequency (LF), and 0.15 to 0.4 Hz as high frequency (HF) [130]. We demonstrated that, in all groups, there were significant differences in phase angle shift, gain, and coherence between the three frequency ranges. Our results show that the VLF component was characterized by an intermediate phase angle shift, low gain and coherence, which indicate poor relation between blood pressure and Vmca oscillations, a characteristic that suggests an active cerebral autoregulation. This finding is also consistent with other previous findings by Diehl *et al.* (1995) and Newell *et al.* (1992) [144, 131]. The LF component was characterized by a higher phase angle shift, higher gain and coherence. These findings suggest that, in contrast to the VLF component, there was greater similarity between the LF components of Vmca and blood pressure. Surprisingly, the phase within the LF range was found to be higher than that in the VLF range, indicating that the Vmca response to LF fluctuations of blood pressure may not be passive. This finding is similar to that observed by Kuo *et al.* (1998) who proposed that in the LF range, fluctuations of

Vmca are partially passive to fluctuations of blood pressure (high gain and coherence) with the *additional* involvement of cerebral autoregulation (higher phase angle shift) [130]. The HF component was characterized by very low phase angle shift, and very high gain and coherence. These findings suggest that, in contrast to VLF and LF, there was considerable similarity between the HF components of Vmca and blood pressure. Also in contrast to the VLF and LF components, the phase angle shift was nearest to zero degree, indicating that the Vmca response to HF fluctuations of blood pressure was almost synchronized, i.e. blood pressure-passive. Such synchronization in the phase, together with the highest gain and coherence, implies an absence of cerebral autoregulation in the HF range, a finding consistent with that observed by Kuo *et al.* (1998) and Zhang *et al.* (1998) [130, 204].

Zhang *et al.* (1998) also found that in the high frequency range (> 0.2 Hz), blood pressure and CBFV varied in parallel (high coherence) with less of a damping effect on changes in blood pressure (high gain) and small phase lead, suggesting that cerebral autoregulation is ineffective in this frequency range. From these results, the authors speculated that the mechanism mediating cerebral autoregulation is likely to be determined by the frequency of blood pressure oscillations, such that myogenic processes is predominant in the high frequency range, and the metabolic processes in the lower frequency range [204].

Our results also show that as time passed from the initial stroke, and with a decreasing frequency, the phase angle shift increased positively. In this study, a positive is defined as output leading input, i.e. Vmca oscillations leading blood pressure oscillations. This positive phase lead of Vmca, which has also been repeatedly shown by other investigators such as Diehl *et al.* (1995) and Birch *et al.* (1994), is a characteristic of a high-pass filter model [144, 145]. In the present study, the phase was negative in the affected hemisphere for visits 1 and 2, indicating that blood pressure oscillations were leading Vmca oscillations. This observation suggests that, after stroke, impairment of cerebral autoregulation may be associated with a disturbance of the polarity of phase (becomes negative). Recovery of cerebral autoregulation may also be associated with restoration of a positive phase. In contrast, the phase in the unaffected hemisphere remained positive throughout the three months. This may suggest that, despite a trend of improving cerebral autoregulation after stroke, the initial impairment of cerebral autoregulation may not be severe enough to disturb the polarity of phase angle. These findings remain to be confirmed.

5.1.3 Usefulness of Rhythmic Handgrip

Over the last few years, investigators have striven to develop the 'ideal' non-invasive bedside technique for the assessment of dynamic cerebral autoregulation. Older techniques of inducing sudden blood pressure changes, such as by repeated inflation and deflation of thigh cuffs, can produce much pain and discomfort that many frail patients find unacceptable. To our knowledge, no other investigator has used rhythmic handgrip as a technique of inducing periodic blood pressure oscillations in the assessment of cerebral autoregulation after stroke. Furthermore, the combination of TCD ultrasonography and rhythmic handgrip has not been tried. Birch *et al.* (1995) used periodic standing and squatting to stimulate blood pressure oscillations (20 seconds period) and found similar forced oscillations of Vmca, the phase of which led that of blood pressure. They also found that the phase decreased as arterial Pco_2 increased, indicating a worse cerebral autoregulation [145]. Our results involving rhythmic handgrip show trends of increasing phase angle shift ($P = 0.04$) and decreasing gain ($P = 0.24$) with subsequent visits, affecting both hemispheres. These trends, however, were not demonstrated in the steady-state transfer function analysis when visits were considered alone.

It is difficult to compare these two techniques (spontaneous blood pressure fluctuation vs. induced blood pressure oscillations by rhythmic handgrip) and validate them against other techniques because the use of rhythmic handgrip is a new approach to the quantitative assessment of dynamic cerebral autoregulation. Only a few investigators, such as Kuo *et al.* (1998) and Diehl *et al.* (1998), have examined dynamic cerebral autoregulation using spontaneous oscillations of blood pressure, and no such study has been conducted in stroke patients during the acute phase of recovery [130, 144]. Dawson *et al.* (1999) used isometric handgrip and the 'thigh cuff' technique to assess cerebral autoregulation in patients with acute ischaemic stroke and found that dynamic, not static, cerebral autoregulation was impaired after acute ischaemic stroke [158].

In the present study, the combination of TCD ultrasonography and rhythmic handgrip appear to be non-invasive, easy to perform at bedside, acceptable to the patient, cheap, and safe. Informal discussions with the subjects and controls have revealed that the commands for rhythmic handgrip were easy to follow, and the handgrips were not problematic or uncomfortable whilst simultaneously trying to

keep still for the continuous TCD monitoring. Moreover, the technique yielded useful and informative results consistent with our hypotheses.

In this study, we found that rhythmic handgrip produced an average change in blood pressure of 5 to 6%. This seems to be a small change, and yet the power of the blood pressure oscillations was about 80%, which means that the induced blood pressure oscillations was mostly of a frequency identical to that of the rhythmic handgrip, i.e. 0.025Hz (or one cycle every 40 seconds). Therefore, the blood pressure oscillations induced by rhythmic handgrip were in the frequency that we desired. We also found no significant difference in the average change in blood pressure or the power of blood pressure oscillations between subjects and controls, which suggests that the cardiovascular (blood pressure) response to rhythmic handgrip may not be influenced by the ischaemic stroke. This finding, however, might also be due to the fact that the controls were selected from a cohort of hospitalised general medical patients rather than healthy volunteers. These interesting hypotheses remain to be explored by future studies.

In retrospect, the reproducibility of this technique could have been examined by repeating the tests in controls. Other investigators have found that many methods of assessing cerebral autoregulation are very poorly reproducible [Professor J. Potter, personal communication]. Further studies using this technique will take this important aspect into account.

From our results, we can conclude that the combined technique of TCD ultrasonography and rhythmic handgrip may be useful in the assessment of the recovery of cerebral autoregulation after stroke. This non-invasive technique deserves further evaluation and refinement.

5.2 Methodological Considerations

Many of the issues concerning the methodology have already been discussed in Section 2.2. This section explores the important aspects that have not yet been covered.

5.2.1 Cerebral Blood Flow Velocity vs. Cerebral Blood Flow

Although TCD ultrasonography permits the temporal resolution necessary to effectively study cerebral haemodynamics after stroke, the use of TCD ultrasonography for cerebral blood flow (CBF) measurements needs to be addressed. Cerebral blood flow velocity (CBFV) that is measured by TCD ultrasonography is not equal to CBF [204]. Since the middle cerebral artery (MCA) carries approximately 80% of the flow volume received by the cerebral hemisphere, MCA flow is generally regarded as a good representative of CBF [130]. Blood flow (F) in a particular artery depends on the artery's cross-sectional area (πr^2) and the mean velocity (V) of the blood flowing through it, such that $F = \pi r^2 \times V$. Hence, a decrease in diameter will result in an increase in the velocity measured, and vice versa, assuming that the actual flow remains unchanged (Poiseuille's law). Changes in CBF are therefore proportional to changes in mean CBFV only if the diameter of the MCA remains constant throughout the autoregulation test [130]. In other words, if the changes in the diameter of the MCA are negligible, then relative changes in Vmca should represent relative changes in CBF. However, small changes in diameter, which are difficult to measure, may produce large changes in CBFV.

There have been many studies that have compared CBF and CBFV measurements, for example, during cerebral vasoreactivity studies. Other investigators have also tried to make direct measurements of the diameters of cerebral arteries during alterations of blood pressure whilst simultaneously monitoring the CBFV using TCD ultrasonography. Changes in MCA diameter have been assessed by simultaneous comparison of CBFV measurements of cerebral arterial inflow and venous return and also by direct correlation with electromagnetic flowmetry [159, 127]. Some of these studies are described here.

Bishop *et al.* (1986) found that, in patients with "cerebrovascular disease", changes in Vmca, as measured by TCD ultrasonography, reliably correlated to

changes in CBF, as measured by intravenous ^{133}Xe studies (r = 0.85). However, there was poor correlation between the absolute Vmca measurements and hemispheric CBF (r = 0.42) [209]. Newell *et al.* (1994) observed that relative changes in Vmca accurately reflected relative changes in the internal carotid artery blood flow during dynamic autoregulation testing in humans using the 'thigh cuff' technique. The authors concluded, "Therefore, alterations in MCA diameter do not occur to the extent that they introduce a significant error in making these comparisons" [146]. Other investigators, such as Larsen *et al.* (1994), Dahl *et al.* (1989, 1992, 1994), Demolis *et al.* (1996), and Kirkham *et al.* (1986) have also demonstrated that changes in Vmca may be used to monitor changes in CBF during cerebral vasoreactivity and autoregulation tests [118, 210, 211, 212, 213, 214].

Giller *et al.* (1993) used direct visualization to examine the diameters of twelve human cerebral arteries during craniotomy with moderate changes in blood pressure and end-tidal CO_2. It was found that for large cerebral arteries, such as the MCA, the mean diameter changed by less than 4% whereas for smaller arteries, such as the anterior cerebral artery, the diameter changed by up to 29%. The authors concluded, "this constancy of (large artery) diameter suggests that the TCD velocities obtained during intraoperative monitoring of craniotomies closely reflect blood flow through the insonated artery" [111]. Angiographic studies have demonstrated that, during a variety of stimuli known to affect CBF, the diameter of the MCA changes by less than 3% [204]. Aaslid *et al.* (1991) also showed that the MCA diameter does not alter in response to a stepwise drop in blood pressure [159]. Clark *et al.* (1996), however, found some evidence of MCA vasodilatation when normal subjects were exposed to progressive hypercapnia and extreme hyperventilation hypocapnia [215]. In the present study, measurements of MCA caliber were not performed, since no reliable non-invasive technique currently exists for doing this. In addition, Pco_2 was not monitored.

There is considerable evidence that MCA diameter remains practically constant during tests of cerebral autoregulation. Even if there were small changes in vessel caliber, they were unlikely to have significant influence on the final results. We have confidence that the beat-to-beat changes in Vmca represent beat-to-beat changes in CBF.

5.2.2 Mean Blood Pressure vs. Cerebral Perfusion Pressure

In the present study, changes in mean arterial blood pressure are used to represent changes in cerebral perfusion pressure. Since cerebral perfusion pressure equals mean arterial blood pressure minus intracranial pressure, this assumption is valid only if changes in intracranial pressure are minimal. As there is currently no reliable non-invasive technique that accurately measures intracranial pressure, it was not measured in the present study. Assumptions were made that intracranial pressure remained constant during rhythmic handgrip, and that there were no significant changes between visits for subjects after ischaemic stroke. It is also probable that the intracranial pressure remained constant whilst the subjects and controls were at rest during the steady-state part of the examination (without rhythmic handgrip). It is uncertain whether there was any change in intracranial pressure during rhythmic handgrip, although breath holding was prevented during handgrip by verbal encouragement. Each examination was performed with the subject or control lying supine and with the head in a horizontal or near horizontal position; any movement was also discouraged. Rosner *et al.* (1986) found that at this position, cerebral perfusion pressure is maximal and any effect on intracranial pressure is minimal. It was observed that the severity and frequency of occurrence of intracranial B-waves are also minimal at this position [216]. None of the subjects who were recruited in this study had devastating stroke, and in particular, none of the CT head scans showed significant oedema or intracranial pressure effect. It can therefore be assumed that, in general, intracranial pressure was normal, and that there was no significant difference in intracranial pressure between subjects at different visits. Similarly, intracranial pressure was presumed not to be significantly different between the affected and unaffected hemispheres, with or without rhythmic handgrip, or between subjects and controls. Only with these assumptions would our comparison of the various groups be valid.

The site of blood pressure measurement is also a limitation. In the present study, it was not possible to continuously measure Vmca and blood pressure *within* the MCA. As a result, the Finapres device was used to monitor finger arterial pressure. As discussed in Section 2.2.2, the Finapres device has been extensively used for continuous monitoring of systemic arterial blood pressure, and its reliability has been demonstrated by numerous studies in the time and frequency domain [168, 174]. The mean and oscillatory components of the arterial blood pressure are tracked well

using this method. If it is considered that the arterial pressure wave moves at a velocity of 5 to 8 m/s in large vessels, the time delay between the finger arterial pressure and cerebral arterial pressure should be small and negligible [204]. Furthermore, although waveforms of arterial pressure differ in different vascular beds because of reflected waves and pulsatility of pressure waveforms increase in peripheral arteries, the mean arterial pressure is likely to be proportional or identical in arteries of similar size [204]. Therefore, despite the fact that the pressure waveform in the finger may be different from the pressure waveform in the MCA, the assumption can be made that the changes in mean arterial pressure in the finger are *proportional* to changes in mean arterial pressure in the MCA. Since we also assumed that intracranial pressure was low and any fluctuation of intracranial pressure was small and negligible, then changes in mean finger arterial pressure, as measured by the Finapres device, should represent closely the changes in mean cerebral perfusion pressure.

5.2.3 Other Limitations of the Study

Despite the findings of significant and non-significant trends from this study, it is not possible to draw firm conclusions about the precise nature and timing of recovery of cerebral autoregulation after acute ischaemic stroke. However, this study may help to highlight certain aspects of cerebral autoregulation with possible clinical importance, as well as to generate new research questions and topics for the future.

The main limitation of the present study is the small number of good quality recordings that could be included in the data analysis [see Section 4.2]. As expected, the controls had more good recordings than the subjects, probably because the controls were in better medical condition, with fewer problems such as cold limbs and hypotension (affecting Finapres recordings), restlessness (affecting ECG, TCD and Finapres recordings), or inability to perform effective rhythmic handgrip due to limb weakness [see later]. In all the observed groups, the standard deviations and standard errors of the mean were large and any effects were less likely to reach statistical significance. In addition, it is difficult to draw definitive conclusion from the significance that was found in the results since they sometimes contradicted with each other. The relationships between blood pressure, Vmca, hemispheres, visits, and frequency ranges, were extremely complex. We speculate that a more detailed study of these complex relationships would therefore require a larger number of subjects

and controls. In the present study, because of the time and practical limitations, it was not possible to recruit a large number of subjects and controls. Although firm conclusions cannot be drawn, the present study has generated some useful questions on which future research can be based [see Section 5.3].

The length of each recording may also have affected the reliability of the results from the steady-state transfer function analysis. The length of each recording determined the number of subintervals, and hence the number of fast Fourier transformation (FFT) spectrums, that were used for transfer function analysis [see Section 3.3.2]. Since two-minute subintervals were used, only three subintervals (each overlapping by 50%) could be obtained from each five-minute recording, that is six per visit (two five-minute recordings per visit). If one-minute subintervals were used, then nine subintervals could be obtained from each recording. This increase in number of FFT spectrums would increase the statistical reliability of the analysis and may reduce the standard deviations and standard errors of the mean. However, two-minute subintervals were chosen to include the blood pressure oscillations in the very low frequency range. Therefore, there was a trade-off between the objectives of the study and statistical robustness.

In general, rhythmic handgrip was found to be a useful non-invasive technique of stimulating blood pressure oscillations, but several aspects of the technique deserves comment. For the subjects, despite using the unaffected (and dominant where possible) hand for rhythmic, some of them were still too weak to perform the task because of general weakness and lethargy after stroke. As a consequence, it seems that more controls were able to generate sizeable blood pressure oscillations (up to about 25 to 30% of the mean) from rhythmic handgrip, although this was not recorded. It is possible that the stroke patients were less (or controls were more) 'sensitive' to rhythmic handgrip, for example due to possible secondary autonomic dysfunction after cerebral ischaemia. Indeed, rhythmic handgrip has been shown to be a useful test of autonomic function, as discussed in Section 2.2.3 [180]. For the assessment of cerebral autoregulation, sizeable blood pressure oscillations may be more effective at stimulating CBFV oscillations. This may be beneficial for the calculation of phase angle shift and gain. In this study, we have not analysed the percentage change in blood pressure from the mean, or the consistency of oscillation, that rhythmic handgrip produced. This information can be used to judge the overall effectiveness of the technique and the applicability to patients after acute stroke.

In retrospect, it would have been appropriate to assess the effect of rhythmic handgrip on the amplitude and frequency of blood pressure oscillations. Future studies in this area will take this important aspect into account. Moreover, it remains to be tested whether induced oscillations of blood pressure are *harmful* to the already vulnerable haemodynamic state at the ischaemic core and the ischaemic penumbra. For example, the induction of high peaks of the blood pressure oscillations may increase the risk of promoting cerebral oedema when cerebral autoregulation is impaired.

In some studies, rhythmic handgrip has been shown to produce a surge of peripheral sympathetic nervous activity [183]. It may be criticized that this sympathetic activity may cause vasoconstriction of the MCA, hence affecting the Vmca. Pott *et al.* (1996), however, found no evidence for a vasoconstrictive influence of sympathetic nervous activity on the cerebral arteries during rhythmic handgrip [190]. Ide *et al.* (1998) demonstrated that rhythmic handgrip increased contralateral Vmca (13%), while the increment was smaller on the ipsilateral side (6%) [179]. It is possible that this type of discrepancy may have affected the findings in the present study. However, since we examined the phase and amplitude relationship between the oscillations of blood pressure and Vmca rather than the absolute values, the differential effect of rhythmic handgrip on each hemisphere may not be significant. Lastly, as Birch *et al.* (1995) also commented, it can be criticized that any mental activity involved with rhythmic handgrip may have affected changes in Vmca [145]. However, mental activation occurred twice in each cycle, manifesting as a second harmonic. Therefore, the effect will be small relative to the first harmonic (or the 'fundamental') at 0.025 Hz, which was the only harmonic used in the Fourier analysis [see Section 3.5]. Even if the mental activity involved with rhythmic handgrip was present, the effect on the first harmonic was likely to be negligible.

This study failed to demonstrate any significant differences in the phase angle shift, gain, and coherences between the affected and unaffected hemispheres, and between the subjects and controls. Furthermore, rhythmic handgrip transfer function analysis revealed smaller-than-expected phase and larger-than-expected gain in controls. This may in fact reflect a degree of background impairment of cerebral autoregulation amongst some subjects and controls. This impairment could have been the consequence of the advanced age of the subjects and controls, and some may have had other pathological conditions such as autonomic dysfunction. It may have been useful to screen out the subjects and controls with abnormal cerebral vasoreactivity

(for example, using 5% CO_2 inhalation) prior to inclusion into the study. However, dissociation between cerebral autoregulation and cerebral vasoreactivity has also been observed by Olsen *et al.* (1983) within the ischaemic penumbra in acute stroke patients [19]. This means that, after stroke, the finding of an abnormal vasoreactivity would not necessary mean abnormal autoregulation. It may also have been useful to examine both the dynamic and static components of autoregulation, such as that carried out by Tiecks *et al.* (1995), in order to make a more precise comparison [149]. However, Dawson *et al.* (1999) concluded from their study that the dynamic, not static, component of cerebral autoregulation was impaired in acute ischaemic stroke, and that this discrepancy implies that they may be distinct entities [158]. This theory remains to be tested and confirmed.

The artefacts that were observed could have influenced the results. For example, the irregular (ectopic) heartbeats in some recordings were not removed and the relevant recordings were not excluded from data analysis. However, there has been no study that has examined the effects of irregular heartbeats on Vmca oscillations or cerebral autoregulation [see Section 3.2.1] and therefore, to our knowledge, it was not justifiable to exclude all irregular heartbeats from data analysis. The electrical spikes that were found were removed by linear interpolation to eliminate their effects on the results. It is also possible that various physiological parameters that were not measured, such as Po_2, end-tidal Pco_2, or critical closing pressure, may have had significant effects on the Vmca oscillations. However, Diehl *et al.* (1991) found that these parameters did not correlate with low frequency fluctuations in Vmca [129]. Overall, the artefacts and the various parameters that were unmonitored were unlikely to have had a significant influence on the final results.

There are also a number of other minor limitations in the present study that indicate that the interpretation given to the results may need to be approached with caution. For example, changes in cerebral metabolism during rhythmic handgrip, or between visits, cannot be ruled out. Changes in metabolism that are concomitant with changes in blood pressure may have modulated the Vmca response thus leading to difficulty in interpreting the results. Furthermore, 'normality' of cerebral autoregulation was poorly defined. Although significant trends were observed for phase angle shift, gain, and coherence, whether these findings represent true changes in cerebral autoregulation remains to be elucidated. The phase angle shift, gain, and coherence index are some of the most researched mathematical representation of the

competence of cerebral autoregulation. We also followed the formulae that were used by other investigators, such as Zhang *et al.* (1998) [204]. Further studies are indicated to explore the complex relationships that have been described.

The differences in the results for phase angle shift and gain may raise questions about the underlying physiological mechanisms that are responsible for cerebral autoregulation. The apparently distinct behaviour of the phase and gain responses may indicate the interplay of two distinct mechanisms involved in cerebral autoregulation. This hypothesis was also proposed by Panerai *et al.* (1998) [13]. To illustrate, the rhythmic handgrip transfer function analysis revealed the significant association between phase angle shift and visits that was not present for gain. With rhythmic handgrip that was performed at a low frequency of 0.025 Hz, a 'phase-related' mechanism may be responsible for a slow Vmca response to changes in blood pressure. Since this association was not present for gain at this frequency, we speculate that the 'amplitude-related' mechanism may be less responsible for the Vmca response to changes in blood pressure at this frequency. We can then hypothesize that the 'phase-related' mechanism, which is responsible for slower responses, may be dependent on metabolic mediators. Conversely, the 'amplitude-related' mechanism may be dependent on other processes such as the myogenic or endothelial processes. More experimental work is required to test the above hypotheses involving the interaction of the various mechanisms.

The synchronization of Vmca oscillations between the two hemispheres was not examined in this study. To do this, it would be necessary to perform transfer function analysis using Vmca of one hemisphere as input and that of the other side as output. The phase angle shift and gain can then be calculated. The phase and amplitude of the Vmca oscillations of one hemisphere in relation to the other hemisphere would indicate the degree of synchronization between the two sides. If there was perfect synchronization, then the phase angle shift would be near zero degree and the gain would be significantly different from zero (closer to one or more). We aim to perform further studies to explore this phenomenon.

One disadvantage of transfer function analysis is the long recording periods, particularly when studying very low frequency oscillations, and complex calculations. However, with refinement and development of better and faster computer programs, the latter problem can be easily overcome.

5.3 Implications for Future Research

5.3.1 Cerebral Haemodynamics

In the past few decades, much research has been carried out in the field of cerebrovascular physiology and haemodynamics, and in recent years there have been dramatic developments in the technology to study the dynamic behaviour of cerebral autoregulation. Despite the high incidence and significant physical and financial burden of stroke, especially ischaemic stroke, much of the research has been performed on patients who have suffered subarachnoid haemorrhage, severe head injury, or other neurological conditions that may be suitable for surgery. Therefore, there is relative inadequacy in the knowledge concerning cerebral haemodynamics, and in particular cerebral autoregulation, after ischaemic stroke.

Given that cerebral autoregulation is a phenomenon that is without measurable units, research in this area has not produced a 'gold standard' method of its assessment or a set of 'normal' values. In fact, as discussed in Section 3.1.1, there is considerable diversity in the methodology and the techniques that are employed to facilitate the examination of the relationships between cerebral blood flow and various physiological parameters. We have shown that the interactions between cerebral blood flow velocity, blood pressure, and frequency of blood pressure oscillations, are extremely intricate, even in healthy subjects. After stroke, parameters such as the time from stroke, side of cerebral ischaemia, and presence of underlying cerebrovascular pathology, may also become relevant. The significance of minor changes in, for example, mental activity, sympathetic nervous activity, Pco_2, and Po_2, remain to be clearly elucidated. Much of this complexity will need to be unravelled in order to increase our understanding of the cerebral haemodynamic disturbances after ischaemic stroke, as well as advancing the development of potential treatments to improve the chance of a better clinical outcome.

The present study raises many interesting questions. We found that cerebral autoregulation behaved as a high-pass filter system by demonstrating that the phase and amplitude of CBFV oscillations (output) were dependent on the frequency of blood pressure oscillations (output). The presence of such a system in patients after ischaemic stroke has not previously been shown. Further studies in this area are needed to confirm this finding and larger number of subjects and controls will be necessary to detect any difference between the affected and unaffected hemisphere, as

well as between subjects and controls. From the results of this study, it is possible to retrospectively estimate the sample size needed for future studies. For this purpose, we can use the results for phase angle shift in the affected hemisphere during rhythmic handgrip. The phase angle shifts for visits 1 and 3 are -26.5^0 and $+33.8^0$ (SD = 55.3^0), and using a power of 80%, α-level of 0.05 and 10% drop out rate, then we estimate that 15 patients will be needed (or 20 patients for a 90% power). We can also use the control data for comparison, even though our control results may not represent a 'normal' population. The phase angle shift for visit 1 is -26.5^0 and for control is 0.5^0, then if a power of 80% is used, we estimate that 73 patients will be needed (or 97 patients for a 90% power). There is a significant difference in the estimated sample size between comparing within patients and comparing patients with controls. This is probably the result of a combination of small phase angle shift found in controls and large standard deviation.

In the future, it will also be interesting to examine the synchronization between the two hemispheres by studying the relationship between the affected and unaffected sides as input and output respectively. It has always been assumed that CBFV in the two hemispheres vary synchronously. However, Diehl *et al.* (1991) has demonstrated asymmetric CBFV oscillations in patients with severe carotid artery obstruction [129]. We are planning to perform studies to explore this interesting area further.

5.3.2 Recovery of Cerebral Autoregulation after Stroke

The results from the present study suggest that a steady recovery of cerebral autoregulation occurs after acute ischaemic stroke. There is, however, no evidence that the rate of recovery decreases during the three months after stroke. More research is necessary to confirm our findings. Our observations also lead to many other interesting questions. Does cerebral autoregulation continue to recover beyond three months? Does cerebral autoregulation ever return to 'normal' after ischaemic stroke? Since cerebral autoregulation can be regarded as a continuum rather than displaying discreet levels of competence, what should be the most appropriate definitions of 'normal' and 'abnormal' cerebral autoregulation for any human being? Does 'abnormal' cerebral autoregulation have important clinical relevance? If so, at what extent of 'abnormality' does it become clinically relevant? How does the brain

compensate for this impairment of cerebral autoregulation after stroke, and are the compensatory mechanisms in the two hemispheres similar?

Similarly, there is lack of knowledge concerning the time around which cerebral autoregulation begins to recover after stroke. Hence, in future studies, it may be helpful to start the serial assessment of cerebral autoregulation within a few days or even hours after stroke. There are, however, potential difficulties with these studies since the 'normal' competence of cerebral autoregulation is unlikely to be known and that the nature of recovery, which may depend on other factors such as age, type of stroke, or underlying cerebrovascular reserve, may be considerably different between subjects. Furthermore, although large numbers of carefully selected subjects and controls are necessary for studies of this kind, the process of selection itself may actually exclude important groups of patients. In the present study, for example, patients and controls with atrial fibrillation were excluded because continuous TCD monitoring would be impossible.

5.3.3 Manipulation of Blood Pressure after Stroke

As already discussed in Section 1.4.2, manipulation of blood pressure after stroke may theoretically be associated with various risks. Reduction of blood pressure soon after stroke may jeopardize the blood flow to the ischaemic area and the surrounding ischaemic penumbra. Increasing the blood pressure soon after stroke may also be harmful by enhancing the development of cerebral oedema and haemorrhagic transformation of infarct. Impairment of cerebral autoregulation is the underlying basis for these risks and our results suggest that the resolution of cerebral autoregulation impairment continues throughout the first three months after acute ischaemic stroke and may not be complete within three months.

Blood pressure reduction in hypertensive patients for the secondary prevention of stroke is highly effective. However, the optimal time to commence antihypertensive therapy, and the most appropriate agent that should be used, remain to be clearly elucidated. The linearity of the trends that was found in the present study suggests that recovery of cerebral autoregulation may continue to take place after three months. Further studies are needed to follow up the subjects for a longer period of time after stroke, for example, up to six months or one year. Our findings also raise the following clinical questions. Does the reduction of blood pressure with a background of impaired cerebral autoregulation worsen the blood supply to the

ischaemic area and the ischaemic penumbra? Is the reduction of blood pressure with a background of impaired cerebral autoregulation associated with a worse clinical outcome (i.e. 'harmful')? Is it 'safe' to administer antihypertensive therapy within three months after stroke? Since it may be difficult to ascertain a threshold of 'normality' of cerebral autoregulation, at what extent of cerebral autoregulation impairment or recovery is it then 'safe' to reduce the blood pressure? Given that impairment of cerebral autoregulation may also affect the hemisphere that is contralateral to the ischaemia, does blood pressure reduction have the same effects in both hemispheres after stroke? What are the physiological effects of blood pressure manipulation on the oscillations of blood pressure and CBFV, phase angle shift, gain, and coherence? Which antihypertensive agents are effective at reducing the blood pressure but without significant disturbance to CBF? Similarly, these questions can be applied to artificially raising the blood pressure after ischaemic stroke.

Further studies are necessary to answer these questions before definitive clinical guidelines can be developed. In the UK, it is currently recommended that antihypertensive treatment may be commenced for stroke patients with persisting hypertension (i.e. no spontaneous decline) about one to two weeks after stroke [76, 5]. Our study highlights some theoretical risks that may challenge this recommendation.

5.3.4 Methods of Assessing Cerebral Autoregulation

Assessment of the dynamic component of cerebral autoregulation has been the subject of much interest for cerebrovascular physiologists in the past decade. Advances in technology have meant that techniques are becoming less invasive and more emphasis has been placed on the use of spontaneous fluctuations of blood pressure and CBFV. Gentle stimulation of blood pressure fluctuations by physiological means has also become more popular.

In this project, both spontaneous fluctuations of blood pressure and rhythmic handgrip were used in the assessment of cerebral autoregulation. The different methods of data analysis have produced different results. For example, while rhythmic handgrip transfer function analysis showed that phase angle shifts for stroke patients at visits 1 and 2 were negative, steady-state transfer function analysis showed that they were positive [see Results]. This is an example of potential conflicting results derived from different methods of assessing phase angle shift, and even cerebral autoregulation itself. Reasons for such conflicts may include differing methods of

calculating phase, gain and coherence, unpredictable blood pressure oscillations from rhythmic handgrip, and variations in other physiological parameters that may affect cerebral autoregulation or confound the interpretation of results.

In general, we found rhythmic handgrip to be a non-invasive technique of stimulating blood pressure oscillations and our study is the first to use this technique in the assessment of cerebral autoregulation after stroke. In the present study, the combination of TCD ultrasonography and rhythmic handgrip appear to be useful in examining cerebral autoregulation after stroke.

Future research in this area should address the following questions. How effective and consistent is rhythmic handgrip at inducing blood pressure oscillations? Is rhythmic handgrip as effective in normal controls as stroke patients? What are the factors that may make rhythmic handgrip more or less effective or consistent at inducing blood pressure oscillations (for example, percentage of maximal voluntary contraction, breath holding, changes in Pco_2, patient's posture, the use of dominant or non-dominant hand)? Does the frequency of rhythmic handgrip, type of stroke, or other factors influence the final results of transfer function analysis? Does the original blood pressure influence the characteristics of the induced blood pressure or the CBFV oscillations? Does antihypertensive therapy influence the responsiveness of the cardiovascular system to rhythmic handgrip after stroke? Is rhythmic handgrip applicable for all patients after stroke? Do changes in phase and gain truly reflect changes in the competence of cerebral autoregulation?

If it is demonstrated that manipulation of blood pressure in patients with impairment of cerebral autoregulation is harmful, then it is likely that the assessment of cerebral autoregulation may become common practice after stroke. We propose that the method of combining TCD ultrasonography and rhythmic handgrip may be used to assess cerebral autoregulation after stroke. With further development, it may become a bedside technique in routine use, especially for patients in whom blood pressure manipulation is considered.

5.4 Conclusion

In the present study, we used cross-spectral transfer function analysis to explore the phenomenon of cerebral autoregulation after acute ischaemic stroke. We found that the relationship between blood pressure and CBFV is very complex. Using rhythmic handgrip, we demonstrated changes in this relationship that may indicate improvement in cerebral autoregulation over the first three months after acute ischaemic stroke. There are also suggestions that these changes may not be limited to the side of cerebral ischaemia.

We have also examined the influence of frequency of spontaneous blood pressure oscillations on CBFV oscillations. The results suggest that cerebral autoregulation behaves as a high-pass filter system, even after ischaemic stroke. Although the results of steady-state transfer function analysis appear to be different from that of rhythmic handgrip, it is difficult to draw definite conclusions because of the small number of good quality recordings that were included in the data analysis. The combination of TCD ultrasonography and rhythmic handgrip appears to be a useful non-invasive method of examining cerebral autoregulation after stroke. When the relationship between blood pressure manipulation and stroke outcome becomes clearer, this method may become a useful clinical tool in the future.

CHAPTER 6 BIBLIOGRAPHY

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CHAPTER 7 APPENDICES

7.1 Appendix 1: Photographs

Photograph No	Description	Page in Thesis
1	Finapres device (Ohmeda 2300)	68
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The following two pages contain the photographs that illustrate some aspects of the study methodology and equipment that were used. All the references to these photographs are located in Chapter 2, 'Methods', the pages of which are shown above. Written consent for the publication of these photographs has been obtained from the patient concerned.

Photo 1. Finapres device (Ohmeda 2300)

Photo 2. Datascope Multicare 3000

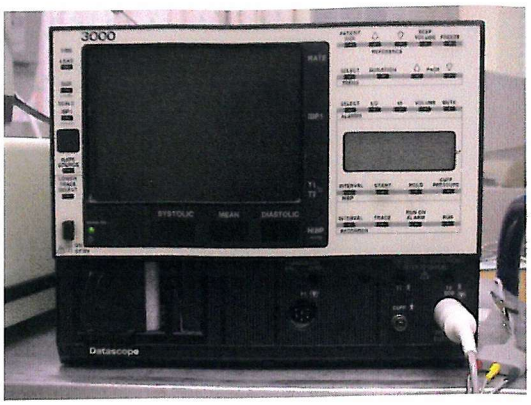


Photo 3. R-wave detector

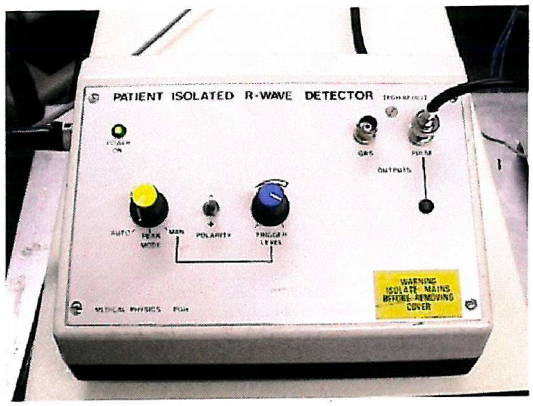


Photo 4. Bilateral TCD monitoring

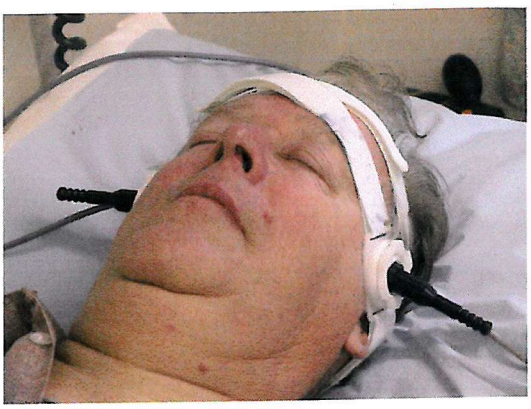


Photo 5. Patient relaxed for continuous TCD monitoring

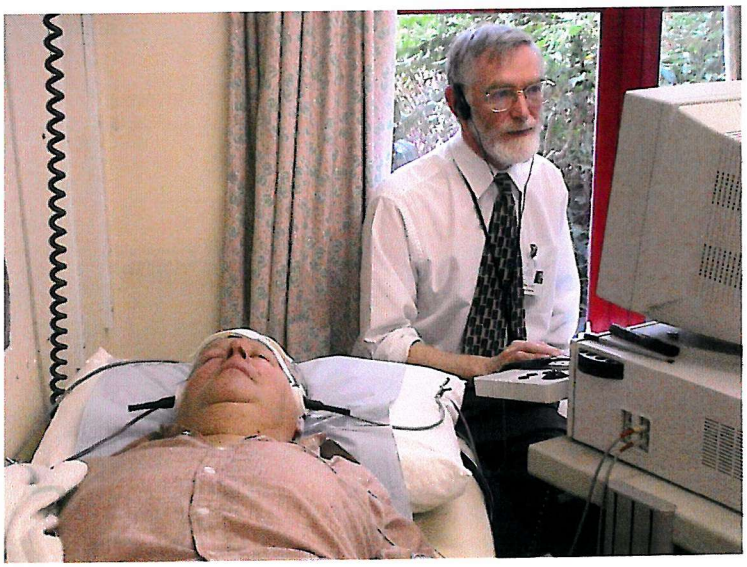


Photo 6. All the equipment for the study

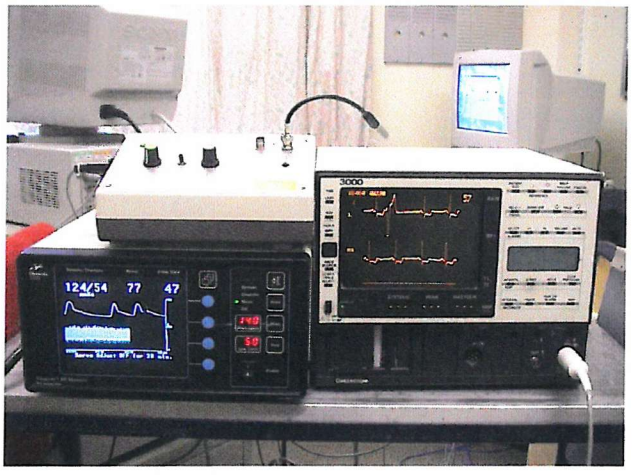


Photo 7. An examination with rhythmic handgrip

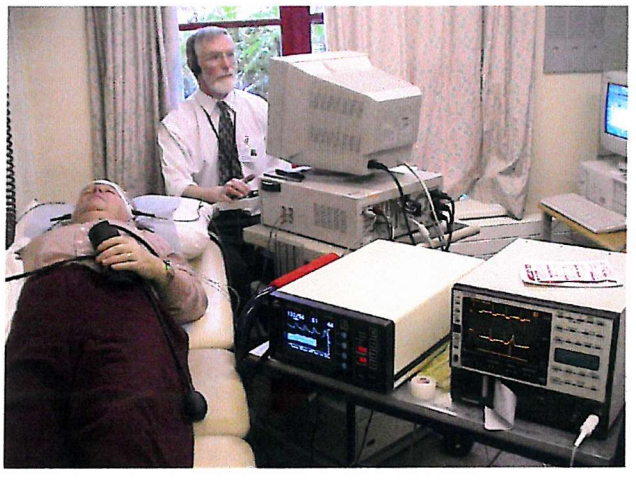
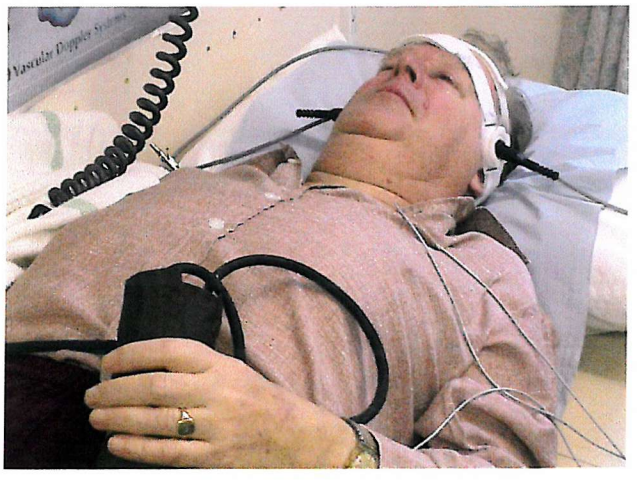


Photo 8. Subject performing rhythmic handgrip by squeezing a sphygmomanometer cuff every 20 seconds



7.2 Appendix 2: Test of Data Distribution

Tables 1 to 5 below show the results of tests for normal distribution (SPSS 9.0) for all 77 data sets in this study. The symbol * against a significance figure represents non-normal distribution of data points. From the Shapiro-Wilk test (suitable for fewer than 50 observations), 67 out of 77 (87%) data sets were found to be normally distributed.

Table 1. Results for gain in rhythmic handgrip data sets

GAIN	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Visit 1 Affected Hemi	.279	6	.156	.886	6	.340
Visit 2 Affected Hemi	.237	6	.200	.909	6	.428
Visit 3 Affected Hemi	.264	6	.200	.894	6	.368
Visit 1 Unaffected Hemi	.194	6	.200	.934	6	.558
Visit 2 Unaffected Hemi	.195	6	.200	.921	6	.475
Visit 3 Unaffected Hemi	.259	6	.200	.852	6	.203
Control	.265	6	.200	.840	6	.156

Table 2. Results for phase angle shift in rhythmic handgrip data sets

PHASE SHIFT	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Visit 1 Affected Hemi	.251	6	.200	.908	6	.424
Visit 2 Affected Hemi	.290	6	.125	.845	6	.174
Visit 3 Affected Hemi	.310	6	.073	.814	6	.085
Visit 1 Unaffected Hemi	.169	6	.200	.989	6	.988
Visit 2 Unaffected Hemi	.218	6	.200	.912	6	.442
Visit 3 Unaffected Hemi	.267	6	.200	.829	6	.111
Control	.393	6	.004*	.702	6	.010*

To be continued next two pages

Table 3. Results for coherence in steady-state data sets

STEADY-STATE COHERENCE	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statist	df	Sig.	Statist	df	Sig.
Visit 1 Affected Hemi V. Low Freq.	.240	7	.200	.905	7	.397
Visit 1 Affected Hemi Low Freq.	.153	7	.200	.980	7	.956
Visit 1 Affected Hemi High Freq.	.201	7	.200	.950	7	.702
Visit 1 Unaffected Hemi V. Low Freq.	.248	7	.200	.882	7	.294
Visit 1 Unaffected Hemi Low Freq.	.215	7	.200	.911	7	.426
Visit 1 Unaffected Hemi High Freq.	.183	7	.200	.895	7	.351
Visit 2 Affected Hemi V. Low Freq.	.206	7	.200	.870	7	.244
Visit 2 Affected Hemi Low Freq.	.297	7	.061	.834	7	.094
Visit 2 Affected Hemi High Freq.	.199	7	.200	.918	7	.455
Visit 2 Unaffected Hemi V. Low Freq.	.180	7	.200	.972	7	.898
Visit 2 Unaffected Hemi Low Freq.	.170	7	.200	.962	7	.808
Visit 2 Unaffected Hemi High Freq.	.188	7	.200	.937	7	.585
Visit 3 Affected Hemi V. Low Freq.	.183	7	.200	.942	7	.626
Visit 3 Affected Hemi Low Freq.	.222	7	.200	.903	7	.390
Visit 3 Affected Hemi High Freq.	.236	7	.200	.871	7	.248
Visit 3 Unaffected Hemi V. Low Freq.	.188	7	.200	.905	7	.399
Visit 3 Unaffected Hemi Low Freq.	.249	7	.200	.857	7	.186
Visit 3 Unaffected Hemi High Freq.	.246	7	.200	.857	7	.184
Control V. Low Freq.	.183	7	.200	.954	7	.736
Control Low Freq.	.227	7	.200	.877	7	.275
Control High Freq.	.310	7	.040	.770	7	.027*

Table 4. Results for gain in steady-state data sets

STEADY-STATE GAIN	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statist	df	Sig.	Statist	df	Sig.
Visit 1 Affected Hemi V. Low Freq.	.346	7	.011*	.643	7	.010*
Visit 1 Affected Hemi Low Freq.	.262	7	.159	.882	7	.294
Visit 1 Affected Hemi High Freq.	.376	7	.003*	.707	7	.010*
Visit 1 Unaffected Hemi V. Low Freq.	.257	7	.178	.922	7	.473
Visit 1 Unaffected Hemi Low Freq.	.216	7	.200	.858	7	.190
Visit 1 Unaffected Hemi High Freq.	.206	7	.200	.963	7	.822
Visit 2 Affected Hemi V. Low Freq.	.240	7	.200	.884	7	.305
Visit 2 Affected Hemi Low Freq.	.170	7	.200	.965	7	.837
Visit 2 Affected Hemi High Freq.	.278	7	.108	.827	7	.085
Visit 2 Unaffected Hemi V. Low Freq.	.258	7	.173	.860	7	.199
Visit 2 Unaffected Hemi Low Freq.	.263	7	.154	.764	7	.023*
Visit 2 Unaffected Hemi High Freq.	.389	7	.002*	.739	7	.013*
Visit 3 Affected Hemi V. Low Freq.	.196	7	.200	.958	7	.772
Visit 3 Affected Hemi Low Freq.	.201	7	.200	.928	7	.503
Visit 3 Affected Hemi High Freq.	.266	7	.144	.827	7	.085
Visit 3 Unaffected Hemi V. Low Freq.	.227	7	.200	.885	7	.309
Visit 3 Unaffected Hemi Low Freq.	.197	7	.200	.892	7	.342
Visit 3 Unaffected Hemi High Freq.	.149	7	.200	.923	7	.478
Control Very Low Freq.	.186	7	.200	.915	7	.440
Control Low Freq.	.355	7	.008*	.634	7	.010*
Control High Freq.	.364	7	.005*	.573	7	.010*

Table 5. Results for phase angle shift in steady-state data sets

STEADY-STATE PHASE SHIFT	Kolmogorov-Smirnov			Shapiro-Wilk		
	Statist	df	Sig.	Statist	df	Sig.
Visit 1 Affected Hemi V. Low Freq.	.172	7	.200	.975	7	.921
Visit 1 Affected Hemi Low Freq.	.265	7	.147	.890	7	.329
Visit 1 Affected Hemi High Freq.	.361	7	.006*	.713	7	.010*
Visit 1 Unaffected Hemi V. Low Freq.	.181	7	.200	.944	7	.648
Visit 1 Unaffected Hemi Low Freq.	.182	7	.200	.889	7	.328
Visit 1 Unaffected Hemi High Freq.	.157	7	.200	.941	7	.617
Visit 2 Affected Hemi V. Low Freq.	.146	7	.200	.962	7	.808
Visit 2 Affected Hemi Low Freq.	.252	7	.198	.936	7	.574
Visit 2 Affected Hemi High Freq.	.225	7	.200	.949	7	.690
Visit 2 Unaffected Hemi V. Low Freq.	.271	7	.130	.858	7	.188
Visit 2 Unaffected Hemi Low Freq.	.230	7	.200	.882	7	.296
Visit 2 Unaffected Hemi High Freq.	.284	7	.092*	.867	7	.230
Visit 3 Affected Hemi V. Low Freq.	.284	7	.092*	.756	7	.019*
Visit 3 Affected Hemi Low Freq.	.180	7	.200	.965	7	.836
Visit 3 Affected Hemi High Freq.	.161	7	.200	.955	7	.749
Visit 3 Unaffected Hemi V. Low Freq.	.203	7	.200	.929	7	.509
Visit 3 Unaffected Hemi Low Freq.	.236	7	.200	.890	7	.331
Visit 3 Unaffected Hemi High Freq.	.192	7	.200	.906	7	.401
Phase Control V. Low Freq.	.195	7	.200	.964	7	.825
Phase Control Low Freq.	.233	7	.200	.880	7	.287
Phase Control High Freq.	.229	7	.200	.950	7	.704

7.3 Appendix 3: Baseline Blood Pressure & MCA Velocity of Subjects

Patient Name	Side of Infarct	Anti-HT Rx[§]	Visit	Baseline BP[¶]	Baseline R Vm[†]	Baseline L Vm[‡]
BL*	Right	Yes	1	135 / 84 (107)	51.4	53.0
			2	133 / 76 (98)	45.9	32.8
			3	114 / 70 (86)	44.7	47.2
AO	Right	No	1	122 / 60 (84)	54.3	48.0
			2	161 / 95 (119)	38.4	41.6
			3	152 / 86 (111)	38.8	38.3
QW	Right	Yes	1	135 / 70 (94)	14.0	14.4
			2	125 / 53 (78)	35.0	32.7
			3	N/A	N/A	N/A
JM*	Left	Yes	1	175 / 83 (115)	41.3	49.5
			2	163 / 73 (101)	44.3	28.7
			3	152 / 52 (83)	45.0	30.9
GD*	Left	Yes	1	174 / 73 (104)	64.1	72.0
			2	157 / 60 (86)	64.7	66.5
			3	146 / 58 (82)	55.3	36.6
HO*	Right	No	1	163 / 87 (117)	55.9	40.6
			2	115 / 61 (82)	60.0	45.3
			3	120 / 62 (83)	54.9	61.9
CM	Right	No	1	158 / 104 (126)	46.6	48.1
			2	N/A	N/A	N/A
			3	N/A	N/A	N/A
OG*	Left	No	1	129 / 66 (89)	40.7	42.1
			2	141 / 70 (99)	39.4	38.0
			3	121 / 63 (85)	38.2	42.6
BP*	Left	No	1	191 / 105 (137)	51.1	49.0
			2	210 / 118 (155)	31.7	39.2
			3	137 / 77 (103)	32.9	38.6
RC	Left	No	1	194 / 106 (143)	36.6	37.3
			2	146 / 80 (107)	30.0	35.5
			3	152 / 62 (96)	44.5	44.5

* Patients whose recordings were included in the final data analysis

§ On antihypertensive agent at the time of visit

¶ Baseline blood pressure (mmHg) by Finapres: systolic/diastolic (mean arterial) blood pressure

† Highest baseline time-averaged mean right middle cerebral artery velocity (cm/s) at each visit

‡ Highest baseline time-averaged mean left middle cerebral artery velocity (cm/s) at each visit

7.4 Appendix 4: Baseline Blood Pressure & MCA Velocity of Controls

Control Name	Anti-HT Rx[§]	Baseline BP[¶]	Baseline R Vm[†]	Baseline L Vm[‡]	Baseline Average Vm[¥]
E F*	No	112 / 62 (79)	43.0	26.0	34.5
C L	No	166 / 81 (113)	56.9	20.9	38.9
G W*	No	152 / 91 (114)	34.2	44.6	39.4
R A*	No	142 / 90 (108)	43.0	42.0	42.5
E H*	Yes	148 / 71 (98)	30.8	31.4	31.1
J B	No	129 / 73 (94)	N/A	N/A	N/A
K A*	Yes	177 / 96 (130)	58.6	59.8	59.2
J D	No	112 / 62 (81)	28.5	37.8	33.2
E T*	No	160 / 77 (105)	48.5	40.1	44.3
H T*	No	128 / 73 (95)	49.8	41.4	45.6
A M	No	169 / 86 (114)	41.5	41.8	41.7

* Controls whose recordings were included in the final data analysis

§ On antihypertensive agent at time of examination

¶ Baseline blood pressure (mmHg) measured with a sphygmomanometer

† Highest baseline time-averaged mean right middle cerebral artery velocity (cm/s) at each visit

‡ Highest baseline time-averaged mean left middle cerebral artery velocity (cm/s) at each visit

¥ Average of the left and right baseline Vm (of previous 2 columns)

7.5 Appendix 5: Changes in BP Induced By Rhythmic Handgrip – Subjects

Patient Name	Visit	% Change In BP[§]	Power of BP Oscillations (%)[¶]
BL*	1	6.0	90
	2	3.2	91
	3	8.7	86
AO	1	3.4	72
	2	4.1	84
	3	16.9	90
QW	1	3.4	91
	2	4.8	74
	3	N/A	N/A
JM*	1	1.5	46
	2	4.4	81
	3	4.8	74
GD*	1	1.7	84
	2	6.6	97
	3	2.4	85
HO*	1	1.7	43
	2	4.6	82
	3	2.9	92
CM	1	1.8	53
	2	N/A	N/A
	3	N/A	N/A
OG*	1	3.5	77
	2	4.8	80
	3	7.0	87
BP*	1	9.3	94
	2	9.5	91
	3	7.0	86
RC	1	3.8	91
	2	5.0	89
	3	4.3	96

* Patients whose recordings were included in the final data analysis

§ Percentage change in blood pressure induced by rhythmic handgrip at each visit

¶ Power (in percent) of the blood pressure waveform. This is the percentage of the average blood pressure waveform made up by oscillations at the fundamental frequency of rhythmic handgrip, i.e. 0.025 Hz.

7.6 Appendix 6: Changes in BP Induced By Rhythmic Handgrip – Controls

Control Name	% Change In BP§	Power of BP Oscillations (%)¶
E F*	12.9	98
C L	3.5	91
G W*	6.6	94
R A*	3.2	52
E H*	3.0	62
J B	11.6	98
K A*	10.3	89
J D	4.1	60
E T*	2.6	67
H T*	3.7	76
A M	4.2	65

* Controls whose recordings were included in the final data analysis

§ Percentage change in blood pressure induced by rhythmic handgrip at each visit

¶ Power (in percent) of the blood pressure waveform. This is the percentage of the average blood pressure waveform made up by oscillations at the fundamental frequency of rhythmic handgrip, i.e. 0.025 Hz.

Comparison of the average % change in BP and power of BP oscillations.

Mann-Whitney test was used for comparison of non-normally distributed data (proven by Shapiro-Wilk test). Both results were non-significant ($P > 0.05$).

Group	Average % Change in BP	Average Power of BP Oscillations (%)¶	Mann-Whitney Test (P value)
SUBJECTS	5.08	81.7	0.735
CONTROLS	5.97	77.5	0.687