UNIVERSITY OF SOUTHAMPTON

A Study of the Effects of Caffeine on Cerebral Blood Flow in Patients Recovering from an Acute Ischaemic Stroke

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ABSTRACT

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by Dr Suzanne Ragab

Caffeine is present in a variety of beverages and food and is widely consumed. Caffeine causes a reduction in cerebral blood flow (CBF) in healthy individuals. A similar reduction in ischaemic stroke patients could theoretically exacerbate pre-existing ischaemia. In a previous study of patients recovering from an acute ischaemic stroke using transcranial Doppler ultrasound a fall in middle cerebral artery (MCA) blood velocity (Vmca) of 12% was demonstrated after ingestion of caffeine. The aim of this study was to investigate if these velocity changes reflected changes in cerebral blood flow.

The study used a randomised, double blind, cross over design. Twenty patients recovering from an acute ischaemic stroke in the MCA territory and ten controls attended two sessions having abstained from caffeine for 48hours prior to each. At each session CBF was measured 4 times using xenon clearance, twice before the oral administration of 250mg caffeine or matched placebo, and twice after. Similarly, three Vmca readings using transcranial Doppler were made prior to administration and four after.

250mg caffeine (equivalent to 2-3 cups of coffee) resulted in a significant fall in CBF and Vmca compared to placebo. This reduction occurred in stroke patients and controls and was similar on the affected stroke hemisphere and the unaffected side. Since caffeine is present in the diet of most patients recovering from an acute ischaemic stroke this effect may have adverse clinical consequences.

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LIST OF ABBREVIATIONS

ACE	angiotensin converting enzyme
ADL	activities of daily living
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BP	blood pressure
CBF	cerebral blood flow
CNS	central nervous system
CO_2	carbon dioxide
CI	confidence intervals
CPP	cerebral perfusion pressure
CT	computerised tomography
CVR	cerebral vascular resistance
HMPAO	hexamethyl propylene amine oxime
ICA	internal carotid artery
LACI	lacunar infarct
LACS	lacunar syndrome
MCA	middle cerebral artery
NMDA	N-methyl-D aspartate
OCSP	Oxfordshire Community Stroke Project
OEF	oxygen extraction fraction
$PaCO_2$	arterial carbon dioxide tension
PACI	partial anterior circulation infarct
PACS	partial anterior circulation syndrome
PET	positron emission tomography
$P_{ET}CO_2$	end tidal carbon dioxide tension

POCI	posterior circulation infarct
POCS	posterior circulation syndrome
rCBF	regional cerebral blood flow
SPECT	single photon emission computerised tomography
TACI	total anterior circulation infarct
TACS	total anterior circulation syndrome
TCD	transcranial doppler
Vmca	velocity in middle cerebral artery
Xe	xenon

CHAPTER 1: INTRODUCTION

Caffeine is one of the most widely consumed drugs worldwide. It is found in a variety of food, drinks and over the counter drugs and is consumed by a large number of people in all age groups. Caffeine-containing beverages and food are regularly served in hospitals and consumed by patients. The use of caffeine continues to provoke controversy as to whether it is associated with any adverse consequences for health.

Caffeine is known to reduce cerebral blood flow in normal healthy volunteers. The aim of this study was to investigate the effect of caffeine on cerebral blood flow in patients recovering from an acute ischaemic stroke. A similar reduction in cerebral blood flow in these patients may exacerbate pre-existing ischaemia with adverse consequences.

In this chapter the classification, clinical features and pathophysiology of ischaemic stroke will be discussed including a review of the regulation of cerebral blood flow and metabolism. A review of the effects and mechanism of action of caffeine will follow this.

1.1. Stroke

The impact of stroke can be devastating both for the individual and for society. In most developed countries it is the largest cause of severe disability (1,2). Worldwide, it is the second leading single cause of death behind ischaemic heart disease (3). In the United Kingdom there are around110 000 first strokes and 30 000 recurrent strokes diagnosed each year. Approximately half of these stroke victims will be physically dependent six months after the event (4). Although normally perceived as a disease of the elderly, 25% of these strokes occur in

people of working age (5). This has huge financial implications for the National Health Service and Social Services, accounting for over 5% of expenditure (6).

1.1.1. Definition

The World Health Organisation (1978) defines stroke as:
A clinical syndrome characterised by rapidly developing signs of focal or global loss of cerebral function with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than vascular origin (7).

The majority of strokes (about 80%) are due to cerebral ischaemia. This is usually secondary to atherothrombosis, thromboembolism or intracranial small vessel disease. The remainder are mainly haemorrhagic in origin, either due to a primary intracerebral haemorrhage (about 10%) or subarachnoid haemorrhage (about 5%).

1.1.2 The classification of ischaemic stroke

The neurological symptoms and signs of a stroke reflect the area of the brain that has been deprived of blood supply or compromised by haemorrhage and oedema. The cerebral circulation is a dynamic system with large inter- and intra-subject variability but can be simply described in two main parts: the anterior (carotid) and posterior (vertebrobasilar) systems (8). For each system there are extracranial arteries (e.g. the common carotid artery), the large intracranial arteries (e.g. the middle cerebral artery) and the small superficial and deep perforating arteries. The larger intracranial arteries have important anastamotic connections over the pial surface and via the circle of Willis at the base of the brain.

Bamford et al (1991) devised a classification of ischaemic stroke based on the clinical features exhibited by the patient (9,10). This has been widely accepted in routine clinical practice and has proved to be a reasonably valid way of predicting the site and size of cerebral infarction on CT scan. In a recent study the Oxfordshire Community Stroke Project (OSCP) classification predicted the site of infarct in three quarters of patients (11). This classification also provides estimation for the prognosis of each subtype in terms of recurrence, mortality rate and probability of functional independence at one year. Using this classification as a framework, the individual clinician can plan appropriate investigations, better predict the outcome and communicate more effectively with the patient and their relatives.

The following four distinct clinical subtypes are described:
Total Anterior Circulation Syndrome (TACS), Partial Anterior Circulation Syndrome (PACS), Posterior Circulation Syndrome (POCS) and Lacunar Syndrome (LACS). Once brain imaging has excluded intracerebral haemorrhage the 4 groups are reclassified as total or partial anterior circulation infarct (TACI or PACI), lacunar infarct (LACI) and posterior circulation infarct (POCI). Each will be discussed in turn.

Patients with TACS present with hemiparesis, with or without hemisensory loss, involving two body parts out of face, upper and lower limbs: homonymous hemianopia and a new cortical deficit such as aphasia. This syndrome is reasonably predictive of a large middle cerebral artery (MCA) territory infarct on CT scan involving both deep and superficial areas. TACS does not distinguish those in which the anterior cerebral artery territory is also affected. There is substantial evidence that the cause will either be occlusion of the MCA trunk (or proximal large branch) or occlusion of the Internal Carotid Artery in the neck. This is commonly due to embolism from the heart or atherothrombosis of the Internal Carotid artery (ICA) and aortic arch. Rare causes include carotid dissection.

Patients with PACS present with only two of the three components of the TACS group, with higher cerebral dysfunction alone or a motor/sensory deficit restricted to one body part. This group predicts a more restricted predominantly cortical infarct on CT scan, due to occlusion of a branch of the MCA, or less commonly of the trunk of the anterior cerebral artery. This syndrome is generally caused by embolism from the heart or from proximal sites of atherothrombosis.

Patients with POCS can present with any of the following: ipsilateral cranial nerve palsy with contralateral motor and/or sensory deficit; bilateral motor and/or sensory deficit; disorder of conjugate eye movement; ataxic hemiparesis or isolated homonymous visual field defect. This group represents infarcts around the brainstem, cerebellum or occipital lobes. It is difficult to correlate the areas of infarction with specific vessel occlusion due to the variable vascular anatomy around the brainstem. The cause is usually in-situ thrombus and less commonly embolism.

Patients with LACS present with pure motor or pure sensory stroke, sensori-motor stroke or ataxic hemiparesis. This syndrome is highly predictive of a small lacunar infarct in the basal ganglia or pons. Most lacunar infarcts are thought to be due to intrinsic disease of a single perforating artery, either by microatheroma or lipohyalinosis. Lacunar infarcts are commonly seen in hypertensive subjects.

The Oxford Community Stroke Project found that 51% of cerebral infarcts are of the anterior circulation subtype (TACS and PACS). These are associated with the poorest prognosis; 96% of patients with TACS and 43% of patients with PACS are dead or dependent at 1 year (9). Patients with PACS are at particular risk of early recurrent stroke. Although the case fatality of LACS is low a large proportion are substantially handicapped. Finally, a few patients in the POCS group will die early presumably due to interference with vital brainstem structures

1.2 Regulation of cerebral blood flow

The survival of the brain is dependent on a continuous and adequate supply of oxygen and glucose. In spite of the brain comprising only 2% of total body weight, it requires 15-20% of the total cardiac output and consumes about 20% of the oxygen and 25% of the glucose at rest. Unlike other organs, it uses glucose as its sole substrate for energy metabolism, via glycolysis and the tricarboxylic acid cycle. Each molecule of glucose yields 36 molecules of adenosine triphosphate (ATP) via aerobic glycolysis but in the absence of oxygen (anaerobic glycolysis) only 2 molecules of ATP. Neurones require a constant supply of ATP to maintain their functional and structural integrity. In normal circumstances the brain receives two to three times its requirement of oxygen per minute and seven times that of glucose so that there is a considerable reserve or margin of safety.

With advances in methods of measuring cerebral blood flow and metabolism, our knowledge of the factors controlling cerebral circulation has increased. These will now be discussed.

1.2.1 Metabolic Regulation

The coupling of cerebral blood flow (CBF) to cerebral metabolic activity was first suspected over 100 years ago (12). Since then numerous studies have confirmed that cerebral blood flow is closely matched to the metabolic needs of the brain tissue both globally and locally (13, 14,15).

Thus, in epileptic seizures CBF is high, and in comatose states it is low. Local blood flow varies directly with local brain function by 10-20% even when global cerebral blood flow stays fairly constant. Metabolically more active regions of the

brain receive proportionally more blood than less active areas (16). Grey matter areas with higher metabolic rates than white matter areas have higher blood flow.

The regional coupling of flow to metabolism can be quantified by reference to the fractional extraction of oxygen from the blood. In normal resting conditions, the fractional extraction of oxygen and glucose from the blood will remain fairly constant throughout the brain. Normally, regional oxygen extraction fraction (OEF) is about one third and the regional extraction fraction of glucose is about one tenth (17). If increases in regional cerebral blood flow (rCBF) occur due to physiological increases in local neuronal activity the oxygen extraction fraction will decrease although glucose extraction appears to remain relatively constant. Conversely, a fall in rCBF will result in a compensatory increase in oxygen extraction to maintain metabolic activity (18).

In the damaged brain the coupling of blood flow and metabolism is lost. This can be seen in conditions such as subarachnoid haemorrhage or around cerebral tumours. Drugs such as barbiturates, which depress both metabolism and flow in equal proportions, can also modify the relationship between metabolism and flow.

For many years it was only possible to measure average blood flow from one hemisphere of the brain. The introduction of the xenon clearance method (see Section 2.2) enabled cerebral blood flow to be measured simultaneously in multiple areas of the brain. Different forms of mental and physical activity such as hand movement, talking and reading were shown to cause local variations in cerebral blood flow (19,20,21,22,23). More recently, positron emission tomography (PET) has confirmed that such increases in cerebral blood flow are indeed linked to increases in local glucose utilisation (24).

1.2.2. Cerebral Autoregulation

Cerebral autoregulation is defined as the ability of the brain to maintain a constant blood flow in the face of varying cerebral perfusion pressure. This homeostatic mechanism allows blood supply to the brain to match metabolic demand during daily activities such as change in posture, or in more critical conditions such as haemorrhagic shock. Autoregulation also protects cerebral vessels against excessive flow during transient or chronic hypertension (25). The first observations of cerebral autoregulation were made by Fog 60 years ago as he studied the pial vessels of the cat through a cranial window (26).

The cerebral perfusion pressure (CPP) and the cerebrovascular resistance (CVR) in the cerebral vascular tree determine cerebral blood flow (CBF). Autoregulation occurs by altering the cerebrovascular resistance imposed by the size of the intracranial arteries. In broad terms, it can be defined as vasodilatation as cerebral perfusion pressure decreases and the occurrence of vasoconstriction in response to increased cerebral perfusion pressure.

The following equation describes the ratio between flow, pressure and resistance.

CBF = CPP/CVR

For the brain the perfusion pressure is the difference between the mean arterial pressure and the venous backpressure. Since venous backpressure is normally negligible the CPP is equal to the systemic arterial pressure. It can be seen that in effective cerebral autoregulation, cerebral blood flow will be kept constant by altering the CVR in proportion to changes in CPP (27,29).

Cerebral autoregulation is effective over a wide range of perfusion pressures, but there are upper and lower limits beyond which the autoregulatory mechanism

fails. In healthy individuals these limits are about 60mmHg and 150mmHg respectively (28). Below the lower limit the capacity for compensatory vasodilatation has been exceeded and further reductions in CPP will now produce decreases in CBF. As the CBF falls the brain will increase the oxygen extraction fraction to maintain cerebral oxygen metabolism. There will come a point when the increase in OEF is no longer adequate to supply the energy needs of the brain and clinical evidence of brain dysfunction will appear. This may be reversible if the circulation is rapidly restored. Further declines in CBF will result in permanent tissue damage.

Above the upper limit, vasoconstriction leads to increases in intraluminal pressure and cerebral blood flow increases. The increased intraluminal pressure leads to a forceful dilatation of the arteriole with damage to the blood brain barrier. As a result, secondary decreases in flow may develop due to brain oedema.

The underlying mechanism of cerebral autoregulation is controversial. The myogenic hypothesis suggests that the smooth muscle of cerebral vessels is intrinsically responsive to changes in transmural pressure. The metabolic hypothesis postulates that changes in cerebral perfusion pressure lead to accumulation of vasoactive metabolites in the perivascular space, which produce vasodilation of the cerebral vessels (30). These metabolites may include adenosine, carbon dioxide, hydrogen ion, lactate or potassium. This metabolic theory is supported by the fact that focal cerebral ischaemia may influence blood flow in remote areas of the brain.

More recently, endothelial cell factors have been suggested as serving an important role in autoregulation, both for detecting and responding to changes in flow and pressure. Autoregulation was impaired in vivo in rats when the cerebral endothelium was removed (31).

The neurogenic theory suggests that changes in vessel diameter are mediated via efferent extrinsic vasomotor nerves, which may be under sympathetic or parasympathetic control (32). This is unlikely to be the major factor though as autoregulation is still preserved after surgical division of aortic and carotid sinus nerves.

On present evidence it seems likely that cerebral autoregulation is due to an intrinsic myogenic reflex, which may be modulated by neurogenic and metabolic factors. Dynamic autoregulation refers to the ability to maintain CBF in the face of blood pressure changes occurring over a matter of seconds and reflects the vasoregulatory capacity of the cerebral vessels (33). Static autoregulation refers to CBF adjustments in response to more prolonged BP changes and is a measure of the overall efficiency of the system.

Losses of cerebral autoregulation results in pressure passive changes in cerebral blood flow i.e. changes in cerebral blood flow follow changes in systemic blood pressure. This may occur in a variety of clinical situations including head injury, carotid artery stenosis and vasospasm secondary to subarachnoid haemorrhage (34). Loss of autoregulation also occurs in acute ischaemic stroke (35). In long-term diabetes mellitus there may be chronic impairment of CBF regulation, probably due to diabetic microangiopathy. Patients in whom autoregulation is impaired are at a much greater risk of cerebral ischaemia or secondary brain damage due to hypo- and hyper- perfusion, since they are unprotected from blood pressure changes.

In chronic hypertension, the limits of autoregulation are shifted towards high blood pressure. Therapeutic intervention with an Angiotensin Converting Enzyme (ACE) Inhibitor shifts the limits of autoregulation towards lower blood pressure values (36). A recent study demonstrated that dynamic autoregulation is unaffected by aging (37).

1.2.3 Cerebral Vasoreactivity

The capacity of the resistance vessels to vasodilate is known as cerebral vasoreactivity and the responsiveness to a vasodilatory stimulus can be used as a test of cerebrovascular reserve capacity (38).

Carbon dioxide is a potent vasodilator stimulus and thus, hypercapnia causes cerebral vasodilatation and a marked increase in cerebral blood flow. The response to hypercapnia is a measure of the amount of pre-existing vasodilataton and can indicate which subjects might be at risk from hypotension or progression of any extracranial cerebrovascular disease (39).

The cerebral vasoreactivity index can be expressed as the percentage increase in cerebral blood flow or velocity per mmHg increase in arterial carbon dioxide tension. In a normal individual a 1mmHg rise in the arterial tension of CO_2 ($PaCO_2$) within the range of 20-60mmHg causes a 2-4% increase in cerebral blood flow. Valdueza (1999) demonstrated that the middle cerebral artery (MCA) undergoes a vasodilatation of 9.5% in maximal hypercapnia (40). Carbon dioxide mediates its effects on changes in the cerebrovascular smooth muscle primarily by altering the pH of the extracellular fluid surrounding the resistance vessels.

Vasoreactivity can be measured by a variety of methods such as positron emission tomography (PET), Xenon-133 inhalation, stable Xenon CT or transcranial Doppler measurements of blood velocity in the MCA (see Section 2). The measurements are made before and after the application of a potent vasodilatory stimulus such as 5% carbon dioxide or acetazolamide. Account has to be taken of age, smoking and conscious level.

Acetazolamide is the selective inhibitor of carbonic anhydrase and causes an increase in arterial carbon dioxide concentration, intracellular carbonic acidosis and a decrease in brain tissue lactate and pyruvate. A standard dose of 1g

acetazolamide is usually administered intravenously although this dose may be too low to give a near maximal effect in some patients' (41). Vasoreactivity is then best assessed 10-30 minutes after administration. The CBF increase that follows acetazolamide injection is dose dependent and the effect of 1g acetazolamide is of similar magnitude to that observed with the inhalation of 7% carbon dioxide.

Percentage increases and not absolute blood velocity values are best used when assessing vasoreactivity with TCD although there is still debate about how to standardize this test. Kleiser (1992) classified vasoreactivity into three groups according to the increase in MCA blood velocity (Vmca) during hypercapnia of 1vol% CO_2 increments. A 10% or greater increase in Vmca per 1%vol change in CO_2 was normal and less than 10% was a diminished response. The response was defined as exhausted if there was less than 5% increase in Vmca during hypercapnia of 1 vol%. During CO_2 reactivity testing blood pressure should be monitored as well as CO_2 since changes in BP can interfere with measurements of cerebral blood velocity due to delay in autoregulation (42).

Dahl et al (1994) assessed vasoreactivity using simultaneous TCD and single photon emission computerized tomography (SPECT) monitoring. They found that changes in side-to-side asymmetry were of more value than changes in absolute values when assessing vasoreactivity using acetazolamide. There was no relationship between the basal rCBF and the rCBF increase, suggesting that absolute rCBF values should be used when defining normal limits for vasoreactivity using SPECT (43).

Studies assessing the relationship between cerebrovascular reactivity and age have shown conflicting results. Some studies have found no change with age in the vasodilatory response to acetazolamide (37, 44). Other authors have found an unchanged or reduced vasoreactivity response with aging when using hypercapnia (45,46).

Information regarding cerebral vasoreactivity has prognostic significance in patients with occlusive carotid artery disease who may benefit from vascular reconstructive surgery. Prospective studies have shown a close correlation between markedly impaired vasoreactivity and increased risk of stroke ipsilaterally to an occluded internal carotid artery (47,48, 49,50).

In contrast to carbon dioxide, changes in arterial oxygen concentration have little effect on CBF until PaO2 falls below 6.7kPa. Thereafter CBF rises markedly.

In focal cerebral ischaemia, cerebral vasoreactivity is virtually abolished within the ischaemic core and may be present but reduced in more peripheral areas. Patients with acute cerebral infarcts have impaired vasoreactivity on the affected side but not on the unaffected side. Follow up studies suggest a spontaneous improvement of formerly impaired vasoreactivity with time. In a small subgroup of patients the predominant mechanism of stroke is low blood flow rather than thromboembolism. This group of 'at-risk' patients is identified by the detection of impaired cerebral vasoreactivity (51,52).

Usually, impaired vasoreactivity is associated with impaired cerebral autoregulation. However, in some circumstances there is disassociation with impairment of autoregulation despite normal cerebral vasoreactivity and vice versa. Olsen (1983) demonstrated this disassociation in acute stroke patients' (53).

1.3 Pathophysiology of ischaemic stroke

Cerebral ischaemia can be defined as the state in which cerebral energy metabolism becomes limited by an inadequate blood supply. The reduction in blood flow is usually due to vascular occlusion or a fall in arterial blood pressure, but it may also be caused by raised intracranial or tissue pressure. The normal brain uses about one third of its energy supply for maintenance of synaptic transmission, one third for transport of sodium and potassium, and one third for preserving structural integrity. During ischaemia, several compensatory mechanisms occur in neurones, which sacrifice electrophysiological activity in order to reduce energy use. This enables membrane ion gradients and cell viability to be temporarily preserved.

Infarction occurs when there is persisting cerebral ischaemia with irreversible neuronal damage and cell death. The size of infarction depends on the site of vascular occlusion, the degree of reduction in cerebral blood flow, and the availability of collateral supply. Infarction should not be seen as a discrete "all-or-nothing" event but rather a dynamic, unstable process in which changes are occurring in neurones and glial cells at a structural and cellular level (4).

1.3.1 Ischaemic Penumbra

Both experimental and clinical studies have shown that there are critical blood flow thresholds for certain brain functions (54,55,56,57). These thresholds show considerable variability according to the technique of flow measurement used and the animal species under study. The following values are approximately correct for humans. At cerebral blood flow rates of about 55ml/100g/minute protein synthesis is inhibited, followed by stimulation of anaerobic glycolysis at flow rates of 35ml/100g/min (61). At cerebral blood flow rates less than about 20ml/100g/minute there is electrical failure of the neurones with flattening of the

EEG. With further falls in CBF below 10ml/100g/minute there is membrane failure with disturbance of cellular ion homeostasis. These critical flow rates can alternatively be expressed as percentages of baseline values. For example, electrical failure of neurones occurs at 40% and membrane failure occurs at 12% of normal control values (58).

The findings of separate thresholds for cessation of electrical signals and for loss of ion homeostasis led to the concept of an ischaemic penumbra (59,61). This represents an area of tissue within and around a core of densely ischaemic brain containing electrically unexcitable but potentially viable cells with maintained ion homeostasis. The penumbra concept focuses the attention on cells suspended in a state somewhere between survival and overt energy failure. Pharmacologically the penumbra can be defined as the part of the infarct that is potentially salvagable. The viability of the penumbra depends on residual flow and the duration and severity of ischaemia (60). The penumbra has been documented in man although it is unclear how long the penumbra remains in this state or how much recovery is likely if cerebral blood flow is restored (62,63). Bowler (1998) demonstrated spontaneous reperfusion occurring within the first week after cerebral infarction (55).

1.3.2 Imaging cerebral ischaemia

Recent technological advances studying the cerebral circulation have had a major impact on our understanding of the pathophysiology of cerebral ischaemia. This has been associated with an expansion in stroke research to search for new and effective strategies in the prevention and treatment of stroke.

Older methods of measuring cerebral blood flow involved using a radioactive tracer such as xenon. Olsen et al (1983) used the xenon intracarotid injection technique to demonstrate that the size and location of the penumbral regions

depended on the site and extent of middle cerebral artery occlusion. (63). Newer techniques of imaging the ischaemic penumbra include Single Photon Emission Computerised Tomography (SPECT) scanning, Positron Emission Tomography (PET) scanning and Xenon computed tomography.

SPECT is an isotope technique in which cross sectional images of the brain are obtained using a SPECT scanner and computerized reconstruction of data from the emitted radiation. Various radiopharmaceuticals are available for SPECT scanning such as hexamethyl propylene amine oxime (HMPAO).

SPECT scanning has demonstrated areas of relative hypoperfusion corresponding to ischaemic tissue within a few hours of stroke onset. In contrast, in the first hours or days after stroke there may be areas of relatively increased blood flow, the "luxury perfusion syndrome" (64). SPECT scanning has also demonstrated changes in blood flow in the contralateral cerebral hemisphere following the acute onset of unilateral cerebral infarction. This phenomenon is termed diaschisis and refers to changes in cerebral blood flow and metabolism occurring in the unaffected hemisphere after a unilateral ischaemic stroke. The exact mechanism is unclear although it has been suggested that contralateral flow changes are due to decreased neuronal stimulation, loss of cerebral autoregulation and release of vasoactive substances into the cerebrospinal fluid (65).

Positron Emission tomography (PET) can be used to display colour coded tomographic pictures obtained by analyzing complicated signals from positron – emitting isotopes. PET has the capability to measure diverse physiological variables such as regional cerebral blood flow and blood volume and the cerebral metabolic rate of oxygen and brain glucose utilization. Experimental studies using PET examined changes in these physiological variables after occlusion of the middle cerebral artery in baboons and cats (66,67). Clinical PET studies provided data on flow and metabolic values predicting final infarction or

suggesting viability at the time of measurement (68). The penumbra was defined as the region around the infarct with misery perfusion i.e. decreased CBF but increased OEF. Penumbral tissue was observed within the first hours of onset of stroke and in some cases was detected for up to 48 hours (69).

1.3.3 The Ischaemic Cascade

At the cellular level a sequence of biochemical events occurs during ischaemia, commonly known as the ischaemic cascade. Central to this is the failure of synthesis of adenosine triphosphate (ATP), which threatens cell survival in three ways (70). Firstly anaerobic glycolysis is stimulated leading to intra and extra-cellular acidosis. The acidosis further exacerbates tissue damage by promoting oedema formation and inhibiting mitochondrial respiration.

Secondly, there is disruption of ion homeostasis with influx of sodium and chloride into cells. Membrane depolarisation causes the opening of voltage operated calcium channels (due to ATP depletion) leading to an increase in intracellular calcium. The calcium activates a number of subsequent enzymes including nitric oxide synthetase, leading to the production of damaging free radicals. Further disturbances in ionic influx occur as a result of the release of glutamate into the extracellular space and its effect on the N-methyl-D aspartate (NMDA) receptor. Increases in other neurotransmitters such as GABA, dopamine and noradrenaline also contribute to the damage.

Thirdly, there is destruction of the structural integrity of the cell. Calcium activates proteases that lyse structural proteins and phospholipases, which hydrolyse membrane lipids.

The following section concentrates on adenosine and it's specific role in the pathophysiology of ischaemia. Since the actions of caffeine are mediated via antagonism of adenosine receptors, this has specific relevance to this study.

1.3.4 Adenosine

An increasingly large body of evidence suggests that adenosine functions as an endogenous neuroprotective substance in brain ischaemia.

Adenosine is formed from intracellular AMP or from the breakdown of extracellular ATP. It is normally present in the extracellular fluid in most tissues of the body, including the brain. The rate of adenosine production is enhanced when the energy demand is larger than the rate of energy supply.

Adenosine receptors are located on cell membranes of the central nervous system and other tissues, including blood vessels, platelets and polymorphonuclear leucocytes. They are subdivided into A1, A2a, A2b and A3 subtypes and are linked via GTP binding proteins (G-proteins) to different effector systems such as adenyl cyclase (71). A1 and A2a receptors in the brain can be localised by autoradiography with radioactive ligands.

The A2a receptor is abundant in basal ganglia, vasculature and platelets and stimulates adenyl cyclase. The A1 receptor is found in the hippocampus, cerebral and cerebellar cortex and thalamic nuclei and inhibits adenyl cyclase. Thus A1 and A2a receptors have partly opposing actions at the cellular level. This is interesting because the two types of receptor are sometimes expressed in the same cell.

During periods of hypoxia or ischaemia, the extracellular concentration of adenosine rapidly increases due to catabolism of intracellular ATP. During

ischaemic stroke endogenous adenosine may be elevated by as much as 50 times the basal level (72). This rise is sufficient to cause an inhibition of platelet aggregation and thromboembolization via adenosine's actions on A2 receptors.

Adenosine receptors are concentrated in those brain regions selectively vulnerable to ischaemic injury. These receptors are significantly and rapidly decreased in number in gerbils and rats subjected to brief periods of cerebral ischaemia (73).

There are several ways in which adenosine may be neuroprotective, and the A1 receptor-mediated pre- and post- synaptic action is of particular importance. By inhibition of neuronal calcium influx, adenosine presynaptically inhibits the release of potentially damaging excitatory neurotransmitters such as glutamate and aspartate. These transmitters increase markedly during ischaemia and may impair intracellular calcium homeostasis or induce uncontrolled membrane depolarisation via ion channel-linked glutamate receptors, especially of the NMDA type. In addition, adenosine directly stabilises the membrane potential by increasing the conductance for potassium and chloride ions, thereby counteracting excessive membrane depolarisation and preventing the vicious cycle of uncontrolled membrane depolarisation and calcium influx which leads to neuronal cell death.In experimental studies, pharmacological manipulation of the adenosine system by adenosine receptor antagonists tended to aggravate ischaemic brain damage, whereas the reinforcement of adenosine action by adenosine agonists showed neuroprotection. In one study rats treated with an acute dose of caffeine (an adenosine antagonist) 30 minutes prior to an ischaemic insult showed accelerated ischaemic brain injury in histopathological analysis and magnetic resonance imaging (74). Conversely, administration of an adenosine receptor agonist, 2-chloradenosine, protected against ischaemic hippocampal cell loss in the rat and gerbil (75,76).

In addition to these neuromodulatory effects, adenosine may improve the cerebral microcirculation via its action on A2 receptors in the cerebral vasculature. Adenosine has been proposed as a regulator of cerebral blood flow (77,78). Adenosine is a powerful vasodilator including the cerebral pial vessels. In experiments in the dog smaller cerebral vessels showed a greater dilatation to topical adenosine than larger vessels. How adenosine elicits relaxation of vascular smooth muscle is unclear. The most likely mechanism is via the production of an increase in vascular smooth muscle cyclic AMP that has been reported to be associated with vascular smooth muscle relaxation. Adenosine acting on A2 receptors may also improve oxygen and substrate supply during ischaemia by its anti-thrombotic effects.

1.4 Caffeine

Since Emperor Shen Nung discovered tea in 2737 BC, caffeine has been a common part of the human diet. After having been limited to the Arab world until the fifteenth century, coffee consumption reached Europe during the sixteenth century and rapidly spread throughout the Continent. Today, caffeine is the most widely used pharmacological substance, with average human caffeine consumption ranging in different cultures and nations from 80 – 400 mg/person per day (79). In several countries caffeine consumption occupies a significant place in national culture with highest caffeine consumption rates being seen in Sweden and Finland. In the United Kingdom average caffeine consumption is 200mg/person/day with 55% of this coming from tea.

The main sources of caffeine are coffee, tea and caffeinated cola drinks. The caffeine content of tea and coffee varies, depending on their method of preparation. For example, a 150ml cup of instant coffee contains about 66mg of caffeine whereas a cup of percolated coffee contains up to 116mg and a cup of filter coffee contains 142mg of caffeine. Similarly a cup of tea made with a bag brewed for 1 minute contains 28mg caffeine and this increases to 44mg after three minutes (80). A 12 oz can of regular coca cola contains 65 mg caffeine and one of pepsi cola contains 43 mg caffeine. Caffeine is also found in other carbonated drinks such as original Lucozade, Tab clear and Dr.Pepper. Plain chocolate contains more caffeine than milk chocolate. Products flavoured with chocolate contain only small amounts of caffeine (e.g. chocolate ice cream has 2-5mg per 50g). Caffeine is also found in many cold and flu medications, many of which are available over the counter.

Drug dosage is also related to body size or weight. For example, when a child drinks a can of cola, caffeine intake is comparable to an adult drinking 4 cups of instant coffee.

1.4.1 Pharmacokinetics

Caffeine is readily absorbed after oral administration and is widely distributed throughout the body. Peak plasma concentrations are reached 15-45 minutes after ingestion (81). The average half-life of caffeine is 5 hours but with considerable variation between individuals (range 3-10 hours). In adult males caffeine half-life is reduced by 30-50% in smokers compared to non-smokers whereas it is approximately doubled in women taking the oral contraceptive (82,83).

Being highly lipid soluble it rapidly crosses the blood-brain barrier by diffusion and by a saturable transport system. Experiments in dogs have shown that caffeine concentration in cerebrospinal fluid reaches one half of its level in plasma within only 4-8 minutes and that brain concentration of caffeine remains stable for at least one hour (84). In humans the concentrations of caffeine in plasma and brain are proportional to the dose of caffeine administered 1 hour before. This correlation between plasma and brain concentrations of caffeine and the administered dose is highly significant (85).

Caffeine is metabolised almost completely in the liver via oxidation, demethylation and acetylation and is excreted in the urine. The metabolism of methylxanthines varies with age; indeed premature babies are able to synthesis caffeine from theophylline.

1.4.2 Mechanisms of action

Caffeine (tri-methylxanthine) is a methylxanthine. Related compounds are theophylline (di-methyl xanthine) and aminophylline (theophylline-ethelyendiamine). The actions of methylxanthines are mediated primarily via competitive antagonism of adenosine receptors (85,86). Four adenosine

receptors have previously been described. Caffeine acts most potently at A2a receptors, closely followed by A1 receptors, then A2b receptors and as a weak antagonist at A3 receptors. At higher doses caffeine inhibits phosphodiesterases, blocks GABA receptors and mobilises intracellular calcium.

Blockade by caffeine of adenosine receptors inhibits the actions of endogenous adenosine. In animals, most pharmacological effects of adenosine in the brain can be suppressed by relatively low concentrations of circulating caffeine i.e. less than 100μM. Adenosine decreases the firing rate of neurones and exerts an inhibitory effect on synaptic transmission and on the release of most neurotransmitters, while caffeine increases the turnover of many neurotransmitters, including dopamine, noradrenaline and 5-hydroxytryptamine.

The inhibition of adenosine by caffeine can be demonstrated elsewhere in the body. Adenosine causes bronchial constriction and therefore methylxanthines are widely used as medication to treat asthma and apnoea in the newborn. Adenosine inhibits platelet aggregation and recently in A2a receptor-knockout mice it was reported that platelet aggregation was increased, indicating the importance of this receptor subtype in platelet function (87).

1.4.3 Effects of chronic caffeine consumption

It is known that tolerance develops to some but not all effects of caffeine with chronic intake. The precise mechanism underlying these effects is not known.

Chronic exposure to caffeine increases the number of adenosine receptors (upregulation) but these observations have previously been mostly limited to animal models that study A1 receptors (88, 89). This receptor upregulation could increase stimulation of inhibitory adenosine pathways resulting in neuroprotection. More recent studies in man have shown that chronic caffeine

consumption also causes a significant upregulation of A2a adenosine binding sites (90, 91). Since activation of A2a receptors causes a decrease in platelet aggregation, it seems likely that chronic caffeine consumption leads to a paradoxical reduction in platelet aggregability.

The adaptive changes to long term caffeine are very dramatic. For example, long-term caffeine treatment leads to a decreased susceptibility to ischaemic brain damage, whereas acute treatment with caffeine exacerbates the damage. To date all these studies have been conducted on animals and are summarised in Table 1.

In contrast to acute administration of caffeine, chronic caffeine treatment significantly reduced the neuronal damage in the gerbil hippocampus following 5 minutes of bilateral carotid occlusion. This can be attributed to increased numbers of A1 receptors. A similar finding occurred in rats subjected to 15-minute bilateral carotid occlusion and hypotension using histopathology and magnetic resonance imaging as indices.

Similarly it is well known that high doses of caffeine can precipitate seizures in man. However, long-term treatment with caffeine leads to a decrease in seizure susceptibility in mice (94).

Table 1. Effect of caffeine on ischaemic brain damage in animal studies

Dose (mg/kg)	Type of Ischaemia	Species	Effects	Ref
30 p.o pre	3 min bilateral carotid occlusion	Gerbil	Increased necrosis	93
10 i.v. pre	10 min bilateral carotid occlusion + hpotension	Rat	Increased hippocampal, cortical and striatal damage	74
10 i.v. pre	3 hour MCA occlusion	Rat	No effect on infarct size	176
0.2% in water 4weeks pre	5 min bilateral carotid occlusion	Gerbil	Reduced necrosis	93
3x 20-30/day p.o. 3 weeks pre	10 min bilateral carotid occlusion + hypotension	Rat	Reduced brain damage	74

1.4.4 Effects of caffeine on the Cardiovascular system

Initial reports indicated that caffeine has a pressor effect, raising the possibility that it might contribute to hypertension. However, further studies have clearly demonstrated that caffeine does not produce a persistent increase in blood pressure. Individuals who do not regularly consume caffeine may experience a slight increase in blood pressure when they are exposed to caffeine, but

tolerance develops rapidly and blood pressure returns to baseline (95). The tolerance to cardiovascular effects of caffeine is paralleled by a decrease in caffeine-induced increases in plasma catecholamines (96).

There is no evidence that restriction of caffeine is beneficial in reducing blood pressure in the non-pharmacological management of patients with mild hypertension (97). Some studies have suggested that caffeine can prevent postprandial hypotension in subjects with autonomic failure and in healthy and frail elderly patients, although the benefit may be less for those with predominantly postural hypotension (98).

There is a large body of epidemiological evidence that caffeine, as an independent risk factor, does not increase the incidence of hypertension (99,100). Caffeine consumption does not increase the risk of coronary heart disease or stroke (101,102). Numerous epidemiological studies considering myocardial function have found no harmful effect of <5 cups of coffee per day although results are controversial at higher intake levels (103).

1.4.5 Effects of caffeine on behaviour

The stimulating action of caffeine on the central nervous system (CNS) is well established. Caffeine elicits a number of responses, including increased vigilance, decreased psychomotor reaction time, and increased sleep latency and waking time and may also influence intellectual performance (104).

The psychostimulant action of caffeine upon the CNS is dose related. In high doses caffeine can elicit anxiety, restlessness, tension, nervousness and psychomotor agitation. These effects are seen primarily in a small group of caffeine sensitive individuals. At very high doses caffeine may potentiate the action of other convulsants and even cause convulsions.

Caffeine dependence and withdrawal have been described (105,106,109). Headache, irritability, restlessness, anxiety, lethargy and depression typify withdrawal. Symptoms usually start within 24 hours of abstinence, peak during the next one or two days and last for up to a week (108). The daily dose level at which withdrawal occurs is roughly equivalent to the amount of caffeine in a single cup of strong brewed coffee or 3 cans of caffeinated soft drink. One of the significant factors motivating caffeine consumption appears to be 'withdrawal relief' (107).

1.4.6 Effects of caffeine on Cerebral Blood Flow and Metabolism

The stimulant effects of caffeine on the central nervous system are associated with changes in local rates of cerebral energy metabolism. Administration of an acute dose of 10mg/kg caffeine induces an increase in the rates of local cerebral glucose utilization. These local increases in metabolism are significant in structures involving the control of locomotor activity such as the extrapyramidal system.

Several studies have examined the effect of caffeine on cerebral blood flow. In 1935 Gibbs was the first to demonstrate that intravenous caffeine caused a decrease in cerebral blood flow as measured by a thermoelectric blood flow recorder introduced into the internal jugular vein (110).

With the introduction of the nitrous oxide technique for the quantitative determination of cerebral blood flow it was possible to obtain more precise information of the effect of caffeine on the cerebral circulation. Shenkin (1951) demonstrated that 0.46g of intravenous caffeine reduced cerebral blood flow by 15.6% after 5 minutes in 9 patients with brain tumour (111). This was consistent

with studies investigating the effect of another methylxanthine, aminophylline on cerebral blood flow (112).

In 1983 Mathew evaluated the effects of 2 doses of caffeine on CBF in normal volunteers using the Xenon inhalation technique. He demonstrated that 250mg caffeine caused a significant reduction in CBF of 22% on the right hemisphere and 18% on the left hemisphere. A further increment in dose to 500mg of caffeine did not produce more CBF reduction (113). Mathew et al followed this up in 1985 with a double blind trial using 250mg caffeine or matched placebo in 45 young healthy volunteers. Caffeine ingestion was found to be associated with significant reductions in cerebral perfusion thirty and ninety minutes later as measured using the Xenon-133 inhalation technique. The mean reduction in CBF 30 minutes after caffeine ingestion was 18% from baseline values on both sides. The CBF changes were observed uniformly across all brain regions bilaterally with the maximum reduction occurring 30 minutes after administration (114).

Cameron et al (1990) used Positron Emission Tomography (PET) to quantify the effect of caffeine on whole brain and regional cerebral blood flow (115). Eight subjects (4 with panic disorder and 4 normal) received intravenous caffeine; 2 additional subjects received oral caffeine. All subjects abstained from coffee consumption for 12 hours prior to the study. They found a dose of 250mg of caffeine whether administered intravenously or orally, caused approximately a 30% decrease in whole brain CBF fifteen and forty-five minutes later. Regional differences in caffeine effect were not observed. Caffeine decreased PaCO2 and increased systolic blood pressure significantly. However, the change in PaCO2 did not account for the change in CBF.

Several investigators have used TCD to assess the effects of caffeine on cerebral circulation. Kerr (1993) examined the effect of caffeine on middle cerebral artery velocity (Vmca) using TCD in 8 young, healthy subjects (116). After 3 days of caffeine abstinence, the ingestion of 400mg caffeine caused a

rapid and sustained 23% decrease in Vmca from 64 to 49cm/s. Debrah et al (1996) performed a similar study on 12 diabetic patients (age range 20 to 48). A dose of 250mg caffeine after overnight fasting caused a sustained and rapid fall in Vmca from 60 to 50cm/s (17%). The investigators also demonstrated enhanced hormonal and symptomatic responses to hypoglycaemia with caffeine and suggested that caffeine may be a potentially useful treatment for diabetic patients who have difficulty recognising the onset of hypoglycaemia (117).

Coutier et al (1996) used transcranial Doppler to study the effect on cerebral blood flow velocity after caffeine was introduced to caffeine users following 24 hours of caffeine abstinence (118). Velocities in the middle cerebral artery (Vmca), the posterior cerebral artery and basilar artery slowed (by 9%, 4% and 3% respectively) 30 minutes after the intake of 150mg of caffeine. Velocities returned to baseline levels 2 hours after intake.

More recently, Jones et al examined the effect of caffeine withdrawal on 10 normal volunteers who were regular caffeine consumers (119). Subjects were examined while maintaining their normal diet (baseline period) and during two 1-day periods in which they consumed caffeine free diets and received capsules containing placebo or caffeine in amounts equal to their baseline daily caffeine consumption. The results showed that abrupt cessation of daily caffeine consumption produced changes in both cerebral blood flow velocity (measured by TCD) and quantitative EEG. For both anterior and middle cerebral artery, placebo administration increased blood flow velocity relative to the caffeine condition. This was accompanied by increases in theta activity on the EEG, which is a sign generally correlated with increased drowsiness. The interpretation was that, relative to acute caffeine administration, caffeine withdrawal increased cerebral blood flow and that cerebral vasodilatation is a possible explanation for the report of headaches that are commonly associated with caffeine withdrawal.

Perod (2000) examined possible caffeine mediated changes in blood velocity in the middle cerebral artery (Vmca) as examined by tests of cerebral vasoreactivity (120). TCD ultrasonography provided bilateral Vmca measures while healthy college students' hypoventilated, hyperventilated and performed cognitive activities. These tests were carried out under non-caffeinated conditions and under two levels of caffeine. The smaller amount of caffeine was obtained from regular cola (45mg caffeine/12oz can) and the larger amount was obtained from coffee (117mg caffeine/8oz cup). The procedure began about 30 minutes after intake and subjects abstained from caffeine for at least 4 hours before a session. The smaller caffeine dose (45mg) had no effect on Vmca but a larger amount (117mg) reduced Vmca by 5.8%. The analysis showed that Vmca increased during hypoventilation, decreased during hyperventilation (with the degree of decrease attenuated under caffeine) and increased over baseline during all cognitive activities (range 3.8-6.9%).

In summary, the existing evidence demonstrates a reduction in cerebral blood flow and velocity with caffeine in healthy volunteers. There is no evidence that these falls in cerebral blood flow after caffeine administration cause any problems in the normal population in whom there is an adequate cerebral reserve. Caffeine containing foods and drink are regularly served in most hospitals and patients with a wide variety of diseases consume it. A similar reduction in cerebral blood flow in patients with impaired cerebrovascular reserve may have adverse clinical consequences.

In a previous study of twenty patients recovering from an acute ischaemic stroke using transcranial Doppler ultrasound a fall in blood velocity in the middle cerebral artery (Vmca) was demonstrated after acute ingestion of 250mg caffeine (121,122,123). Sets of Vmca measurements were obtained prior to caffeine abstention and then at 30, 20 and 10 minutes prior to ingestion of the medication (caffeine or placebo) and at 30, 60 and 90 minutes after ingestion. The average reduction in Vmca was 13% from a baseline of 47cm/s (-6.3cm/s [95% CI –3.8 to

–8.7cm/s], p<0.001) on the side of the brain affected by the stroke, and 12% from a baseline of 47cm/s (-5.6cm/s [95% CI –2.6 to –8.6cm/s], p<0.001) on the unaffected side.

These changes in blood velocity are likely to indicate a similar reduction in cerebral blood flow; the basic assumption being that changes in cerebral blood velocity represent relative changes in cerebral blood flow as long as there is no change in arterial diameter. Blood flow (F) in a particular artery depends on the cross sectional area (πr^2) and the mean velocity (V) of the blood running through it. The flow can be calculated from

$F = \pi r^2 \times V$

Changes in CBF are therefore proportional to changes in mean velocity provided the vessel diameter remains constant

In this study our aim was to investigate if the cerebral blood velocity changes previously demonstrated with caffeine reflect changes in cerebral blood flow. The primary aim was to assess the effect of caffeine on global cerebral blood flow. A further aim was to investigate the effect of caffeine on regional cerebral blood flow, especially around the infarct area. Finally, we sought to identify a subgroup of patients with impaired vasoreactivity to determine whether they responded differently to the caffeine.

1.5 Summary

Stroke is the third most common cause of death and the leading cause of disability in the developed world. Recovery after an ischaemic stroke is a complex multifactorial process, with restoration and maintenance of regional cerebral blood flow appearing an important factor. In some patients with major cerebral occlusion the cerebrovascular bed is already under chronic haemodynamic stress and further falls in cerebral blood flow after ischaemic stroke may worsen the extent of neuronal damage or limit recovery.

Caffeine is a widely consumed pharmacological substance, which produces a variety of effects in humans through blockade of adenosine receptors. Acute ingestion of caffeine in normal individuals appears to reset the coupling between cerebral blood flow and metabolism by decreasing blood brain flow while simultaneously increasing brain glucose utilization. This reduction in cerebral blood flow is unlikely to cause ischaemia in the normal healthy brain. However it is unclear whether caffeine increases the risk for ischaemia in individuals with established cerebrovascular disease who may have impaired cerebral reserve.

In this study the aim was to investigate the effect of caffeine on the cerebral circulation in patients recovering from an acute ischaemic stroke. A previous study demonstrated that the acute ingestion of caffeine reduced middle cerebral artery blood velocity in patients recovering from an acute ischaemic stroke. The objective of this study was to determine if this fall in blood velocity reflected a similar fall in cerebral blood flow. Cerebral blood flow was measured by the xenon clearance method and middle cerebral artery blood velocity was measured using transcranial Doppler ultrasound. Both these techniques will be discussed in the next chapter.

CHAPTER 2: SPECIALIST INVESTIGATIONS

2.1 Transcranial Doppler Ultrasonography

Transcranial Doppler ultrasonography is a non-invasive technique for measuring the velocity of blood flow within the larger cerebral arteries. It was introduced in clinical practice by Asalid *et al* in 1982 and provides unique information concerning the velocity, pulsatility and routes of blood flow through the Circle of Willis and vertebrobasilar system (124).

Historically, early investigators using ultrasound to study the cerebral circulation concluded that recording from the intracranial arteries would be impossible due to the barrier imposed by the human skull. It was, however, possible to evaluate the cerebral circulation by recording from the carotid arteries in the neck and this application has been widely used in clinical medicine. Transcranial Doppler ultrasonography was made possible by improvements in Doppler equipment along with the use of a relatively low frequency (2MHz) and the introduction of spectral analysis. More recent advances have included vessel mapping as well as transcranial colour coded real time ultrasonography techniques.

In this section the principles, technique and clinical applications of transcranial Doppler ultrasonography (TCD) will be described.

2.1.1 Principles of TCD

Ultrasound refers to sound frequencies higher than 20, 000 cycles per second. The typical TCD ultrasound transducer (or probe) is a piezoelectric crystal that is stimulated to emit pulses of ultrasound of a known frequency (2mHz). The Doppler effect causes ultrasound waves directed at a stream of moving red blood

cells to be scattered back with a changed frequency and the Doppler frequency shift is automatically calculated. The velocity of blood flow can be calculated from the frequency shift according to the following equation (125):

$$V = Fs.c / Fo.Cos\theta$$

where Fs = frequency shift

Fo = original frequency

V = velocity of blood flow (cm/sec)

c = velocity of sound in human soft tissues

θ = angle between ultrasound beam and axis of blood flow

Even when the Doppler spectrum is generated by laminar flow in a single artery, it represents a range of velocities (flow is usually slower at the edges and faster at the centre), which vary throughout the cardiac cycle. The computation of meaningful velocity data by analysis of that spectrum is not straightforward. For the calculations the angle between the ultrasound beam and axis of blood flow is assumed to be 0.

To simplify the analysis, a Fast Fourier Transform algorithm (FFT) is used to convert frequencies to velocities, separate the different blood velocities and to identify the maximum velocity envelope (126). This maximum blood velocity is then averaged over 3 or 4 cardiac cycles to obtain the time averaged maximum velocity (Vm). Peak systolic velocity (Vs) and end diastolic velocity (Vd) can also be calculated. The spectral analysis is displayed on a xy graph so that the x-axis represents time and the y-axis represents velocity (Figure 1).

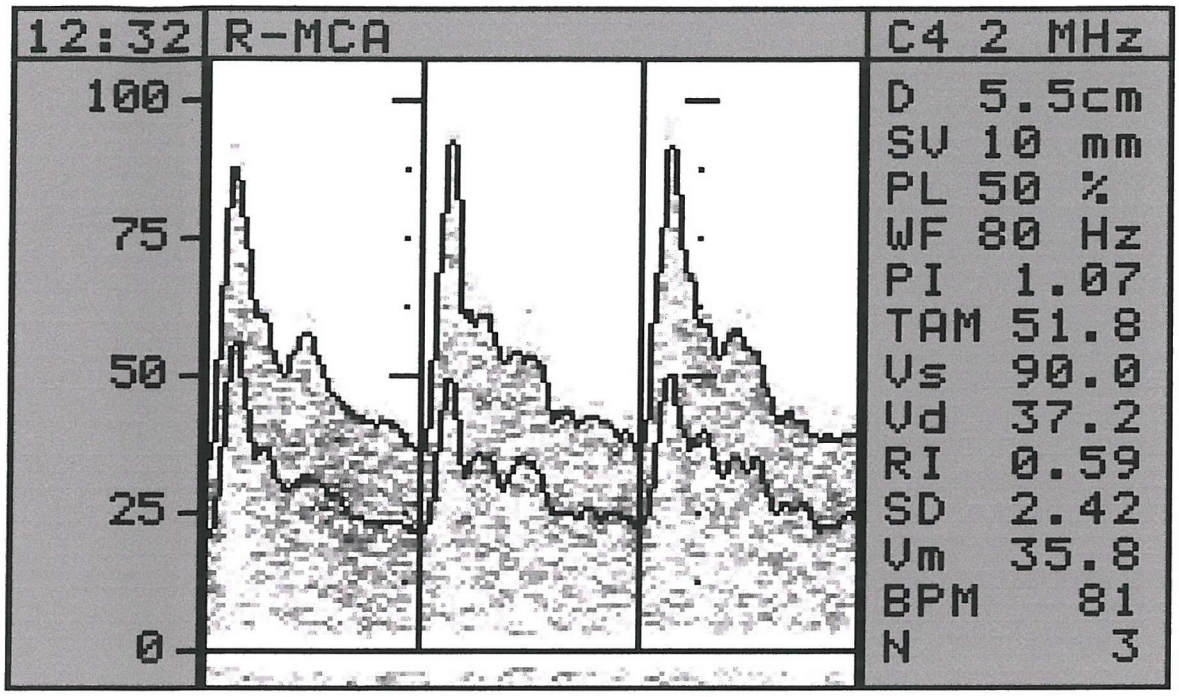

Figure 1. TCD Waveform

The Gosling pulsatility index (PI) can also be calculated by the following formula:

PI = (Vs – Vd)/ Vm

The PI gives an indication of vascular resistance and is influenced by many factors, including cardiac contractility.

Normal cerebral blood velocities vary considerably and absolute values are rarely helpful except in extreme cases. The strength of TCD lies in its ability to detect changes in blood velocity.

Interpretation of cerebral blood velocity depends on assumptions made about local haemodynamic changes. Changes in absolute blood flow will only follow those in the blood velocity envelope if the cross sectional area of the vessel remains constant (127). For example, assuming constant steady flow, a 10%

diameter decrease in the middle cerebral artery will yield a 23% velocity increase. Unexpectedly high velocities may indicate either arterial narrowing due to spasm, stenosis or higher volume flow as may be found with an arterio-venous malformation. Low velocities may indicate low volume flow distal to a stenosis or global low tissue flow. The relationship between cerebral blood velocity and cerebral blood flow is complex under pathological conditions.

There has been much discussion in the literature about the stability of the cross sectional area of large vessel diameter. On one hand several investigators suggest that the diameter of the middle cerebral artery remains relatively constant despite changes in arterial blood pressure and blood gases (128,129, 130). On the other hand some studies claim that the cross sectional area of large vessels can change for example due to sympathetic stimulation or infusion of drugs such as nitroglycerin (131).

2.1.2 TCD Examination Technique

Transcranial Doppler examination can be performed on any patient able to remain stationary in the supine or semi-recumbent position.

The preferred position for the examiner is at the head of the bed to allow an assessment of each cerebral hemisphere. The patients head should face forwards during insonation.

A hand held, low frequency (2-MHz), pulsed Doppler transducer (probe) is placed on the side of the patient's skull (Figure 2). Stereo headphones and a quiet environment allow localisation of the high frequency, low amplitude Doppler signal. The position and angle of the probe can be varied by hand until the target vessel is identified with the strongest possible Doppler signal. During a long examination the probe can be held in position by using a head strap. Vessel

identification is based on standard criteria, including the cranial window used, transducer position, depth of sample volume, direction of blood flow, and relationship to other vessels.

Transcranial Doppler examinations are performed through four naturally occurring cranial windows: transtemporal, transorbital, transforaminal and submandibular. The transtemporal window is most commonly used and occurs at regions of cortical thinning in the temporal bone. This is routinely 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. Transcranial doppler examinations without a specified window will refer to the transtemporal window. The transorbital window is used to examine the ophthalmic artery and the cavernosal portion of the ICA. The transforaminal window is accessed when the patient's head is slightly flexed forward and allows examination of the vertebral and basilar arteries. The submandibular window is accessed just below the angle of the mandible and allows assessment of the extradural section of the ICA.

Patient comfort is very important during TCD to minimise head movements, which may alter the probe position, and to avoid anxiety induced changes in blood flow, blood pressure or respiration.

There are several practical advantages in using TCD compared to other methods of studying the cerebral circulation. The procedure is non-invasive, non ionizing and is performed with relatively inexpensive equipment. Since the equipment is portable TCD can be readily performed by the bedside, in Intensive Care, or during radiological and surgical procedures.

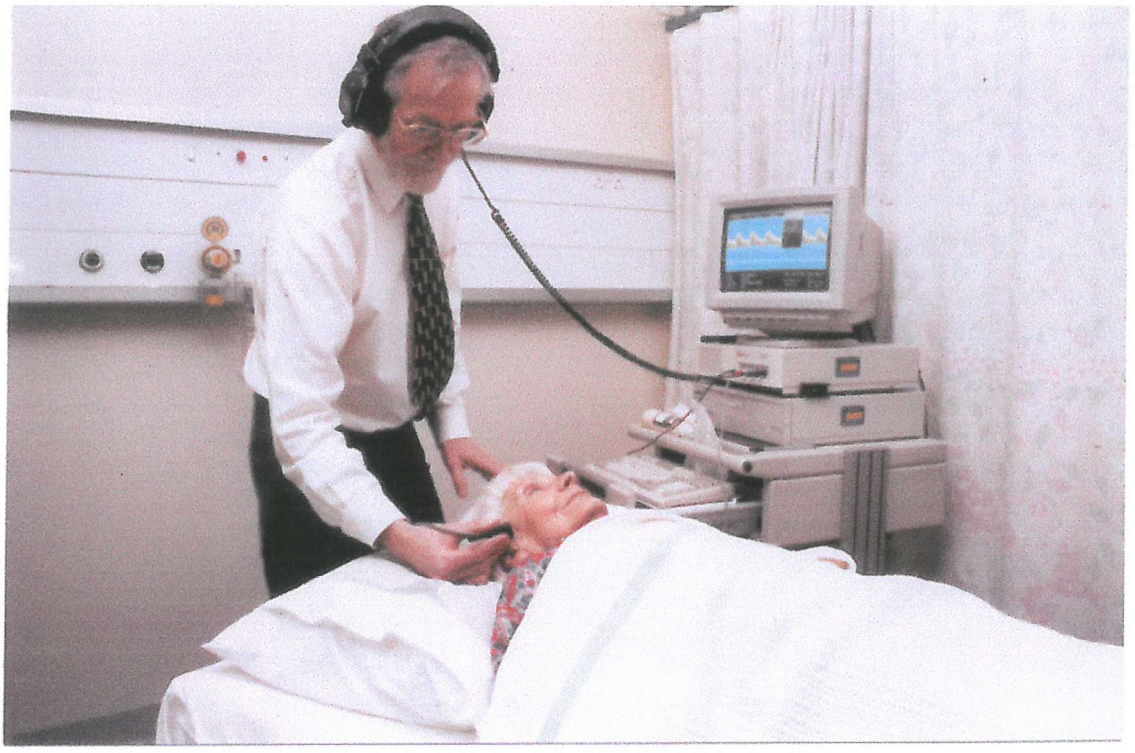

Figure 2. Transcranial Doppler examination

2.1.3 Physiological Factors affecting blood flow velocity

Several factors may alter cerebral blood flow velocity. There is a fall in flow velocity with increasing age. Other variables include haematocrit, carbon dioxide and oxygen concentration and intracranial pressure (132). Increases in cerebral

metabolism associated with functional or cognitive activities may also increase Vmca (19,133).

2.1.4 Repeatability of measurements

Reproducibility of trancranial doppler measurements allows repeated measurements in clinical studies. For example, there is only a 5% probability for two measurements of Vmca performed by the same observer using a fixed probe to differ by more than 9.66cm/sec. In other words, if such a difference occurs, it is unlikely that it is due to the inter-observer variability and can reasonably be attributed to other factors such as a physiological stimulus (134). TCD measurements are best compared if they are performed at the same hour due to diurnal variation (135).

2.1.5 Limitations of TCD

TCD is an operator dependent technique and there is a significant learning curve before an examiner can perform reproducible studies. 5-10% of patients will have inaccessible ultrasonic windows and this failure rate may approach 30% in women over the age of 60. Another issue is that the displayed Doppler spectra represent flow detected anywhere within the sample volume of the ultrasound beam. This means that the Doppler signal for vessels lying distal or proximal to the displayed depth may be detected. Finally there may be problems with radiofrequency interference from surrounding equipment, such as ventilators or intravenous pumps.

2.1.6 Clinical Uses of TCD

There are numerous clinical applications for TCD ultrasonography. A few of these will be described here.

TCD can be used to detect vasospasm after subarachnoid haemorrhage or head trauma (136,137). Comparisons between vessel diameter on cerebral angiography and elevation of TCD velocities have revealed close correlation. For example a study by Lindegaard et al revealed that TCD had a sensitivity of 94% and a specificity of 90% for detecting angiographic spasm of the middle cerebral artery (138). Knowledge of the time course of vasospasm can be clinically useful in determining the risk of delayed ischaemic neurological deficit and when it is safest to operate. TCD can also detect other causes of raised blood flow velocity such as an intracranial stenosis and may be useful in the assessment of the functional effects of carotid lesions (139).

TCD can also be used to reflect various haemodynamic changes in the cerebral circulation that occur with raised intracranial pressure. In patients with intact cerebral autoregulation, increases in ICP will cause an increase in the pulsatility index and no change in the mean blood flow velocity within the autoregulatory range. Below the range of autoregulation and in patients with absent autoregulation, increases in ICP will cause a decrease in blood velocity unless matched by an increase in arterial blood pressure. Characteristic changes in the velocity waveform have been described in patients who have progressed to brain death. TCD has been shown to be a reliable technique in determining brain death with 91% sensitivity and 100% specificity (140).

TCD is a useful tool in the assessment of cerebral autoregulation and cerebral vasoreactivity. Within 6 hours of ischaemic stroke TCD examination can help predict early deterioration and early improvement (141,142).

2.2 Measurement of Cerebral Blood Flow by Xenon Clearance

Technological advances in the measurement of cerebral blood flow, including the use of radioisotopes have contributed significantly to our understanding of brain pathophysiology.

Historically, Kety and Schmidt (1948) pioneered the nitrous oxide method of cerebral blood flow measurement (143). This was based on the Fick principle; namely, that the rate of uptake and clearance of an inert diffusible gas is proportional to blood flow in the tissue. Based on the concentration of nitrous oxide gas in arterial and jugular venous blood it was possible to yield global estimates of cerebral blood flow.

In 1961 Lassen and Ingvar were the first to employ radioisotopes for the assessment of cerebral blood flow (144). This had the advantage that by introducing collimated scintillation detectors externally placed over the skull it was possible to obtain a degree of regional blood flow measurement. These early measurements were obtained by intracarotid injection using Krypton. The more efficient gamma emitter Xenon soon replaced this (145,151). Because of the risk entailed by intracarotid injection however, Mallet and Veal (1966) proposed inhalation of the isotope while Agnoli et al (1969) and Austin et al (1972) employed intravenous injection (146,147,148). Obrist et al (1975) and Risberg (1975) later improved these minimally invasive methods (149,150).

2.2.1 Xenon-133 Characteristics

Xenon is a weakly soluble, readily diffusible inert gas. It passes through cell membranes and freely exchanges between blood and tissues. Xenon-133 is a radioactive material, produced in a nuclear reactor. During decay to stable Cesium-133 it emits a 81keV gamma photon and an X-ray of 31 keV.

The physical half-life of xenon is 5.27 days. Inhaled xenon passes though the alveolar wall and enters the pulmonary venous circulation. Most of the xenon that enters the blood circulation is returned to the lungs and exhaled after a single pass through the peripheral circulation. Therefore the effective biological half life of Xenon-133 is very short.

Several investigators have studied the tissue radiation doses from β and γ radiation when using Xenon-133. These have shown that there is no significant radiation hazard to the patient even for a relatively great number of repeated measurements.

2.2.2 The Equipment

Commercial equipment is available with typically 16 small gamma ray detectors positioned in a helmet type arrangement placed over the head. This instrument allows cortical perfusion to be assessed with some degree of regionality. The instrument is readily portable, thereby permitting examinations in special environments such as Intensive Care facilities. Results can be displayed using circles representing different flow bands superimposed on a brain outline.

2.2.3 Intracarotid rCBF Method

The intracarotid method of CBF measurement is the most accurate but its use has been limited by the invasiveness of the procedure.

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Xenon-133 in saline is injected as a bolus into one internal carotid artery. The xenon diffuses very rapidly and equilibrates between the blood and brain tissue. Within a few seconds xenon activity in the brain reaches a maximum and then

fresh arterial blood begins to wash out the isotope present in the brain tissue. The Xenon-133 emits gamma photons that are monitored with multiple collimated external detectors, placed over the injected hemisphere to record the rate of clearance. A rapid rise in count rate occurs as the bolus enters the brain, followed by a slower decline over the next 10-15 minutes as the isotope clears. Little re-circulation of Xenon occurs because the cerebral venous blood is diluted by isotope-free blood from the rest of the body, and because of efficient elimination of the gas by the lung.

The washout of the xenon from the brain can be approximately represented by two separate exponential components. The fast component corresponds to blood flow in grey matter and the slow compartment corresponds to the white matter and extracerebral tissue. The mathematical equations used to compute the blood flow values can be read in Appendix 1. This analysis yields the coefficients P1, K1, P2, K2 corresponding to the size and clearance rate of the fast and slow components. The calculations assume almost instantaneous diffusion between blood and brain. This means that the gas will distribute itself at all times in accordance with its equilibrium concentration, dependent on its solubility in blood and brain. This can be expressed as the partition coefficient (λ), defined as the ratio of blood and brain concentrations at equilibrium (152). As well as the blood flow in the grey and white matter it is possible to calculate the relative weight of the grey matter component and the hemispheric mean flow.

Owing to the weak γ radiation of Xenon 133 the underlying cortex will be seen better than the tissues at depth. Extraction of the fast component of the clearance curve provides a good estimate of cortical flow. The measurements are expressed as absolute flow values in ml/100g/min. Typically grey matter flow flows are between 60-80ml/100g/min and white matter flows are between 10-20ml/100g/min. The disadvantages of this technique are its invasive nature and that it is not possible to obtain simultaneous bilateral examinations.

2.2.4 Non Invasive rCBF Method

To avoid the need for intracarotid injection Xenon can also be administered either intravenously (via a peripheral or central vein) or by brief inhalation (1-2min). In Figure 3 the inhalation method of measuring CBF though xenon clearance is displayed.

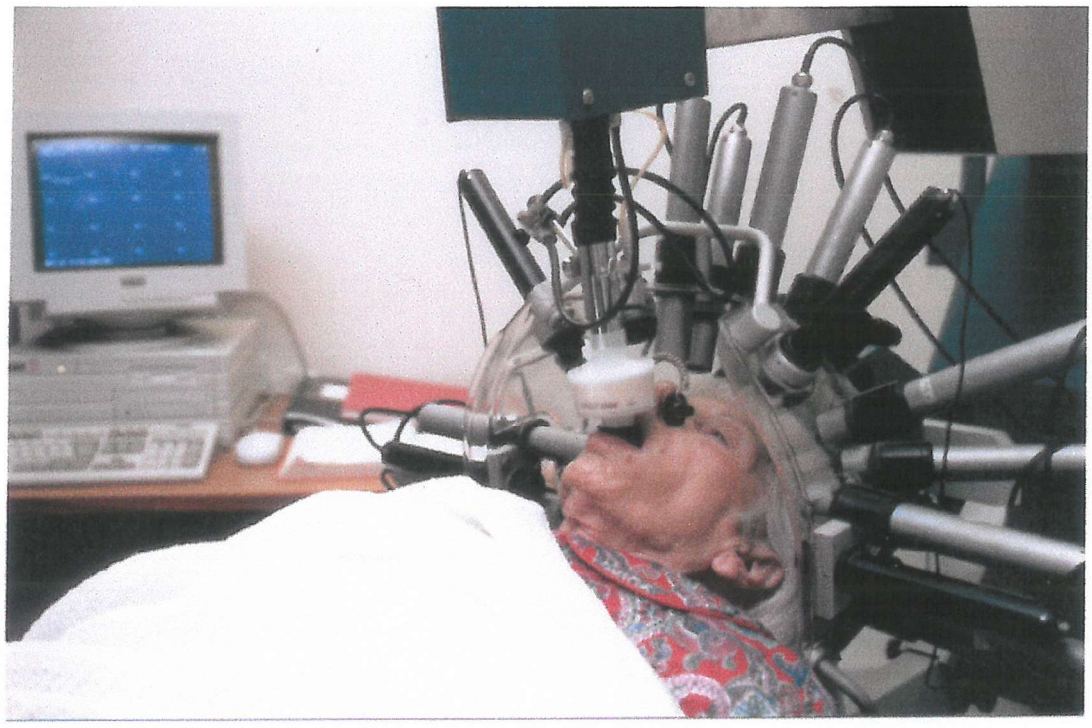


Figure 3. Measurement of CBF via inhalation of xenon

Two major difficulties beset the inhalation method that are negligible in the intracarotid technique. These are recirculation of the xenon and extracerebral contamination (153). Both will be considered in turn.

With direct intracarotid xenon injection a brief bolus directly enters the brain, from which it clears exponentially with minimal recirculation. An example of a

clearance curve from a brain detector is shown in Figure 4. A simple mathematical model can be employed to analyse the clearance curves. During inhalation or IV injection of xenon the effects of recirculation of the Xenon need to be taken into account (154). Failure to make such a correction may produce a 20-30% underestimation of CBF values. Even with a rapid intravenous injection, the bolus requires 30 seconds or more to reach the brain. Since the isotope is taken up and released by tissues throughout the body, a large amount of recirculation occurs that further prolongs the input. The xenon clearance curves measured from the brain are a convolution of the desired exponential washout curves with a curve representing the input arterial concentration of xenon.

Veall and Mallettt first proposed monitoring of end tidal air as a means of correcting Xenon Clearance Curves for recirculation (153). They demonstrated that the end tidal Xenon peaks correspond well with measured arterial concentrations provided the patient has normal pulmonary function. By drawing a fraction of the patients expired air past a radiation detector; the end tidal values can be used as the 'arterial input function' for defining the variable input of xenon to the brain in the flow calculation (Figure 5: air curve). (This procedure will fully correct for the effects of re-circulation except in patients with severe pulmonary disease).

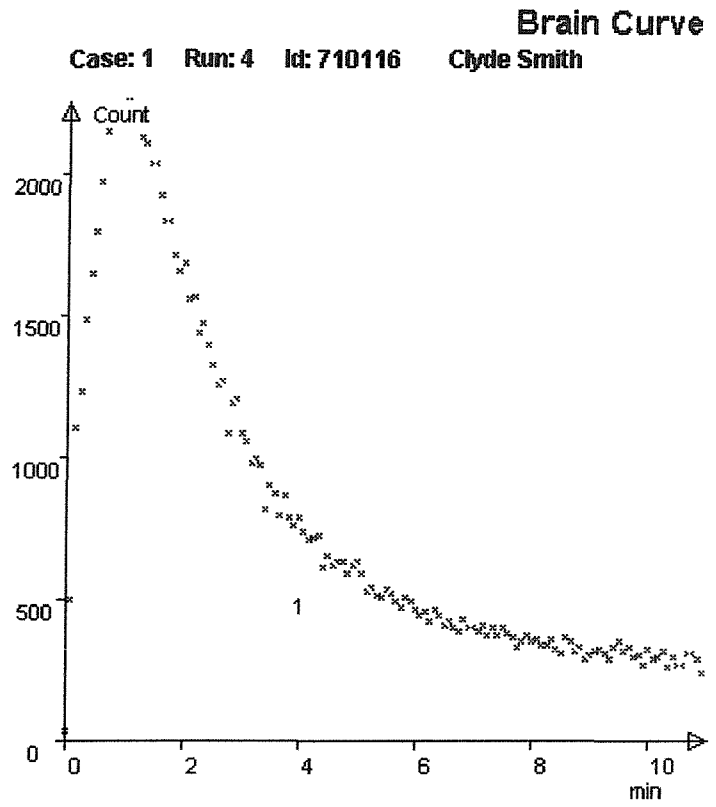


Figure 4. Clearance Curve from a single detector during inhaled xenon measurement

The input function so determined is used to deconvolve the head clearance curve to obtain the exponential washout curves from which regional cerebral blood flow (rCBF) values are derived. The deconvolution extracts clearance curves which would have been obtained had a single arterial injection been made at the beginning of the measurement period.

A second disadvantage of the intravenous or inhaled administration of xenon is increased contamination of the clearance curves by radioactivity from the scalp and extracranial sources, including the air passages and sinuses (155). Activity

in the scalp washes out relatively slowly. Although this activity is minimised by the brief administration of isotope, the clearance curves are nevertheless distorted by a variable, usually small percentage of extra -cerebral counts. The extra cerebral count rate has a short-lived component from activity in the airways and sinuses. The curve fitting process is delayed until the Xenon-133 activity of the end tidal air curve has decreased to 20% of its maximum value to minimise this extracerebral contamination. The curves recorded over the head are corrected for background activity and in serial examinations for remaining activity.

A biexponential analysis is then performed on the clearance curves. However, unlike the intracarotid method, the slow compartment values may be contaminated by the influence from extracerebral tissue and therefore underestimate white matter flow. The grey matter flow is, however, accurately estimated within the limits of the mathematical model. When fast flow coefficients approach slow flow values the compartments become difficult to separate and results may be unreliable. A 3 compartmental analysis of the curves was suggested by Obrist in which the first compartment of flow represents grey matter, the second compartment represents white matter and the third compartment represents extracerebral tissues (149). While this 3 compartmental analysis provided reliable measurements of both grey and white matter flow the extended time period of 40 minutes required for curve fitting limited the clinical practicality of this approach.

Risberg (1975) devised a new index of flow, which was not so dependent upon the separation of the components (154). This was the Initial Slope Index and refers to the slope of a curve reconstructed from the grey and white matter components over the first 90 seconds of clearance. He demonstrated that the initial slope index, although not ideal from the theoretical standpoint, produces measurements that are reproducible and robust. This index reflects clearance from both compartments but is dominated by the fast compartment. In clinical

situations an Initial Slope analysis is preferable to the bicompartmental model because of its superior stability. Extended measurement times with 3 compartment analysis is not practical; it is difficult for patients to remain still for this length of time and CBF cannot be considered constant over longer time periods in sick patients, particularly intraoperatively.

Compared to the intracarotid method, these less invasive methods have the advantage of being repeatable a number of times with minimal patient discomfort and risk. They also allow simultaneous measurement of both cerebral hemispheres. Results obtained with the inhalation and the intra-arterial injection techniques on the same group of patients have shown a very high correlation (156).

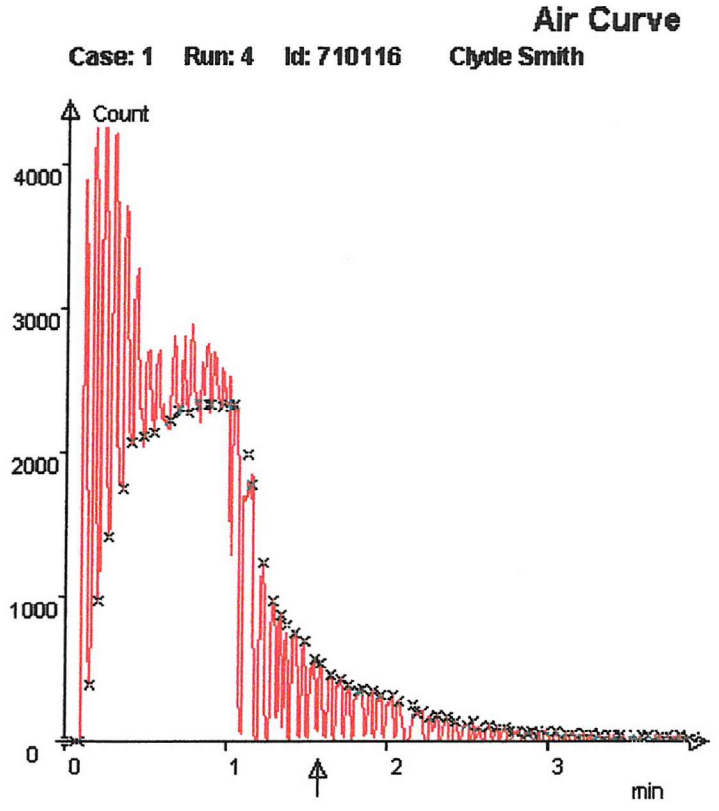


Figure 5. Air curve during inhaled xenon clearance

2.2.5 Serial Applications of the Xenon-133 inhalation Technique

In typical applications of the Xenon-133 inhalation technique CBF is measured in consecutive tests in a single session on the same day. During serial measurements a correction is necessary to prevent the slow washout components of a previous measurement from contaminating subsequent measurements. This is often done by measuring slow washout before each new measurement and extrapolating exponentially forward. This is then subtracted from the new data.

There is little information available about reproducibility of the test using such a design. Prencipe (1972) estimated the reproducibility of serial measurements as ranging from 15-20% depending on the mathematical methods used to analyse the xenon-activity tracing (157). Similarly Blauenstein (1977) demonstrated fluctuations from subject to subject and in subsequent measurements in the same subject ranging from 5 to 20% (158). Studies have shown high correlations between serial resting measurements with a trend towards lower CBF in the second of two resting measurements (159,160). These effects are attributed to habituation and are likely to reflect changes in metabolic demands (161).

2.2.6 Limitations of Xenon Inhalation Technique

The Xenon inhalation technique is a safe and validated technique for measuring regional cerebral blood flow. It lacks the ability for three-dimensional presentation of rCBF as shown by SPECT or PET and lacks the high resolution achieved by CT scanning during inhalation of stable xenon. Other methodological shortcomings include scattered radiation and uncertainty of the partition coefficient for diseased tissues.

This technique also suffers from a limitation known as the look through phenomenon. This can be illustrated using the extreme case of a region of infarction with no perfusion. There will be no uptake of Xenon-133 into this region and therefore the signal received by the gamma ray detector positioned over this region will be totally from perfusion in other overlying or underlying regions (the contra lateral hemisphere and also scatter from surrounding tissue). Flow values in focal ischaemic areas can therefore be overestimated and hemispheral cross talk can have an effect on sensitivity. In practice this is not a problem if the xenon clearance method is combined with CT scan or other non invasive procedures.

2.2.7 Clinical Applications of Xenon-133 Clearance

A wide range of pathological conditions can be studied within the fields of neurology, neurosurgery and psychiatry using the Xenon clearance technique.

When the intracarotid method became available for the first time it was possible to demonstrate focal decreases or increases in blood flow that could meaningfully be related to neurological and angiographic findings. Investigations were carried out in a variety of disease states including acute stroke, subarachnoid haemorrhage, brain tumours, epilepsy and dementia. It was clear that these CBF measurements provided clinically relevant information beyond that obtained by angiography. Furthermore, it was possible to investigate cerebral autoregulation and vasoreactivity. Following subarachnoid haemorrhage, measurement of vasoreactivity by xenon clearance either pre-operatively or intra-operatively allowed prognostic clues as to which patients were at risk of developing delayed cerebral ischaemia. Similarly, assessment of vasoreactivity could be used to identify whether patients were safe to undergo major surgery after a cerebral insult such as a stroke or head injury. The

occurrence of luxury perfusion was first demonstrated in man by the intracarotid Xenon technique (161).

With the introduction of the minimally invasive method advances were made in several areas. Cross sectional CBF studies were carried out that defined age and sex variations and their relationship to risk factors. It became possible to investigate special subgroups such as children and patients undergoing intensive care. In addition it was possible to do longitudinal studies that described the natural history of pathological CBF changes and assessed the long-term effects of therapy. For example, longitudinal studies revealed that treatment of hypertension can retard and possibly reverse age –related decline in cerebral blood flow (162).

CHAPTER 3: METHODS

The study was carried out at Southampton General Hospital between February 2000 and January 2001. Recruitment and screening of subjects and analysis of the results occurred at The Royal Bournemouth Hospital. The Southampton and South West Hants Joint Research Ethics Committee and the East Dorset Local Research Ethics committee granted ethical approval.

3.1 Study Objectives

The primary aim of this study was to investigate the effect of caffeine on cerebral blood flow in patients recovering from an acute ischaemic stroke. In a previous study using transcranial Doppler ultrasound, caffeine caused a fall of 12% in middle cerebral artery blood velocity in ischaemic stroke patients (121,122,123). Our intention was to determine whether these velocity changes reflected changes in cerebral blood flow.

Our study objectives were:

1. To assess the effect of caffeine on global cerebral blood flow in patients recovering from an acute ischaemic stroke using Xenon-133 clearance.

2. To establish the relationship between caffeine induced changes in middle cerebral artery blood velocity as measured by transcranial doppler ultrasound and cerebral blood flow as measured by Xenon-133 clearance.

3. To investigate if there are regional differences in the effect of caffeine on cerebral blood flow

4. To investigate if patients with impaired cerebrovascular reactivity had an altered blood flow response to caffeine. Cerebrovascular reactivity was measured by inhalation of 5% carbon dioxide.

3.2 Subjects and Controls

The subject group was recruited from the Royal Bournemouth Hospital Acute Stroke Unit, the Stroke Rehabilitation Unit at Christchurch Hospital, and the Acute Stroke Unit at Southampton General Hospital. All subjects were recovering from an ischaemic stroke in the middle cerebral artery territory. The time period selected was 2 to 10 weeks after the acute stoke, since this was considered to be the time that most patients were stable and in a period of recovery.

3.2.1 Selection Criteria

In this study the patient group will be referred to as subjects and the control group will be referred to as controls.

The subjects were included or excluded according to the criteria outlined below.

Inclusion Criteria:

1. Absence of exclusion criteria
2. The subject was over the age of 18 years.
3. The subject had symptoms and signs of an acute stroke, involving the territory of the middle cerebral artery i.e. Total or Partial Anterior Infarct according to the OSCP definitions.
4. The onset of the stroke occurred in the two to ten weeks prior to entry into the study.
5. CT or MRI brain scanning did not refute the clinical diagnosis of acute ischaemic stroke in the MCA territory.
6. The subject was able to lie still for at least 40 minutes without discomfort.
7. The subject was able to breathe through the mouthpiece during the Xenon studies.

8. The subject was willing and able to give signed informed consent.

Exclusion Criteria:

1. Absence of inclusion criteria
2. The subject was pregnant
3. The subject was unable to comprehend the study
4. The subject had an extracranial severe stenosis (> 75%) or a complete occlusion on carotid duplex scanning
5. The subject had respiratory difficulties or an unusual breathing pattern, which would have made interpretation of the xenon clearance difficult.
6. The subject was intolerant of dietary caffeine.
7. The subject required a hoist for transfer.

Subjects who fulfilled the inclusion and exclusion criteria but were found to have temporal windows through which TCD ultrasonography could not be consistently carried out were excluded from TCD measurements but included in the Xenon arm of the trial.

A control was defined as a person who had no indication or history of cerebrovascular disease. Controls were not screened for asymptomatic cerebrovascular disease. Controls were age and sex matched with the patient group. The control group had to satisfy the inclusion criteria apart from points 3 to 5 of the inclusion criteria that are stroke related.

3.2.2 Calculation of Sample Size

The total number of participants in the study was 30. This consisted of 20 subjects and 10 controls.

The sample size was determined by a power calculation based on the reproducibility of cerebral blood flow and velocity measurements and the anticipated treatment effect (Table 2).

From a previous transcranial doppler study performed at Bournemouth the mean change in blood velocity after taking caffeine is 6.3cm/s (3.8 –8.7, 95% CI) from a baseline of 47cm/s (123). Error in an individual measurement (1 SD) was estimated to be 5.5 cm/s. This corresponds to a variability of 12%, which is consistent with estimates of variability in the literature (134). For the power calculation we fed in a range of effect sizes between 3.8 and 5.0cm/sec. The lowest effect size corresponded to the 95% Confidence Interval given above.

Table 2: Power Calculation for transcranial doppler measurements

Effect Size (cm/s)	Equivalent % change	n for 80% power
5.0	10.6	12
4.5	9.6	14
4.0	8.5	17
3.8	8.1	19

A similar calculation was performed for the xenon cerebral blood flow measurement, assuming a 5% error in an individual measurement and effect size between 5 and 13% (Table 3).

Table 3: Power Calculation for Xenon measurements

Effect Size (%)	n for 80% power
13	4
10	5
7	7
5	10

Assuming the smallest effect size the minimum number of patients needed is 10 for the xenon measurement and 19 for the transcranial doppler. We therefore chose 20 patients for the study group. A separate group of 10 controls was used in order to have equal numbers of measurements on affected cerebral hemispheres, unaffected hemispheres and control hemispheres.

3.2.3 Screening of Subjects

Subjects were screened for suitability to enter the study at the Acute Stroke Unit and the Stroke Rehabilitation Unit, Royal Bournemouth Hospital, and the Stroke Unit at Southampton General Hospital.

During screening subjects were asked to breathe through a mouthpiece similar to the one used with the Xenon measurements. Most subjects also had trancranial doppler measurement of Vmca during the screening process to assess whether consistent signals could be obtained. If subjects fulfilled all the selection criteria they were invited to enter the study. In total 44 subjects were screened of which 20 participated in the study.

Controls were recruited in three ways. Firstly, relatives of subjects participating in the study were assessed for eligibility and invited to join the study. Secondly, information letters about the study were circulated to members of the local Stroke carer group. Thirdly, hospital staff that were aware of the study volunteered to be controls. Controls were not recruited from other hospital patients.

3.2.4 Subject Consent

Written informed consent was obtained from all participants in the study. A patient information sheet supported the consent process. The subjects' General Practitioners were sent a letter detailing the purpose of the study.

3.3 Study Protocol for Subjects and Controls

The study was carried out in the Wessex Neurological Centre at Southampton General Hospital. Subjects recruited from Bournemouth and Christchurch Hospitals were transferred to Southampton via car or ambulance depending on their level of disability.

The same research room was used throughout the study. This room had Xenon inhalation equipment, a transcranial Doppler unit, a manual sphygmomanometer, carbon dioxide monitoring equipment and a Douglas bag containing 5% carbon dioxide.

The study used a double blind, randomised, cross over placebo control design. Each subject attended for two studies, 1 or 2 weeks apart, and was given 250 mg caffeine in one study and matched placebo in the other. The dose of caffeine used was comparable to the quantities in which it is typically consumed (roughly equal to two cups of brewed coffee).

For 48 hours prior to each study the subjects abstained from caffeine and were given specific dietary instructions as to which food and drink to avoid (appendix 2). Decaffeinated tea and coffee was allowed although it has been shown to contain traces of caffeine and therefore is not strictly caffeine free.

The subject's Barthel Index, which is a validated score of general disability based on activities of daily living, was recorded at the beginning of the first visit (163,164).

Two baseline measurements of cerebral blood flow (CBF) using Xenon 133 clearance were made under identical laboratory conditions. This enabled simultaneous measurement of cerebral blood flow in 16 homologous regions of

both hemispheres by scintillation detectors. Two measurements were made in order to reduce error and to study reproducibility.

Three baseline Middle Cerebral Artery Blood Velocities (Vmca) were obtained bilaterally using transcranial doppler. This was similar to a previous study and enabled reduction of error and estimation of reproducibility (121,122,123).

Subjects were then given a capsule containing either 250mg of caffeine or placebo. Subjects were assigned caffeine/placebo on a random basis independently determined by the pharmacy department. The caffeine and placebo tablets were identical in appearance. The caffeine/placebo administrations were completed in less than 2 minutes.

Blood flow measurements were repeated twice more, 40 and 60 minutes after the administration of the caffeine/placebo. Two repeat measurements of Vmca were recorded 30 minutes post ingestion of caffeine/placebo and a further 2 measurements at 120 minutes.

Venous blood samples were obtained 40 minutes prior and 65 minutes after ingestion of caffeine/placebo. These were analysed for blood concentration of caffeine by an enzyme immunoassay technique (EMIT; Behring Diagnostics, Milton Keynes, UK) on an Olympus AU560 autoanalyser (Olympus Optical, Eastleigh, UK). Blood Pressure measurements using a sphygmomanometer were recorded at the beginning and end of each pair of xenon measurements (i.e. four times).

Additional tests of cerebral vasoreactivity were carried out at the end of the study using transcranial doppler ultrasound and the inhalation of 5% carbon dioxide.

Each study visit lasted for three to four hours. The protocol enabled two subjects to be studied simultaneously and this was done wherever possible. The protocol was identical for controls.

3.3.1 The Barthel Index

The Barthel index is a functional scale used to measure the level of disability or dependence in activities of daily living (ADL). Each subject's Barthel index was scored at the beginning of Visit 1 and calculated as follows:

Bowels:
0 = incontinent
1 = occasional accident
2 = continent

Bladder:
0 = incontinent or catheterised and unable to manage
1 = occasional accident (maximum 1per 24 hours)
2 = continent (for over 7 days)

Grooming:
0 = needs help
1 = independent face/hair/teeth/shaving

Toilet use:
0= dependent
1 = needs some help
2 = independent (on & off, dressing, wiping)

Feeding:
0=unable
1 = needs help cutting, spreading butter etc
2 = independent

Transfer:
0= unable
1 = major help (1-2 people, physical)
2 = minor help (verbal or physical)
3 = independent

Mobility:
0=immobile
1= wheelchair independent including corners etc.
2 = walks with help of 1 person (verbal or physical)
3 = independent (but may use any aid e.g. walking stick

Dressing:
0=dependent
1 = needs help, but can do about half unaided
2 = independent

Stairs:
0 = unable
1 = needs help (verbal, physical, carrying aid)
2 = independent up and down

Bathing:
0=dependent
1=independent

The purpose of measuring the Barthel Index was to give a quantitative figure of the level of disability in our subject population. Our intention was to study 20 subjects with stroke of varying levels of severity. However, since the study involved transferring onto a couch, the subject group was weighted towards those with less mobility impairment. For example, we were unable to study patients who required a hoist for transfer.

3.3.2 Cerebral Blood Flow measurements

Each cerebral blood flow measurement was measured using a Novo Cerebrograph 32c instrument, (manufactured by Novo Diagnostic Systems, Denmark). This is a compact, mobile unit containing an automated Xenon administration and retention system, a helmet assembly with 16 head detectors

and all associated mechanical and electronic equipment. The Cerebrograph output is connected to a computer, which provides on-line numerical and graphical documentation.

The apparatus enabled the operator to supervise all relevant indices during the investigation. Patient respiration was continuously monitored by CO_2, O_2 and Xenon-133 meters. Instant signals warned of abnormalities in respiration, CO_2 and O_2 levels. A highly sensitive leak detector monitored the mouthpiece seal during the study. The build up of all regional clearance curves was displayed during the investigation, giving continuous information about the progress and technical quality of the measurement.

During each visit the subject had four xenon studies (2 prior to administration of drug/placebo and 2 after). Each study was carried out as follows with the subject lying supine.

A tightly fitting mouthpiece was carefully placed in the mouth and nose clips were applied to prevent nasal breathing. The subject was allowed several minutes to adapt to the breathing system and to obtain normal and regular breathing. The 16 external collimated scintillation head detectors (sodium iodide crystals and photomultiplier tubes) were placed in parallel at right angles to each lateral side of the head. Of the 8 head detectors that monitored each cerebral hemisphere, 3 were frontal, 1 central, 2 temporal, 1 parietal and 1 occipital corresponding to the area of the brain they were overlying.

The patient inhaled air containing Xenon 133 in a concentration of 3mCi (110MBq)/litre by means of the re-breathing Xenon-133 administration system for one minute. After one minute of inhalation the system automatically switched to normal air. The subject then continued to breathe normal air for the next 4 minutes with the mouthpiece in to collect exhaust xenon. A leak detector continuously monitored the mouthpiece seal during the examination. At 3

minutes the end tidal CO_2 tension was recorded as measured by the integrated CO2 analyser.

Throughout inhalation and then for a further 6 minutes after the mouthpiece was taken out the 16 detectors continuously monitored the γ radiation emitted by the Xenon-133 as it cleared form different regions of the brain. The rate of washout of the xenon formed the basis for the flow calculations. A separate scintillation detector monitored the concentration of Xenon-133 in the expired air. The end tidal isotope concentrations were used as an estimate of the arterial concentration of xenon-133 to correct the head detector curves for recirculation of the tracer (see Section 2.2.4).

The clearance curves were subject to a two-compartment analysis although for the purpose of this study the Initial Slope Index was used to express CBF results. This was chosen as the most reliable measure of CBF because it is independent of the partition coefficient of xenon (which is unknown for ischaemic tissue) and is little affected by the extra-cerebral matter. This is a modification of the ISI described by Risberg (154). A deconvoluted clearance curve is constructed from the biexponential analysis. On this curve the ISI is defined as 100 x the monoexponential slope between 0.5 and 1.5 minutes. This index reflects clearance from both compartments but is dominated by the fast compartment.

As a secondary measure grey matter flow was calculated using the bicompartmental curve analysis. This permits the separation of the highly perfused grey matter from white matter and extracranial tissues. The coefficients P1, K1, P2, K2 were obtained from this corresponding to the size and clearance rate of the fast compartment (representing grey matter) and slow compartment (representing white matter). The fast compartment flow (F1) representing flow in the grey matter was calculated from the equation:

$F1 = K1 \times \lambda g \times 100$

where λg is the partition coefficient for xenon perfusion in the grey matter. F1 is expressed as ml/100g brain tissue/minute. As previously stated the F1 may be subject to errors since the exact partition coefficient of infarcted tissue is unknown.

During data processing, corrections were applied for the influence of scattered radiation from the air passages and remaining activity from previous measurements. The clearance curves were checked to enable reconstruction or exclusion of bad data that may have been caused by technical problems or patient movement.

Correction for background activity was carried out as follows. On starting the instrument a background measurement of radioactivity was performed. Background activity was measured in sampling intervals of 5 seconds during 30 seconds prior to studies 1 and 3. A mean background value was then calculated and subtracted from subsequent data for correction of head curve data. Studies 2 and 4 followed on closely from previous studies and therefore a correction for the decay of residual radioactivity from the previous measurements was necessary. This was obtained by measuring the residual and background activity for 5 minutes. An exponential fit of this data was then extrapolated through the subsequent measurements and correction for remaining activity was made by subtracting the obtained remaining activity count rate values from the primary head curve data.

3.3.3 Middle Cerebral Artery Blood Velocity Measurements

Each middle cerebral artery velocity (Vmca) measurement was carried out using a transcranial doppler unit (PCDop842, Scimed Ltd, Bristol, UK).

During each visit, the subject had 7 measurements performed (3 baseline measurements, 2 at 30 minutes and 2 at 120 minutes after ingestion of tablet). The number of measurements was identical to a previous TCD study investigating the effect of caffeine in stroke patients, which demonstrated that the maximum reduction in blood velocity was stable between 30 and 120 minutes (123). Each measurement consisted of 3 Vmca readings from each side and was carried out in the following way.

For five minutes prior to each measurement the patient was asked to lie down on the couch to ensure they were as relaxed as possible and to avoid any sympathetic influence on the spontaneous variation of cerebral blood velocity. Initially the MCA on each side was insonated through the temporal window and the operator manipulated the probe to find the position and depth of the best signal (usually around 50mm). The skin at this position was then marked with a marker pen for easy identification during subsequent measurements.

During the measurement the operator manipulated the probe to find the maximum signal at the marked position. From a previous study this has been shown to be the optimal method of providing consistency (121, 122, 123).

Each measurement consisted of three Vmca readings on each side, of which the maximum value was used for analysis. Vmca refers to the time averaged maximum velocity over 3-4 cardiac cycles and is calculated using a Fast Fourier Transformation (see Section 2.1.1).

3.3.4 Cerebral Vasoreactivity to Hypercapnia

In healthy subjects an increase in arterial carbon dioxide concentration produces an increase in cerebral blood flow (see Section 1.2.3). This reactivity to hypercapnia may be diminished or abolished in patients with cerebral vascular

disease. In this study our aim was to identify if there was a subset of stroke patients with impaired vasoreactivity and to determine whether these patients responded differently to the caffeine. Theoretically, patients with impaired cerebral vasoreactivity are at most risk from fluctuations in cerebral blood flow since they have impaired cerebral reserve.

The methods for measuring cerebrovascular reactivity have already been described. In this study we chose to measure reactivity by inducing hypercapnia by inhalation of 5% carbon dioxide and measuring changes in Vmca with transcranial Doppler ultrasound.

At the end of each study we recorded blood flow velocities in the MCA of both hemispheres in turn using a Doppler probe attached by an elasticised head strap. The probe was adjusted until strong MCA signals were obtained and the head strap maintained the probe in this position to allow continuous monitoring. The patient was asked to breathe from a hand held mouthpiece with an integrated CO_2 analyser, which measured end tidal CO_2 tension. Breathing through the nose was avoided through use of a clip. After a one-minute baseline recording each subject had a further 1-minute breathing from a reservoir bag filled with 5% carbon dioxide, 30% oxygen and the remainder nitrogen.

Two readings of Vmca were taken at baseline once a steady state of end tidal CO_2 had been obtained. Two further Vmca recordings were made during CO2 inhalation. The 2 baseline readings were averaged, as were the 2 readings during $C0_2$ inhalation. The CO_2 tension was measured twice during baseline and twice during CO_2 inhalation. Reactivity was calculated using the averaged data for each middle cerebral artery as the percentage increase in velocity per mmHg increase in end tidal CO_2.

The cerebral vasoreactivity to carbon dioxide was then correlated with the subjects' response to caffeine.

All vasoreactivity measurements were carried out with the patient in a sitting position.

3.4 Other methodological considerations

Our aim in this study was to have 2 study visits per subject which were identical in every factor apart from the administration of caffeine one week and placebo the other.

Cerebral Blood Flow and Velocity show a diurnal variation. We therefore scheduled the study visits to occur at approximately the same time of day, for example if the subject attended in the morning on their first week they were scheduled to return in the morning for their second week.

Since the study involved travel from Bournemouth to Southampton for some patients, this automatically excluded patients who were unwilling to travel or suffered from car sickness. It also meant that recruitment of patients was weighted towards those with less disability who would be more easily able to tolerate the journey.

To prevent inter-operator variability, the same person carried out the TCD or the Xenon Studies in consecutive subject visits.

Transcranial Dopppler ultrasonography is an operator dependent technique. To minimise intra-operator variability the position of maximal Doppler signal was marked with a marker pen at the beginning of each visit for easier subsequent identification. This mark was then removed at the end of the first visit and each subject was re-marked at the beginning of Visit 2. During the measurements, the

operator angled the probe to find the maximum velocity at the marked position and to obtain consistency between measurements.

During the study subjects had to transfer between two beds for the TCD and then Xenon measurements. Subjects with higher levels of disability found this tiring and in those cases we opted to move the beds around rather than the subjects.

3.5 Problems Encountered

The problems encountered during the study can be divided into subject-related and technical problems.

3.5.1 Subject-related Problems

Subject related problems were mainly difficulties with breathing though the mouthpiece, irregularities in respiration or heart rate, subject movement and withdrawal of consent.

For the Xenon studies subjects had to maintain a good seal on the mouthpiece whilst breathing though the mouth. This meant that patients who had severe facial palsy encountered problems with the device. Leaks through the mouthpiece during the measurement cause inaccuracies in CBF values, which may be artificially raised or decreased. Other subjects disliked breathing through the mouth for various reasons such as anxiety, mouth ulcers, and sinusitis. Overall, 4 screened and consented subjects were not included in the study as they were unable to co-operate with the xenon measurements.

It was important during both xenon measurements and transcranial Doppler measurements for patients to have a regular breathing pattern. With the xenon measurements an abnormal breathing pattern may cause fluctuations in areterial CO_2 tension with consequent changes in cerebral blood flow.

Transient cardiac arrhythmias such as atrial fibrillation or ventricular ectopic activity may have an effect on TCD measurements. However, in this study only 1 of the subject group and 1 of the controls were not in constant sinus rhythm. In both cases the TCD measurements had to be recorded over longer periods of time to average the maximum velocity over more heart beats and therefore obtain a more accurate reading. In 3 other cases there were only minor

paroxysmal runs of arrythmia such as ectopic beats or a missed beat and in these cases it was possible for the operator to wait for a period of regularity before taking the measurement.

Finally, subject movement during both Xenon and TCD studies can cause inaccuracies in measurement. During Xenon studies it is important that the head is kept still since movement toward/away from detectors will cause artificial increases/decreases in CBF values. During TCD movement is less of a problem, as in this study the operator is able to wait until the patient is still before recording. With a fixed probe such as during the vasoreactivity measurements subject movement can be more of a problem.

Only 1 out of the 20 patients and none of the controls withdrew their consent between study visits.

3.5.2 Technical problems

In 4 out of the 20 patients we were unable to obtain consistent TCD measurements and therefore these patients only participated in the xenon arm of the trial. In a further patient, the operator was aware half way through the study that there was a change of probe position such that velocity in another vessel was being measured. This data was therefore not included in the analysis. Although the operator aimed to record maximum velocities at the identical position each time, in some subjects this was difficult due to tortuosity or stenoses of the vessel being insonated.

3.6 Statistical Analysis

Data was analysed on 19 subjects and 10 controls. The 20th subject withdrew consent between study visits and therefore we were unable to use the data collected from her first visit. In 5 of the 19 subjects and 1 of the 10 controls we were unable to obtain consistent Vmca readings. Data was therefore analysed on CBF measurements using Xenon clearance for 19 subjects and 10 controls and on Vmca measurements using TCD for 15 subjects and 9 controls. Vasoreactivity was measured on 15 subjects and 9 controls. In 9 of the subjects and 6 of the controls vasoreactivity was measured bilaterally.

Data were stored on a spreadsheet programme in 'Excel' files. Data evaluation was carried out by standard statistical techniques. The distribution of data was tested to see if it was normally distributed in order to apply parametric tests. Measurements of changes in blood flow between the caffeine and placebo group were compared using the paired T test. Analysis of variability was measured by calculating the mean and standard deviation of differences between consecutive measurements.

CHAPTER 4: RESULTS

4.1 Characteristics of Subjects and Controls

4.1.1 Subjects

Twenty subjects were studied. One subject withdrew her consent between the study visits and therefore has not been included in the data analysis. The remaining nineteen subjects displayed the following characteristics.

Table 4. Demographic Characteristics of Subject Population

ID	SEX	AGE	SIDE INFARCT	OCSP	WEEKS FROM STROKE	BARTHEL INDEX
1	Male	77	Right	TACS	6	18
3	Female	81	Right	PACS	5	19
4	Male	85	Right	PACS	5	17
5	Female	54	Left	PACS	2.5	20
6	Female	77	Left	PACS	3	18
8	Female	73	Right	TACS	4	20
9	Male	78	Left	TACS	8	9
10	Male	86	Left	TACS	2	17
11	Female	69	Right	TACS	7	12
12	Male	48	Left	TACS	5	19
13	Female	64	Right	TACS	5	13
14	Male	52	Left	PACS	2	15
15	Female	62	Left	TACS	2	10
16	Female	85	Left	PACS	3.5	19
18	Male	69	Right	PACS	2	20
19	Male	68	Right	TACS	5	6
20	Male	77	Left	PACS	2	19
21	Male	85	Left	TACS	8	18
23	Male	64	Right	TACS	9	12

The mean age of the subjects was 71 years with a range from 48 to 86 years.

4.1.2 Controls

Ten controls were examined with the following characteristics.

Table 5. Demographic Characteristics of the Control Population

ID	**SEX**	**AGE** (years)
2	Female	75
7	Male	67
17	Female	74
22	Female	72
24	Male	58
25	Male	85
26	Female	75
27	Male	52
28	Male	66
29	Male	64

The mean age of the controls was 69 years with a range from 52 to 85 years.

4.13 Study Visits

Subjects and controls were randomly assigned to receive caffeine on visit 1 or visit 2. The concentration of caffeine before administration of the tablet was <0.1mg/l for all subjects and controls with the exception of subject 11 on the first study visit who had a pre tablet caffeine concentration of 2.4 and a post tablet concentration of 1.8. The concentration of caffeine in the blood sample taken after administration of the tablet for each visit can be seen in Table 6.

Table 6. Caffeine concentration in blood samples

ID	Caffeine concentration mg/l (post tablet) visit1	Caffeine concentration mg/l (post tablet) visit 2
Subject 1	2.9	<0.1
Subject 2	<0.1	6.0
Subject 3	1.0	<0.1
Subject 4	<0.1	3.4
Subject 5	4.2	<0.1
Subject 6	<0.1	4.4
Subject 7	3.1	<0.1
Subject 8	3.5	<0.1
Subject 9	<0.1	4.1
Subject 10	<0.1	2.7
Subject 11	1.8	4.9
Subject 12	<0.1	3.1
Subject 13	4.7	<0.1
Subject 14	3.2	<0.1
Subject 15	<0.1	5.3
Subject 16	2.5	<0.1
Subject 17	1.7	<0.1
Subject 18	3.3	<0.1
Subject 19	<0.1	3.1
Control 1	<0.1	4.2
Control 2	<0.1	2.6
Control 3	4.0	<0.1
Control 4	<0.1	2.5
Control 5	2.3	<0.1
Control 6	<0.1	2.8
Control 7	4.5	<0.1
Control 8	0.1	0.9
Control 9	3.4	<0.1
Control 10	<0.1	3.0

4.2 Distribution of Data

Tests for normal distribution were applied to the Cerebral Blood Flow data. The skewness statistic was 0.06 with a standard error of skewness of 0.43. Since the skewness statistic is <2x standard error of skewness this suggests the data is symmetrical in a normal distribution. We also calculated the kurtosis statistic for this data, which was 0.52 with a standard error of kurtosis of 0.85. Since the kurtosis statistic is <2x the standard error of kurtosis this tells us the tails of distribution are not fatter/thinner than a normal distribution.

4.3 CBF Measurement

4.3.1 Effect of caffeine on global cerebral blood flow

For each subject the mean of the CBF values for the 8 detectors on each side of the brain was calculated. Measurement 1 and Measurement 2 were averaged to obtain the baseline CBF value. Measurement 3 and 4 were averaged to obtain the after tablet CBF value for each patient. The mean CBF values were then averaged for the 19 subjects to calculate the overall mean and SEM. This data can be seen in Table 7 and Figure 6. The mean CBF values were averaged for the 10 controls and this data can be seen in Table 8 and Figure 7.

The effect of caffeine was calculated for each subject as the difference in CBF before and after caffeine. The placebo effect was calculated as the difference in CBF before and after placebo. The Stroke and Non Stroke sides of the brain were analysed separately for the subjects. For the controls the Right and Left sides of the brain were averaged since there was no significant difference between the 2 sides. The change in CBF can be seen in Table 9 and Figure 8.

The analysis showed that caffeine reduced Cerebral Blood Flow compared to placebo in subjects recovering from an acute ischaemic stroke with an average decrease in CBF of 10ml/100g/min from a baseline of 43ml/100g/min (-24% [95% CI 18% to 30%], $p<0.000001$). Caffeine reduced cerebral blood flow compared to placebo in the control group with an average decrease in CBF of 9ml/100g/min from a baseline of 49ml/100g/min (-19% [95% CI –12% to –26%], $p<0.001$). This effect of caffeine has been calculated according to the formula:

Effect of caffeine = (change in CBF after caffeine) – (change in CBF after placebo).

There was no significant difference in the effect of caffeine between the subjects and the controls ($p=0.23$).

Table 7. Mean CBF Measurements in Subject Group

	Baseline CBF * ml/100g/min (SEM)	CBF After Tablet* ml/100g/min (SEM)
Caffeine	43.66 (1.86)	34.55 (1.45)
Placebo	42.14 (1.72)	43.22 (1.68)

* global CBF = mean of 16 detectors and average of 2 measurements for 19 subjects

Table 8. Mean CBF Measurements in Control Group

	Baseline CBF ml/100g/min (SEM)	CBF After Tablet ml/100g/min (SEM)
Caffeine	48.56 (1.6)	38.0 (1.4)
Placebo	49.17 (3.03)	47.84 (1.77)

Table 9. Mean Change in CBF

Change in CBF	Affected Side ml/100g/min (SEM)	Non Affected Side ml/100g/min (SEM)	Controls ml/100g/min (SEM)
After caffeine	-9.02 (0.93)	-9.21 (0.93)	-10.59 (0.72)
After Placebo	1.01(0.83)	1.13(0.72)	-1.33(1.63)

4.3.2 Effect of caffeine on regional cerebral blood flow

The effect of caffeine was not significantly different between the affected and unaffected cerebral hemisphere in the subject group ($p = 0.47$). The effect of caffeine was calculated as the change in CBF after caffeine minus the change in CBF after placebo. On the affected side, caffeine caused an average decrease in CBF of 10ml/100g/min from a baseline of 43ml/100g/min (-24% [95% CI –18% to –30%], $p<0.000001$). On the unaffected side, caffeine caused an average decrease in CBF of 10ml/100g/min from a baseline of 43ml/100g/min (-24% [95% CI –19% to –29%], $p<0.0000001$).

The effect of caffeine was not significantly different between the right and the left hemisphere in the control group, although the controls possibly had higher baseline values in the right compared to the left ($p=0.064$).

To analyse regional effects of caffeine on cerebral blood flow the measurements were divided between those looking predominantly at the fronto-temporal and parietal regions and those looking at the occipital region. 6 detectors looked at the front of the brain and 2 detectors looked at the rear.

Caffeine reduced cerebral blood flow in the front and the rear of the brain in both subjects and controls ($p<0.001$). In the subject group caffeine had a bigger effect in the front of the brain (mean change in CBF–10.3mls/100g/min) compared to the rear (mean change in CBF –8.5mls/100g/min) ($p=0.007$). This regional variation on the effect of caffeine was not seen in the control group where caffeine affected the front and the rear of the brain equally ($p=0.54$).

4.3.3 Effect of other variables on CBF measurements

Barthel Index

There was no significant correlation between Barthel Index with overall change in CBF following administration of the tablet between caffeine and placebo, r = -0.05 (p = 0.83)

Effect of Gender

There was no significant difference in the effect of caffeine on CBF in males compared to females in both the subject and the control group (p=0.16). Combining subjects and controls the average change in CBF with caffeine in males was –8.7ml/100g/min (SD 5.4) and in females was –11.5ml/100g/min (SD 4.8).

Effect of side of stroke

There was no significant difference in the effect of caffeine between subjects who had a stroke affecting the right side of the brain and subjects whose stroke affected the left side (p= 0.59). If the stroke was on the left side there appeared to be higher baseline blood flow values (p=0.06).

Effect of carbon dioxide

Table 10. End Tidal Carbon Dioxide Measurements during Xenon Studies

Mean CO_2 mmHg (SEM)	**Subjects**	**Controls**
Before caffeine	25.5 (0.7)	24.7 (1.3)
After caffeine	23.3 (0.5)	22.0 (1.6)
Before placebo	24.7 (0.7)	23.5 (1.4)
After placebo	23.1 (0.8)	22.9 (1.5)

The change in end tidal carbon dioxide after administration of the tablet was not significantly different in the caffeine compared to the placebo group (p=0.06). Measurements of end tidal CO_2 made during the Xenon studies

tended to be lower after administration of the tablet whether it was caffeine or placebo.

Effect of Blood Pressure

Administration of 250mg caffeine did not significantly alter blood pressure (p=0.5 for Systolic BP, p= 0.96 for Diastolic BP). The effect of caffeine on blood pressure was the same for patients and controls (p=0.37 for Systolic BP, p= 0.62 for Diastolic BP).

There was a trend towards higher BP on Visit 1, which may be a reflection of anxiety.

4.3.4 Effect of caffeine on grey matter flow

For all measurements the grey matter flow (fg) was also calculated. The results in the subject group are summarised in Table 11.

In the 19 subjects reduction after caffeine was about 16ml/100gm/min on the affected and unaffected cerebral hemisphere. Combining both sides, reduction was 16mls/100g/min from a baseline of 57mls/100g/min (28%, 95%CI 18% to 37%, $p<10^{-5}$).

Table 11. Effect of caffeine on grey matter flow (fg) -subjects

	Stroke side	Non Stroke side	Both sides
Absolute Change in fg (caffeine – placebo) mls/100g/min	-15.5 [95%CI –9.5 to – 21.5]	-16.2 [95% CI –11.1 to – 21.3]	-15.9 [95% CI –10.4 to – 21.3]
% change in fg (caffeine – placebo)	-27.3 [95% CI –16.7 to – 37.9]	-28.3 [95% CI –19.4 to – 37.2]	-27.8 [95% CI –18.3 to – 37.3]

The results of the grey matter flow in the control group is summarised in the following table. In controls (n=10) the reduction was 12ml/100g/min from a baseline of 66ml/100g/min (18%, 95% CI 7% to 28%, p<0.005).

Table 12. Effect of caffeine on grey matter flow (fg) - controls

	Right side	Left side	Both sides
Absolute Change in fg (caffeine -placebo) mls/100g/min	-11.9 [95% CI –4.5 to – 19.2]	-11.3 [95% CI –4.0 to – 18.6]	-11.6 [95% CI –4.5 to – 18.6]
% change in fg (caffeine – placebo)	-17.7 [95% CI –6.7 to – 28.8]	-17.4 [95% CI –6.1 to – 28.6]	-17.5 [-6.9 to –28.2]

4.4 Effect of caffeine on Vmca Measurements

4.4.1 Effect of caffeine on Vmca in subject group

The maximum of the three Vmca recordings per measurement was used for the analysis. The average of the 3 measurements was calculated to obtain the baseline value. Similarly the average of the 4 measurements taken after the tablet was used. The average of the 2 sides before and after the tablet was taken across the 14 subjects and mean and SEM was calculated. This data is displayed in Table 13 and figure 10.

Caffeine reduced Vmca compared to placebo in the subject group. The average decrease in Vmca with caffeine was 8cm/sec from a baseline of 54 (-16% [95% CI –11% to –21%], $p<0.0001$)

In the subject group the affected and the non-affected hemisphere were analysed separately. On the affected side caffeine caused an average decrease in Vmca of 9cm/sec from a baseline of 56cm/sec (-16% [95% CI –10% to –23%], $p<0.001$). On the unaffected side caffeine caused an average decrease in Vmca of 8cm/sec from a baseline of 52cm/sec (-15% [95% CI –10% to –19%], $p<0.000001$). There was no difference between the effects of caffeine on the stroke side compared to the non-stroke side ($p=0.34$)
There was no change in the results after adjusting for the order in which the subject received caffeine and placebo.

Table 13. Mean Vmca measurements in Subject Group

	Vmca at baseline cm/sec (SEM)	Vmca after tablet cm/sec (SEM)
Caffeine	54.34 (4.16)	46.04 (3.49)
Placebo	53.51 (3.87)	53.53 (3.96)

Table 14. Mean Vmca measurements in Control Group

	Vmca at baseline cm/sec (SEM)	Vmca after tablet Cm/sec (SEM)
Caffeine	53.03 (3.72)	46.8 (2.63)
Placebo	53.81 (3.70)	53.54 (3.63)

Table 15. Mean Change in Vmca

	Vmca on affected side cm/sec (SEM)	Vmca on non affected side cm/sec (SEM)	Controls cm/sec (SEM)
After Caffeine	-8.65 (1.66)	-7.96 (1.3)	-6.24 (1.59)
After Placebo	0.35 (0.67)	-0.29 (0.85)	-0.27 (1.59)

4.4.2 Effect of caffeine on Vmca in control group

Caffeine reduced Vmca compared to placebo in the control group ($p<0.02$). Caffeine caused an average decrease in Vmca of 6cm/s from a baseline of 53cm/sec (-12% [95% CI –3% to –21%], $p<0.02$).

There appeared to be a difference in the effect of caffeine on Vmca between the left and right sides. Caffeine reduced Vmca by 8.7cm/s on the right and by 3.3cm/s on the left ($p=0.044$). However the results on the left side were not normally distributed because of two anomalous readings showing an apparent large increase following the caffeine. These have been included in the analysis but are probably distorting the results.

4.4.3 Comparison of effect of caffeine on Vmca in Subjects and Controls

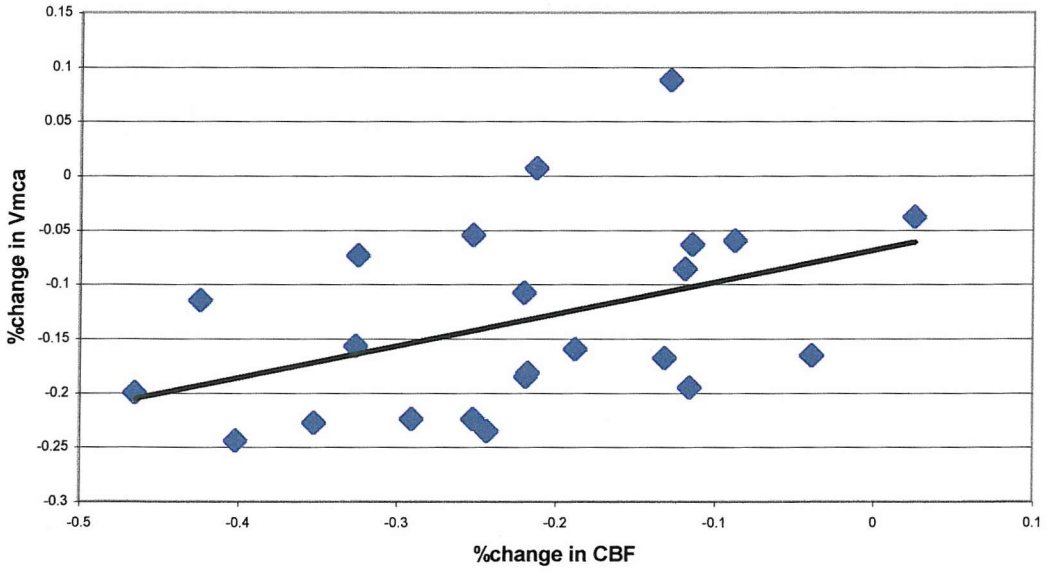
For both patients and controls caffeine significantly reduced the Vmca ($p<0.001$). There was no significant difference in the effect of caffeine in stroke patients compared to controls ($p=0.28$). Analysing subjects and controls together the mean change with caffeine on the Vmca (i.e. change with caffeine – change with placebo) was –7.4cm/sec [95% CI -9.6 to –5.2, ($p<0.001$)]

4.5 Correlation of CBF with Vmca measurements

The absolute change in cerebral blood flow after caffeine was compared with the change in Vmca on the same side in both subjects and controls. There was poor correlation between absolute change in cerebral blood flow and change in Vmca ($r = 0.28$, $p=0.06$). This may be a reflection of the error in individual measurements.

Similarly, the overall change with caffeine (i.e. change following caffeine – change following placebo averaged across both sides) expressed as a fraction of the baseline was compared for CBF and Vmca. There was better correlation with fractional change ($r = 0.42$, $p=0.045$). The line of best fit had a slope of 0.29 and intercept of –0.07. This can be seen in figure 12.

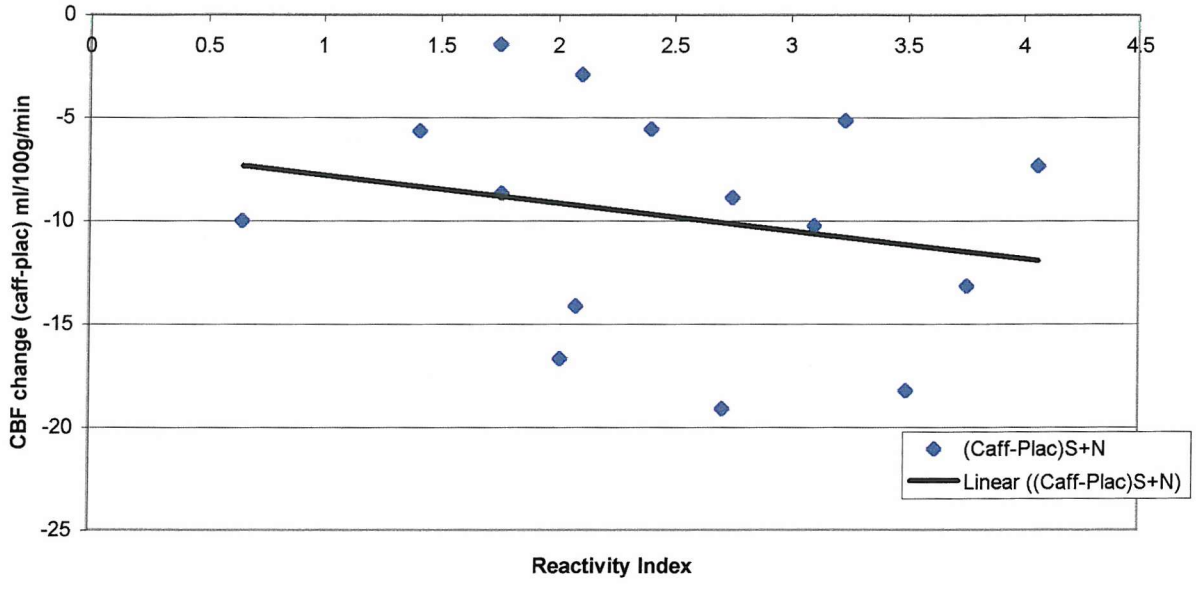
Figure 6. Correlation of Percentage change in Vmca with % change in CBF



4.6 Vasoreactivity

The effect of caffeine on cerebral blood flow (i.e. change in CBF with caffeine –change in CBF with placebo) was correlated with subjects vasoreactivity to carbon dioxide. The vasoreactivity measured after placebo and not after caffeine administration was used. The reactivity index was calculated as the percentage increase in Vmca per mmHg increase in end-tidal CO_2. The results in the subject group can be seen in Figure 13.

Figure 7. Correlation of effect of caffeine on Vmca with vasoreactivity in subjects



The correlation coefficient was r=-0.23, p= 0.3 in the subject group. In the control group the correlation coefficient was r=0.08, p=0.8.

As an incidental finding, there was a significant difference between vasoreactivity measured after caffeine administration and vasoreactivity after placebo. The mean change in Vmca was 1.97% per mmHg change in $P_{ET}CO_2$ in the caffeine group compared to 2.48% change in Vmca per mmHg

$P_{ET}CO_2$ in the placebo group. The difference was highly significant ($p<0.001$).

There was no significant difference in reactivity on the stroke side compared with the non-stroke side in the subject group ($p=0.24$). There was no difference in vasoreactivity between patients and controls ($p=0.35$).

4.7 Variability of Measurements

For both the Xenon and the TCD measurements variability was calculated for 2 consecutive measurements and for 2 consecutive study visits.

The mean difference in blood flow between pairs of xenon readings on the same side of the brain (average of 8 detectors) obtained immediately after each other was calculated to be –0.14ml/100g/min (p=0.62). The standard deviation was 4.1ml/100g/min, which equates to 9.1% of the baseline mean pre-tablet value of 45.1ml/100g/min. From this the variability of a single xenon measurement is 2.9ml/100g/min (6.4%). This is comparable to the 5% variability that was used in the initial power calculation for the study.

The mean difference between pairs of the same baseline xenon measurements on the same side obtained on successive visits one week apart was –1.0ml/100g/min (p=0.14). The standard deviation was 6.6ml/100g/min (14.6%).

For the transcranial Doppler measurements the mean difference between pairs of successive measurements on the same side obtained immediately after each other was 0.26cm/sec (p=0.36). The standard deviation was 4.1cm/sec, which equates to 7.7% of the baseline value of 53.4cm/sec. From this the variability of a single measurement is 2.9cm/sec (5.4%).

The mean difference between pairs of baseline reading on the same side obtained one week apart was 2.0cm/sec (p=0.07). The standard deviation of this is 6.2cm/sec (11.6%).

Figure 8. Cerebral Blood Flow - Subjects (both hemispheres)
Mean +/-1SEM

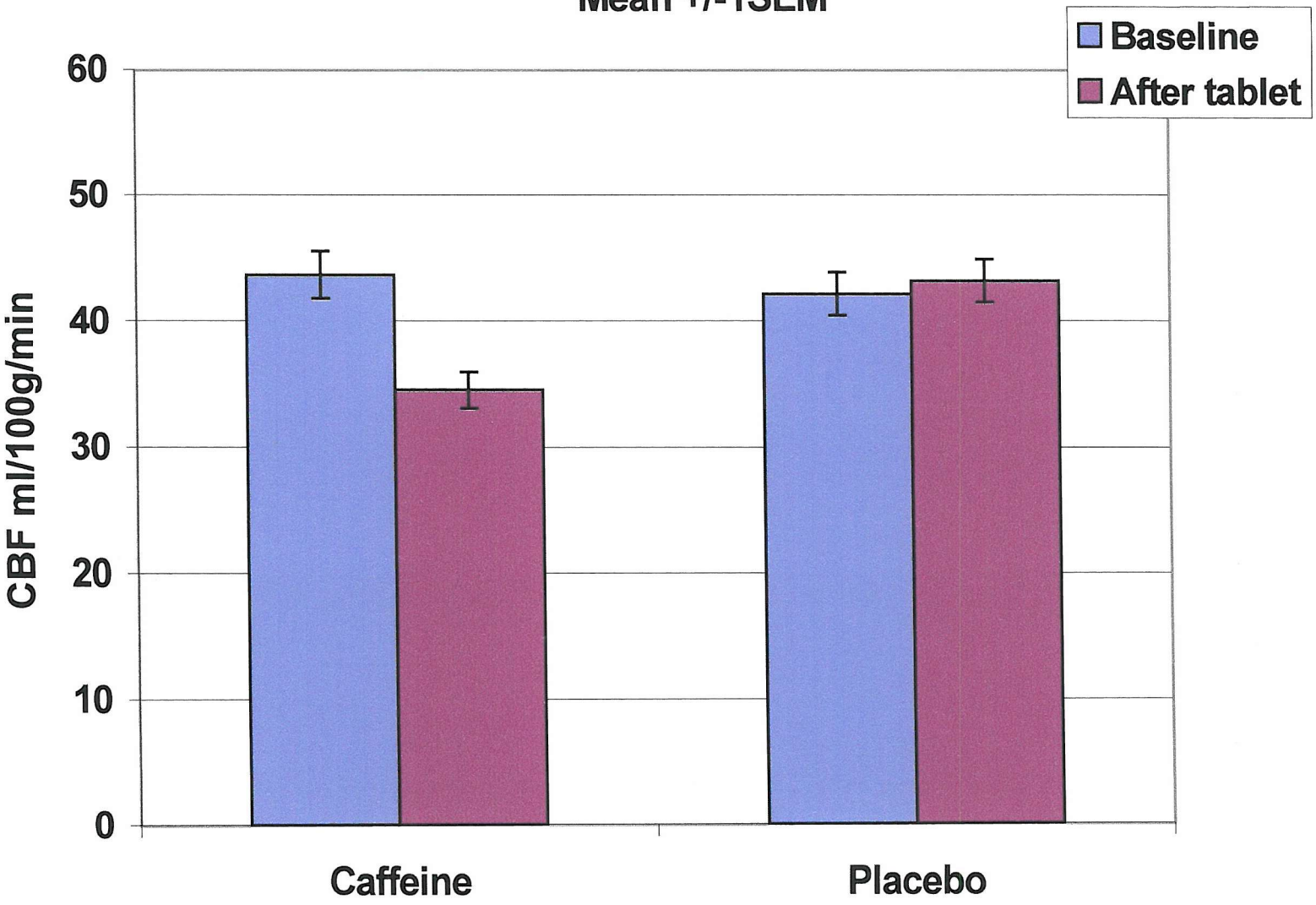


Figure 9.Cerebral Blood Flow - Controls (both hemispheres)
Mean +/-1SEM

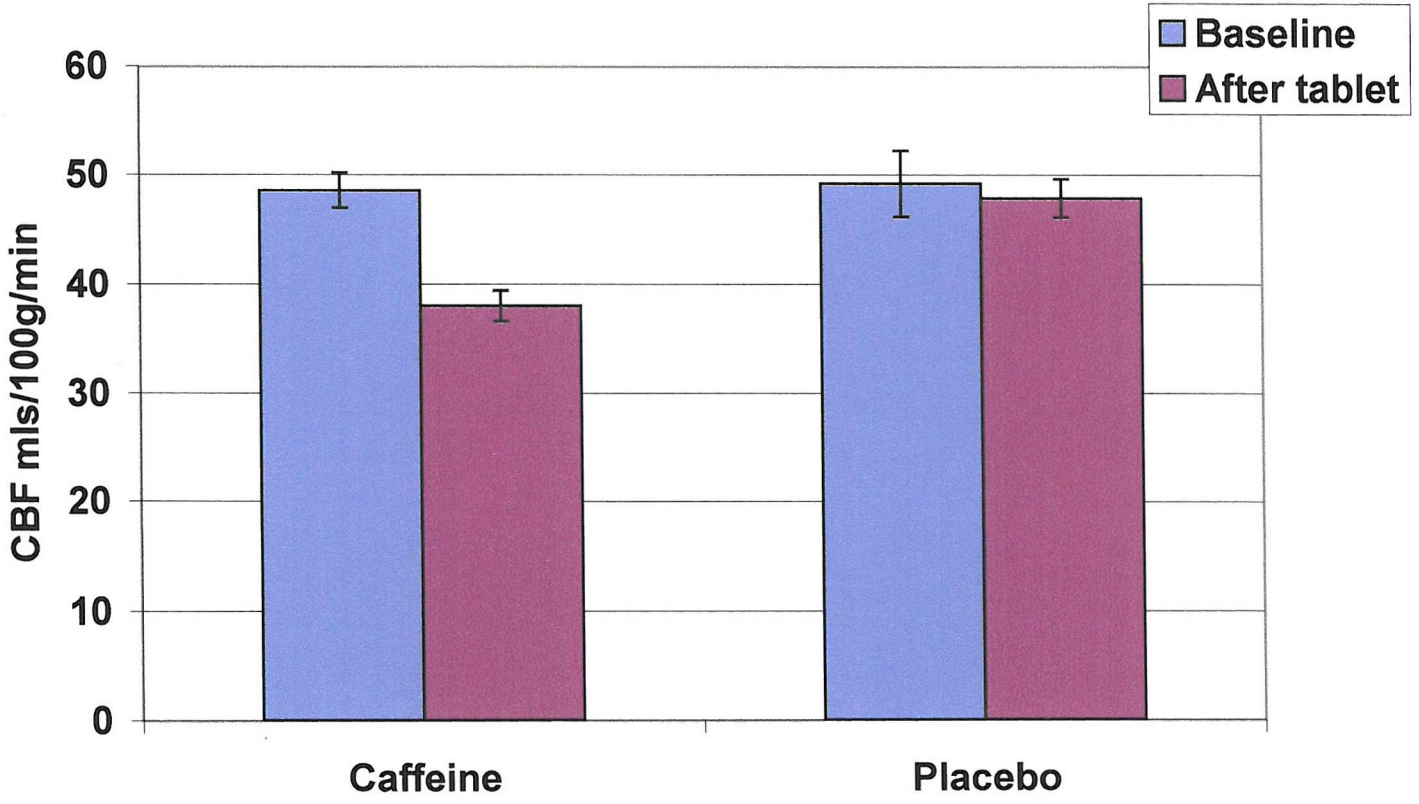


Figure 10.Change in CBF after tablet - subjects and controls
Mean +/- 1SEM

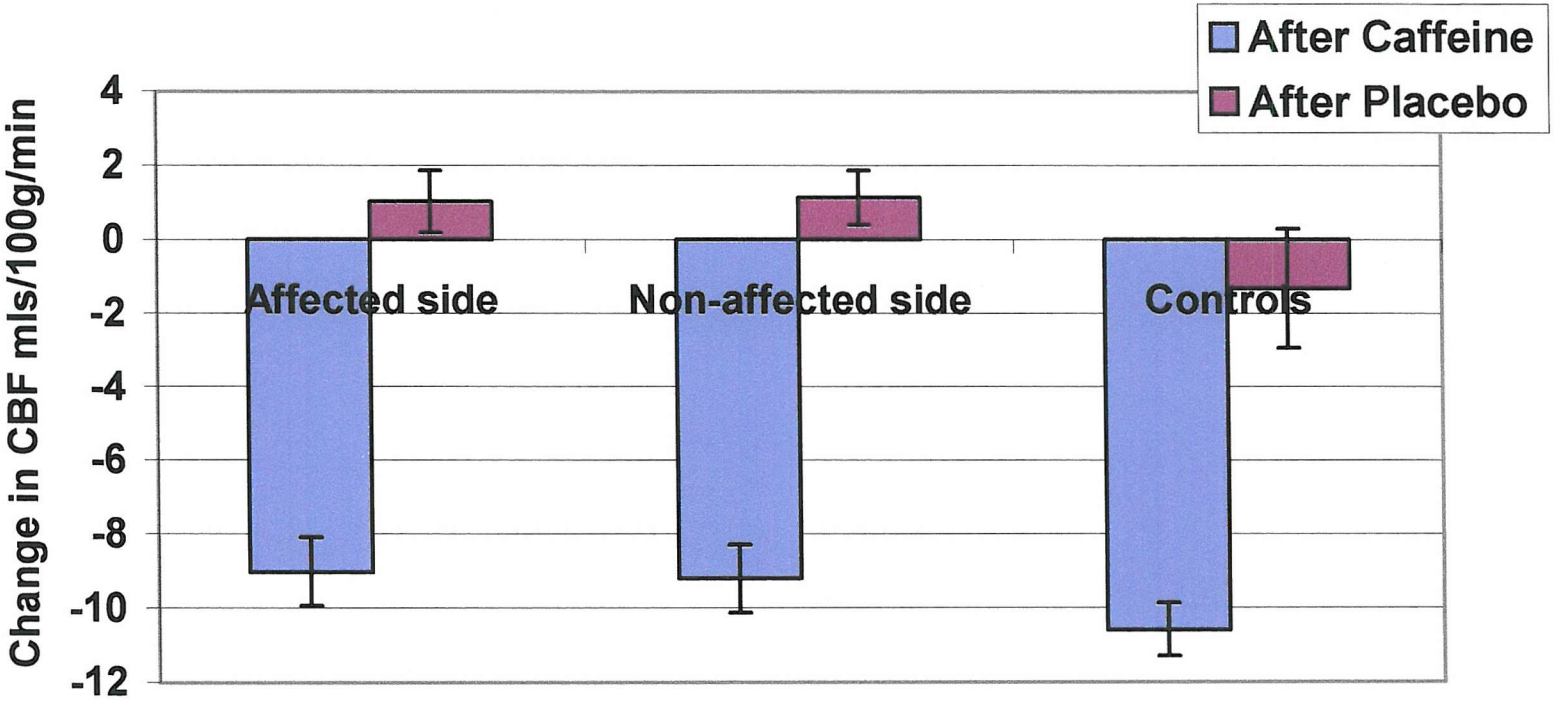


Figure 11. Middle Cerebral Artery Blood Velocity - Subjects (both hemispheres) Mean +/-1SEM

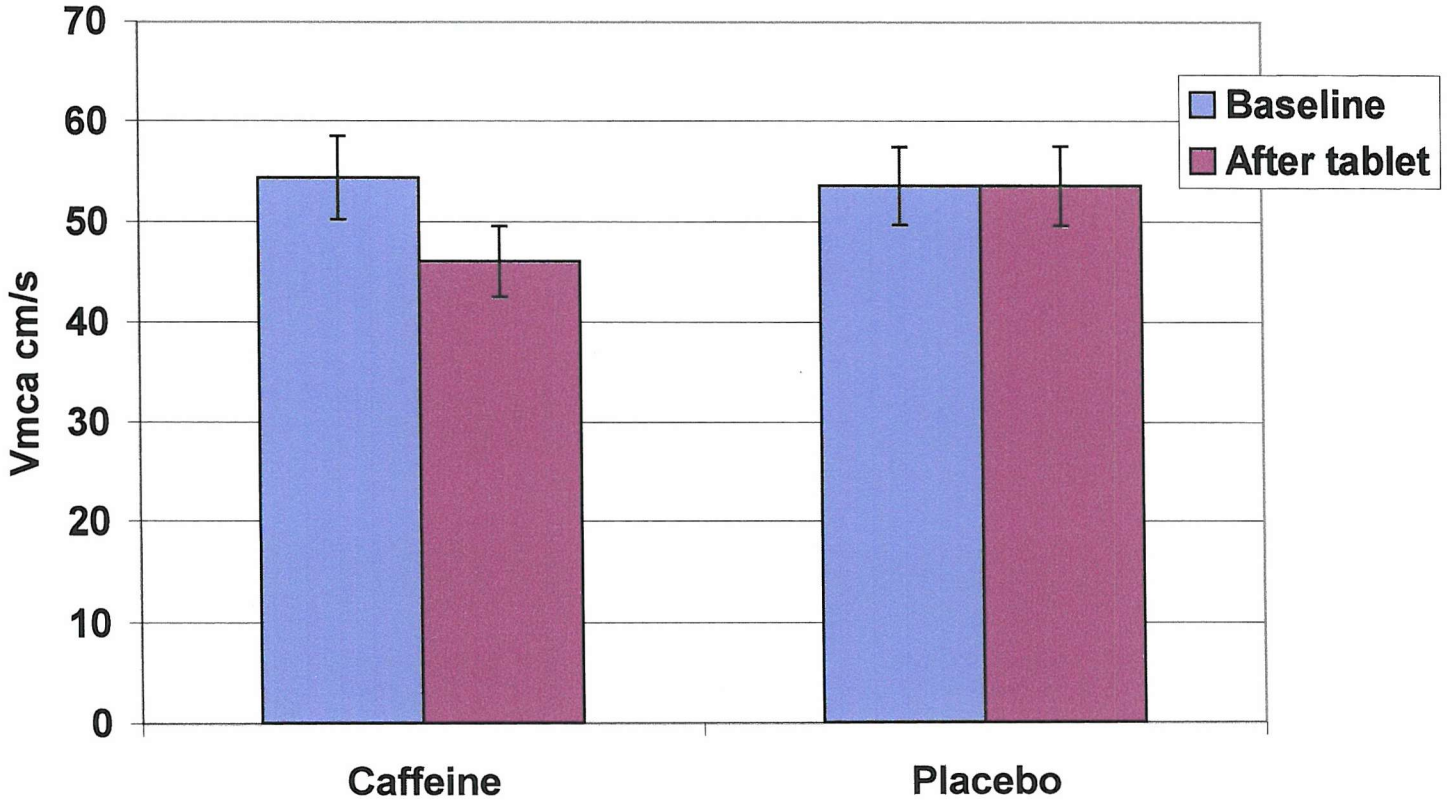


Figure 12. Middle Cerebral Artery Blood Velocity - Controls (both hemispheres)
Mean +/-1SEM

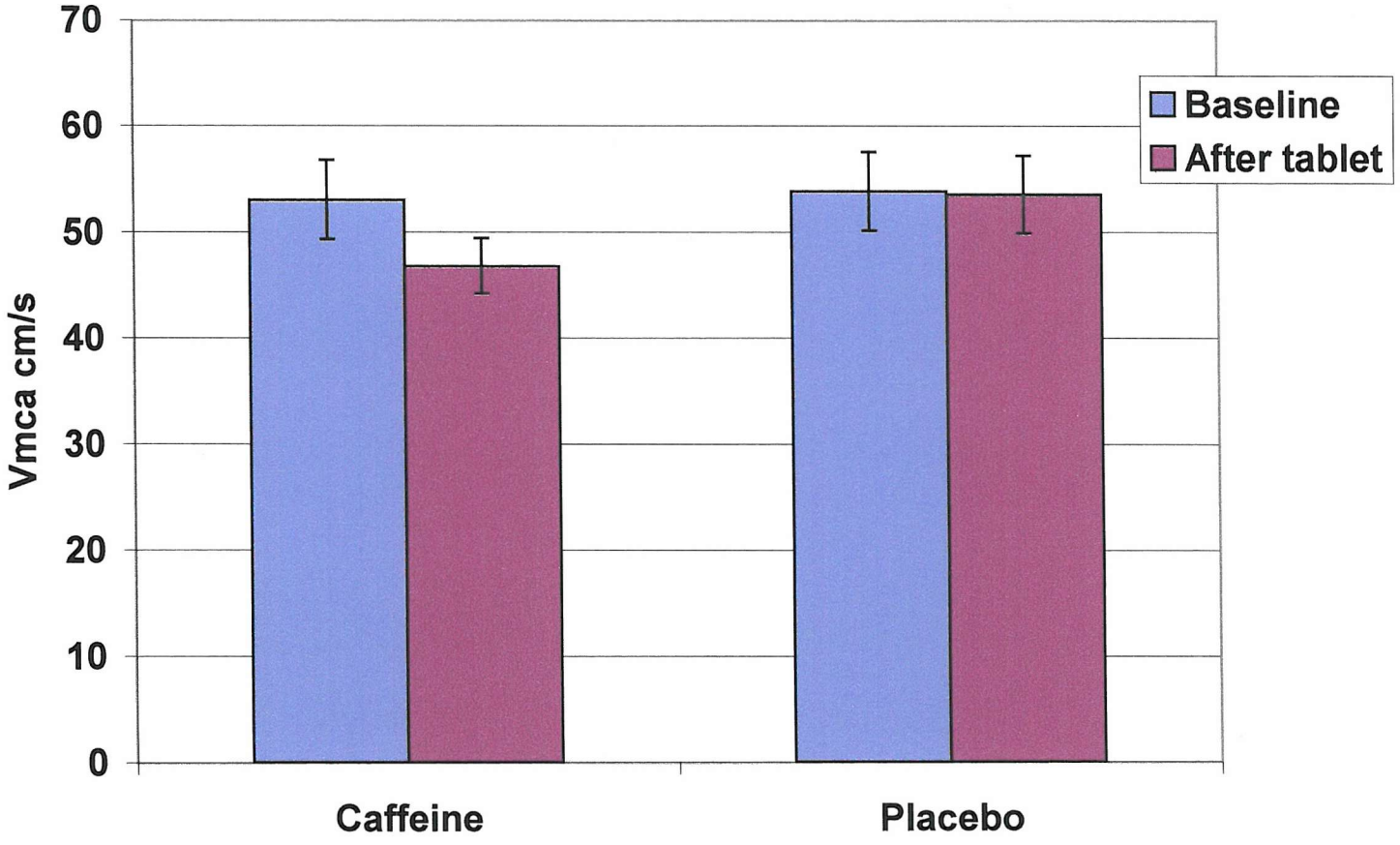
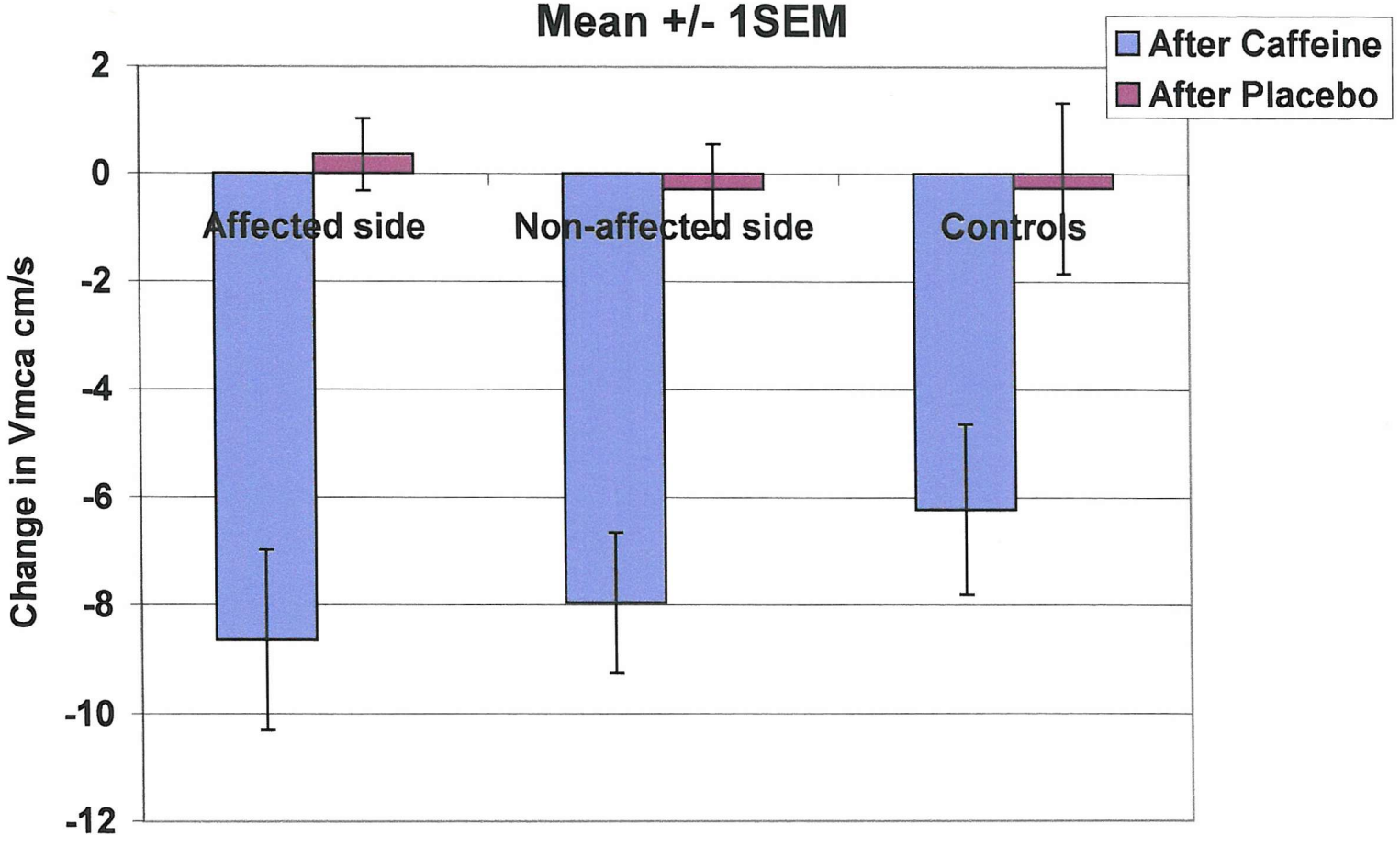


Figure 13. Change in Vmca after tablet - Subjects and Controls
Mean +/- 1SEM



CHAPTER 5: DISCUSSION

In this chapter the interpretation of the results will be discussed. This will be focussed around the stated initial aims of the study but will then proceed to the wider implications of the results. In particular, the possible clinical relevance of our findings will be discussed and potential further studies will be outlined.

5.1 Interpretation of Results

5.1.1 Effect of caffeine on global cerebral blood flow

Several investigators have demonstrated a reduction in cerebral blood flow after the administration of caffeine in normal healthy volunteers (110,111,113, 114, 115). There have been no previous studies examining the effect of caffeine on cerebral blood flow in patients recovering from an acute ischaemic stroke. Though the CBF reduction after caffeine administration is unlikely to cause symptoms of cerebral ischaemia in normal individuals, patients recovering from an ischaemic stroke may have less cerebrovascular reserve and therefore be at more risk. The primary aim of this study was to investigate the effect of caffeine on CBF in ischaemic stroke patients.

The results of this study clearly demonstrated significant reductions in global cerebral blood flow up to 90 minutes after the oral administration of 250mg caffeine as measured by the xenon inhalation technique in patients recovering from an ischaemic stroke. The dose of caffeine used was equivalent to 2-3 cups of instant coffee. Both males and females showed similar CBF responses to caffeine. Similar results were obtained with both compartmental and non-compartmental indices of cerebral blood flow and identical flow changes were found in the affected stroke hemisphere and the unaffected side.

The average reduction in CBF with caffeine was 24% (95% Confidence Interval –18% to –30%, $p<0.00001$) in the subject group. This reduction was similar in the control group although the baseline CBF values were higher in the control group compared to the subjects. This may be due to increased metabolic brain activation in the controls associated with entering the research room with unfamiliar equipment. It could be hypothesized that the subject group who were mostly hospital in-patients would be more used to having hospital investigations and consequently less stressed by the procedure.

From these results it can be concluded that caffeine significantly reduces cerebral blood flow and the effect of caffeine on CBF is similar in stroke patients and controls. The reduction in CBF after caffeine in this study was comparable with previous clinical studies in newborns treated with caffeine for apnoea where CBF reductions of up to 21% have been reported. This study is also comparable with the previous studies conducted in normal healthy volunteers. Reductions of CBF of 18% after 250 mg caffeine were demonstrated with Xenon-133 clearance (113,114). A further PET study demonstrated 30% reductions in CBF after 250 mg caffeine (115). The latter study differed from this study by having a considerably smaller sample size of 8 subjects and by administering caffeine as an intravenous infusion. It has been demonstrated that the pharmacokinetics of caffeine are comparable after oral or intravenous infusion (165).

The mechanism of CBF reduction with caffeine is likely to be due to a global effect on the cerebral vasculature since there were no differences between the affected and unaffected cerebral hemisphere in the subject group or between the right and left hemisphere in the control group. At physiological doses caffeine is a competitive antagonist of adenosine receptors. Since adenosine is a potent vasodilator of cerebral blood vessels the most plausible explanation for this effect is a direct vasoconstriction due to adenosine receptor blockade.

5.1.2 Effect of caffeine on Cerebral Blood Velocity in Stroke Patients

The second objective was to validate the results of a previous transcranial Doppler study that examined the effects of caffeine on cerebral blood velocity in ischaemic stroke patients (121,122,123). The aim was to determine if the changes in middle cerebral artery blood velocity reflected changes in blood flow.

This study confirmed that caffeine caused a reduction in middle cerebral artery blood velocity of 16% (95% CI -11% to –21%, $p<0.0001$) in patients recovering from an acute ischaemic stroke. This effect was similar in both hemispheres. These results were comparable to a previous transcranial Doppler study in which caffeine caused a 13% decrease in Vmca on the affected side (95% CI –8% to –19%) and a 12% decrease on the unaffected side (95% CI –6% to –18%) in 20 patients recovering from an acute ischaemic stroke.

Cafffeine caused a reduction in Vmca of 12% in the control group although the 95% CI were fairly wide, possible reflecting the smaller sample size in the control group and error in individual measurements. The conclusion was that caffeine has a similar effect on middle cerebral artery blood velocity in both subjects and controls.

5.1.3 Correlation between CBF and Vmca measurements

This study showed a significant positive correlation between percentage changes in middle cerebral artery blood velocity as measured by TCD and changes in cerebral blood flow as measured by xenon -133 ($r=0.42$, $p=0.045$). Theoretically, if all measurements had no error and changes in TCD exactly followed changes in CBF it would have been expected to show identity in the percentage changes with both techniques (i.e. perfect correlation, $r=1$). There was no correlation between absolute values of measurements with TCD and Xenon.

Our results on correlation between CBF and Vmca measurements are consistent with other studies in the literature. 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 xenon studies (r=0.85). However there was poor correlation between the absolute Vmca measurements and hemispheric CBF (128).

Sorteberg demonstrated a positive and highly significant correlation between MCA velocity and regional CBF in the MCA territory ($r=0.63$, $p<0.001$) in normal subjects after the data were normalized to a standard pCO_2 (166). Larsen used SPECT scanning to demonstrate a good correlation between CBF (percent baseline) and TCD mean flow velocity in the MCA (percent baseline) below the lower limit of autoregulation (167). Dahl *et al.* (1989, 1992, 1994), Demolis *et al.* (1996), and Kirkham *et al.* (1986) have also demonstrated that changes in Vmca correlate well with changes in CBF during cerebral vasoreactivity and autoregulation tests (43,168,169,170,171).

In our study the changes in CBF were of larger magnitude than the changes in Vmca. There are three possible explanations for this. Firstly, there may be systematic over/underestimation of blood flow by one technique compared to the other. Both TCD and Xenon techniques have limitations of measurement, which will be further discussed in section 5.2. It seems likely that some of the poor correlation between the two techniques may be explained by the variability within each measurement.

Secondly, caffeine may be having a direct effect on the middle cerebral artery diameter. It is known that the relationship between Vmca and CBF is a complex one and as previously discussed blood flow (F) in a particular artery depends on the cross sectional area (πr_2) and the mean velocity (V) of the blood running through it. Flow is also dependent on the weight of the perfusion territory and can be calculated from the formula

$CBF = v \times \pi r^2/M.$

where M is the weight of the perfusion territory and CBF is flow (ml/100g/min). Changes in CBF are therefore proportional to changes in mean velocity provided the weight of the perfusion territory and the vessel diameter remains constant (166).

Caffeine is unlikely to have an effect on tissue weight but may have an effect on the diameter of the middle cerebral artery although there is no direct evidence of this in the literature. A decrease in diameter would result in an increase in the velocity measured provided the actual blood flow remains the same. It therefore follows that with a reduction in blood flow and a vasoconstrictive effect at the MCA, CBF changes would be of greater magnitude than Vmca changes.

Thirdly, differences in the regional effect of caffeine could account for the discrepancy between the TCD and Xenon measurements. TCD measures middle cerebral artery blood velocity, whereas the xenon is measuring the flow from all 3 major arteries. The MCA carries approximately 80% of the flow volume received by the cerebral hemisphere and is therefore generally regarded as a good representative of cerebral blood flow (172). After an ischaemic stroke cerebral haemodynamics may change so that the anterior and posterior cerebral arteries contribute to blood supply of areas of the brain normally perfused by the MCA (173).

5.1.4 Effect of caffeine on regional cerebral blood flow

Analysis of the data in the subject group revealed that the reduction in cerebral blood flow after caffeine ingestion is bigger in the front of the brain compared to the back. In the front of the brain the mean change in CBF was –10.3mls/100g/min whereas in the rear it was –8.5mls/100g/min ($p=0.007$).

All the subjects had infarcts situated in the front of the brain therefore it may be expected that caffeine causes a greater reduction in CBF in the area around the infarct. However, the regional difference was observed in both the affected and unaffected hemisphere. The changes at the front of the brain were analysed from 6 detectors and the changes at the back of the brain were taken from 2 detectors. However, the standard deviations were not lower in the front compared to the back so the difference was probably not due to a difference in number of measurements.

This regional difference was not observed in the control group. This may be a reflection of the smaller sample size of the control group. Alternatively a different mechanism may be occurring in the stroke patients, which is not seen in the controls. In previous human studies examining the effect of caffeine on CBF in normal volunteers the CBF changes were uniform across all brain regions (115).

Caffeine is known to reset the coupling between cerebral blood flow and metabolism and the regional variations in CBF induced by caffeine may reflect local variations in cerebral metabolism. Animal studies have demonstrated that caffeine induces a decrease in local CBF mainly in the area where it increases metabolism (e.g. in the motor and limbic systems and in the thalamus) (174,176).

5.1.5 *Variability of measurements*

During the analysis variability was calculated for 2 consecutive TCD or Xenon measurements. The analysis showed a variability of 9.1% between pairs of CBF measurements (i.e. a standard deviation of 6.4% for a single xenon measurement). This is comparable to the 4-7% variability between pairs of CBF measurements found by Obrist (149). Risberg found the variability of a single xenon measurement to be 4% (159).

The variability for a single Vmca measurement with TCD was 5.4% which is similar to that obtained with Demolis (5-7%). The Demolis study used the same criteria in that comparisons of 2 measurements were performed at the same hour with a 1-week interval by one observer, although in that study a fixed TCD probe was used (134). This would suggest that by using a hand held probe it is possible to obtain the same consistency as with a fixed TCD probe.

5.1.6 Correlation of effect of caffeine with impaired vasoreactivity

Subjects with impaired vasoreactivity to hypercapnia are in a state of maximal cerebral vasodilatation and might be at risk from reductions in cerebral blood flow. It was therefore intended to identify within our subject and control group, a subgroup of individuals with impaired vasoreactivity and to correlate this to the effect with caffeine on cerebral blood flow.

It may have been expected from our results that there would have been a difference in the vasoreactivity between subjects and controls. In fact we were unable to show any statistical difference in vasoreactivity to hypercapnia between subjects and controls.

There was no significant correlation between vasoreactivity to hypercapnia and the effect of caffeine on cerebral blood flow. A further analysis compared the vasoreactivity after caffeine and after placebo in those subjects who had reactivity measured on both study visits. The increase in CBF with hypercapnia was significantly smaller after caffeine than after placebo (2.0% per mmHg $PaCO_2$ after caffeine and 2.5% after placebo, $p<0.001$)

This effect has been previously demonstrated in rats where the administration of caffeine (10mg/kg intraperitoneally) 15 minutes prior to hypercapnic challenges significantly decreased the peak increases in CBF (74). This observation

suggests that at least part of the increased CBF during hypercapnia is due to increased extracellular levels of adenosine in the brain. Further support for the hypothesis that adenosine is responsible for the regulation of CBF in the hypercapnic brain was demonstrated when dipyridamole (an adenosine uptake inhibitor) enhanced the peak increase in CBF during hypercapnia (174).

During the vasoreactivity testing in this study blood pressure was not monitored although variability in blood pressure may have influenced the results. Parallel measurement of blood pressure may have increased the reliability of the test (175).

5.2 Methodological Considerations

5.2.1 Study Design

The randomized double-blind cross over experimental design was used to reduce the influence of chance and non-specific factors. The xenon inhalation technique was chosen as the method of measurement of CBF as the robustness of the technique has previously been demonstrated by reports of high degrees of test/retest stability (157,158). A cross over design was employed so that the patients were acting as their own controls. In addition, a separate group of 10 healthy controls was studied.

Several limitations of the method have already been described in Section 3.4. In this section we shall concentrate on other important aspects of the method that have not yet been reviewed.

5.2.2 Limitations of Xenon measurements

As previously described the Xenon-133 inhalation technique suffers from a limitation known as the look through phenomenon (see Section 2.2.6). The main perquisite for measuring blood flow by this technique is that the tracer (Xenon-133) gains access to the tissue being studied, in other words that the tissue is perfused. This implies that in an extreme case of a region of infarction with no perfusion the signal received will be totally from perfusion in other overlying or underlying regions. In a less extreme example if there is an area of high flow and an area of low flow in the region of a detector, the measurement will be biased towards the higher flow.

It could be argued therefore that our measurements are biased towards blood flow in the normal brain tissue and not in the core area of the infarct. This raises

the question whether we are witnessing a steal phenomenon in which caffeine reduces cerebral blood flow in the normal tissue in order to divert it towards the area of the infarct. For example, Magnussen demonstrated that 250mg of aminophylline administered in the acute stages of stroke decreased cerebral blood flow in the non ischaemic areas and increased collateral blood flow surrounding the ischaemic region (177).

If this were the case in our study, it would be hard to hypothesise that caffeine is having an adverse effect on the patient. However, while acknowledging this is a limitation of this study, a steal phenomenon is unlikely for two reasons. Firstly in order for a steal mechanism to occur vasodilatation is required and it is probable that the vessels around the ischaemic area are already in a state of maximal vasodilatation. Secondly, if there were increased flow towards the area of infarct this increased flow would be more likely to contribute to the measurement.

Finally, it may be possible that the xenon inhalation is a learned technique and that on subsequent measurements the cerebral blood flow will fall due to habituation. With this in mind we analysed the effect of order in which the patients received the caffeine/placebo to assess if this affected our results. Adjusting for the group (i.e. order of caffeine/placebo) did not significantly affect any of the results obtained for Xenon measurements.

5.2.2 Limitations of Vmca Measurements

The power calculation for transcranial Doppler measurements calculated a sample size of 19 subjects in order to detect the smallest effect size. Unfortunately, during the study we were only able to obtain reliable TCD measurements on 14 subjects due to technical difficulties. Despite this we were able to demonstrate a significant effect in our subject group. This was because the effect of caffeine was greater than assumed for the original power calculation.

Similarly to the Xenon measurements there did not appear to be a significant learning effect between visits. Adjusting for the order in which they received caffeine and placebo did not alter the results.

5.2.3 Effect of other variables

In interpreting the results an assumption is made that the decreases seen in both CBF and Vmca are due to the effect of caffeine since these decreases are not seen with placebo. Both CBF and Vmca can be altered by other variables. In particular carbon dioxide tension, blood pressure and changes in metabolic activity in the brain will affect blood flow. The effect of each of these variables will be considered in turn.

Carbon dioxide is well recognised to be one of the most powerful agents to influence cerebral blood flow. There could be a bias if caffeine was causing a change in pCO_2. In this study there was no continuous recording of respiratory rate or end tidal carbon dioxide. One recording of end tidal carbon dioxide ($P_{ET}CO_2$) tension during each xenon measurement was made. The effect of changing CO_2 levels was investigated by comparing the average change following placebo with average change following caffeine.

There was no significant change in $P_{ET}CO_2$ in subjects or controls after caffeine compared to placebo. The mean change in $P_{ET}CO_2$ after caffeine in the subject group was –1.2mmHg. In a normal brain cerebral blood flow will decrease by 2-4% for every mmHg decrease in arterial CO_2 tension. If the assumption is made that $P_{ET}CO_2$ is a good estimate of arterial CO_2 tension then the anticipated effects of the changes in CO_2 measured in the subject group are a 2.4% - 4.8% reduction in cerebral blood flow. It can be concluded therefore, that changes in carbon dioxide probably cannot account for the 25% reduction in CBF seen after caffeine. Interestingly, in a previous study investigating the effect of caffeine on

CBF in normal volunteers, a significant reduction in carbon dioxide after caffeine was observed but the authors concluded that the changes in CO_2 tension did not account for the changes in CBF (115).

From studies dealing with cerebrovascular reactivity to induced alterations in carbon dioxide tension correction factors have been applied to values for blood flow to standardize them for an arterial Pco_2 of 40mmHg. These correction factors were not applied to this data since they are only meaningful in connection with artificially altered CO_2 tension and cannot be used to standardise regional flow data under normal conditions. It cannot be assumed that blood vessels in different regions of the brain respond to increases in arterial carbon dioxide concentration equally or even in the same direction. For example, the grey matter flow in response to the inhalation of carbon dioxide increases more than that of white matter.

Blood pressure changes may have an effect on CBF since dynamic cerebral autoregulation is impaired after acute ischaemic stroke (37). Blood pressure was monitored during this study. Previous studies have demonstrated an acute pressor effect of caffeine in caffeine naïve subjects. However, in this study administration of 250mg caffeine did not significantly alter blood pressure and therefore changes in blood pressure are unlikely to contribute to the changes in cerebral blood flow seen after caffeine.

It is known that variations in mental activity such as talking or thinking can affect local cerebral blood flow. Each measurement was taken with the patient lying supine in a quiet room. No instructions were given as to whether the patient's eyes should remain closed or open. Some patients engaged in conversation during the measurements whereas others fell asleep. This may have increased the overall variability, and therefore the 95% CI, but should not affect the overall change since we used the randomised crossover protocol.

5.2.5 Other limitations of the study

The dose of 250mg caffeine used is equivalent to 2-3 cups of instant coffee and was judged to be a representation of average daily caffeine intake. However, this study did not address the issue of whether this dose ingested in increments (as would occur by drinking cups of tea/coffee over the day) would cause similar reductions in CBF.

The clinical information collected during the study related to subjects age, the clinical (OCSP classification) and CT diagnosis of stroke and the Barthel index of current disability. In retrospect, it would have been interesting to collect more clinical information about the subject group. Information such as whether this was their first or a recurrent stroke or whether there was a history of chronic hypertension, would have given us further information about their cerebrovascular reserve capacity. Documenting whether they were right or left-handed would have enabled us to assess blood flow changes in the dominant versus the non-dominant hemisphere. A re-assessment of the Barthel index a month after participation in the study would have yielded information about whether the subject group were still in a period of recovery from their acute stroke.

A drug history was not taken for the patients during the study. This may have been relevant as for example, dipyridamole is an uptake inhibitor of adenosine and therefore might interfere with the effects of adenosine antagonists such as caffeine (178). However, dipyridamole does not readily cross the blood brain barrier and therefore such effects are likely to be minimal. A history of antihypertensive drug intake may have been helpful due to the effects of chronic hypertension on cerebral autoregulation.

Finally, we did not take a caffeine history from subjects or controls. Since we know that the effects of caffeine are different in caffeine naïve people compared

to regular caffeine users this might have been a factor in the variability seen. This issue shall be discussed in more detail in Section 5.3.2.

5.3 Clinical Implications

5.3.1 Clinical Implications of acute caffeine consumption in stroke patients

This is the first study to demonstrate significant reductions in CBF using Xenon-133 after the administration of caffeine in patients recovering from an acute ischaemic stroke.

This finding raises several important and interesting questions. Does this represent a true reduction in blood flow in the ischaemic areas of the brain? Does the reduction in blood flow reflect a reduction in cerebral oxygenation? Is this effect likely to have adverse clinical sequelae?

Recovery after an acute stroke is a complex multi factorial process. Most of the recovery of motor function in patients suffering a cerebral infarct takes place during the first two to three months although improvement may continue in some patients for up to 1-2 years.

Several mechanisms have been suggested to explain recovery. Following an infarct four tissue subtypes can be identified through PET scanning. The already irreversibly damaged 'core', the at risk 'penumbra', the mildly hypoperfused 'oligaemic' area and the unaffected tissue (179). In the first few days after a stroke, neurones in the area of the ischaemic penumbra that are not irreversibly damaged may start to function again. This process may occur due to improved blood supply, reversal of metabolic problems or resolution of cerebral oedema. SPECT studies have demonstrated reactive hyperaemia in the area of the infarct occuring between day 6 and day 15. Most studies identify spontaneous recanalisation of an occluded artery occurring within the first one to two weeks after an acute ischaemic stroke. Early spontaneous recanalisation (within 24 hours) is associated with a lower fatality rate (180).

Caffeine induced falls in cerebral blood flow during the period where the penumbral and oligaemic tissues are potentially viable may jeopardize blood flow to the ischaemic area with harmful consequences to the patient. The duration of the penumbra is unknown although it may last as long as 5-7 days (55). In our study our subject group were all at least 2 weeks after their stroke so it is unlikely that the penumbral and oligaemic regions of the infarct were still viable.

It is well recognised that some patients with major cerebral arterial occlusion may continue to be under chronic haemodynamic stress (181,182). In these patients the cerebral vascular bed has reached a maximum vasodilatation to preserve blood flow in compensation for the reduced perfusion pressure. These areas will have reduced vasoreactivity to a vasodilatory stimulus such as hypercapnia or acetazolamide. PET can detect this state of 'misery perfusion' where blood flow is decreased but oxygen consumption is preserved at a level sufficient for tissue survival for a limited period of time. Areas of misery perfusion will have an increased oxygen extraction fraction up to 80% -100% from the normal value of approximately 40% (183).

These haemodynamically-compromised areas are exposed to a high stroke incidence and a drop in blood flow in these areas has been postulated as a risk factor for stroke evolution. Therefore, theoretically, caffeine induced falls in cerebral blood flow during the acute stage of recovery may exacerbate pre-existing cerebral ischaemia in areas of misery perfusion.

A limitation of the xenon measurements is a lack of cerebral metabolic information. In interpreting the clinical implications of the results an assumption is made that CBF changes represent neuronal function abnormality. Ideally, this study should be repeated with PET scanning which as well as measuring CBV and CBF can give us information about the oxygen extraction fraction (OEF) and metabolic rate of oxygen consumption (CMRO2) and glucose utilization.

Wise (1983) studied the stages of recovery in acute stroke with PET scanning. He demonstrated that in half of their patients with an acute infarct there was a greater reduction in regional CBF than in $CMR0_2$, with consequent increases in OEF. The other half had regional $CMRO_2$ reduced as much as or even more than CBF and therefore the OEF was normal or low. By one month CBF and $CMRO_2$ were relatively matched with a normal or slightly reduced OEF (183).

A PET study with caffeine would assess the effect of caffeine on CMRO2 and OEF as well as CBF. This would give us further information about whether caffeine is likely to be of clinical harm to stroke patients. However, PET scanning does involve greater expense and technical complexity. SPECT, Xenon CT and diffusion/perfusion MRI may be more accessible alternatives but more work is needed to validate them.

A reduction in CBF is a normal response to a reduction in cerebral metabolism. It could be hypothesised from our results that caffeine is causing a reduction in cerebral metabolism with a secondary decrease in cerebral blood flow. In fact the evidence points towards caffeine increasing cerebral metabolism. Administration of an acute dose of 10mg/kg caffeine induces an increase in the rates of local cerebral glucose utilization particularly in structures involved in the control of locomotor activity (176). Caffeine increases synaptic activity, enhances firing of neurones and stimulates release of neurotransmitters causing a net increase rather than a decrease in brain glucose requirements (71).

Further research is needed to address whether the caffeine induced reductions in cerebral blood flow after an acute ischaemic stroke are of clinical detriment to the patient. A distinction needs to be made between caffeine administration in the hyperacute and acute stage and during the subsequent period of recovery. A second distinction would need to be made between subjects who are caffeine naïve and subjects who are regular caffeine consumers since we know that tolerance develops rapidly to caffeine and up-regulation of adenosine receptors

occurs in chronic caffeine consumers. This shall be discussed further in the next section.

There have been no randomised controlled trials investigating the clinical effect of caffeine administered acutely to stroke patients. There have been two randomised controlled trials investigating the effects of a related methylxanthine (aminophylline) given acutely to patients with acute ischaemic stroke. Geismar (1976) administered aminophylline within 96 hours of stroke onset and Britton (1980) administered aminophylline within 18-114 hours of stroke onset (184,185). These trials showed no definite evidence that aminophylline caused harm or benefit when administered at doses suitable for the treatment of asthma (186). However, the numbers of patients studied was small.

There is limited data on the influence of caffeine on outcome from ischaemic stroke in experimental animal models. Sutherland (1991) demonstrated that rats treated with an acute dose of caffeine of 10mg/kg 30 minutes prior to an ischaemic insult show accelerated ischaemic brain injury. It is generally assumed that 10mg/kg in a rat represents about 250mg of caffeine in a human weighing 70kg (3.5mg/kg) since the half life of caffeine is much shorter in rats (0.7-1.2hours) than in humans (2.5-4.5hours). These accelerated ischaemic brain changes were demonstrated in magnetic resonance imaging and histopathological analysis at 24 hours post ischaemia (74).

Consistent with this, Rudolphi et al (1987) demonstrated that acute treatment with an active caffeine metabolite (theophylline), aggravated histological damage in a gerbil model of cerebral ischaemia (92).

Strong (2000) investigated the effect of a combination of low dose ethanol and caffeine on cerebral infarct volume in rats. They demonstrated that the combination of 10mg/kg caffeine and 10% ethanol given 3 hours and 1 hour before MCA occlusion dramatically reduced infarct volume by approximately

83%. The same dose of caffeine given alone did not affect infarct volume. *Chronic administration of ethanol and caffeine daily for three weeks abolished* this neuroprotective effect (187).

5.3.2 Clinical Implications of chronic caffeine consumption in stroke patients

Most patients admitted to hospital with acute ischaemic stroke will be chronic caffeine consumers. In hospital they are likely to be served caffeine-containing food and drink regularly. Unless they have a period of being 'nil by mouth' (40% are likely to have some dysphagia acutely) there will not be a period of caffeine abstinence as modelled in our experimental design. This raises the question 'Are the effects of caffeine ingestion on cerebral blood flow likely to be the same in chronic caffeine consumers as in caffeine abstainers?'

It is well known that caffeine tolerance occurs rapidly and the adaptive changes to long-term caffeine are very dramatic. For example, caffeine causes an acute rise in blood pressure in caffeine abstainers but does not appear to affect blood pressure in people who are regular caffeine users. Long term treatment with *caffeine causes a reduction in locomotor activity whereas acute* treatment stimulates motor activity in rats (188).

The mechanism underlying these effects is likely to be an upregulation of adenosine receptors with chronic caffeine consumption. Most of the evidence for this is found in animal studies where chronic exposure to caffeine has led to an increase in number and G-protein coupling of the receptors (189).

Rudolphi (1989) randomly divided 14 gerbils into 2 groups. One group received caffeine (0.2% as caffeine sodium benzoate) over 4 weeks and the other group acted as controls. At the end of the treatment all the gerbil brains were examined

by histopathological analysis (93). Chronic treatment with caffeine caused a small but significant increase in receptor density in the frontoparietal cortex and the hippocampus. Boulanger (1983) also demonstrated that administration of caffeine for 7-14 days increased the total number of A1 receptors in the rat brain (191).

Can this upregulation in receptors with chronic caffeine protect against brain ischaemia? In the second part of Rudolphi's experiment the gerbils were subjected to 5 minutes of bilateral carotid occlusion. Seven days after ischaemia the animals were killed and the brains histologically examined. The chronic caffeine treated group had a significantly reduced degree of ischaemic hippocampal damage.

In a further study rats chronically pre- treated with caffeine (up to 90mg/kg/day for 3 consecutive weeks) were compared to rats given 10mg/kg caffeine prior to an ischaemic insult. The rats given the chronic administration of caffeine showed significantly less ischaemic brain damage than the rats given an acute dose (74).

These observations support the idea that activation of adenosine receptors (chronic treatment with caffeine) reduces ischaemic damage while inhibition of the adenosine pathway (acute treatment with caffeine) augments such damage.

Can these results from the animal experiments be extrapolated to human studies? To date there have been no studies examining the effect of chronic caffeine consumption on brain ischaemia in humans. There have been studies that show that upregulation of adenosine receptors does occur with chronic caffeine intake in man.

Baggioni (1991) found that withdrawal from chronic caffeine consumption in normal subjects resulted in sensitisation to adenosine's actions on platelets (192). In a further study (Paul 1993), withdrawal from chronic caffeine (250mg 3

times a day for 7 days) in normal subjects resulted in upregulation of A2 receptors in platelets. This was demonstrated at 12 and 60 hours after caffeine withdrawal. Furthermore, chronic caffeine consumption resulted in sensitisation not only to adenosine but also to the actions of prostacyclin in human platelets (90).

More recent studies (Varani 2000) have confirmed that chronic caffeine intake (600mg for 2 weeks or 400mg for 1 week) leads to upregulation of adenosine A2 receptors in healthy human volunteers (91). A significant increase in the number and sensitivity of A2a receptors was demonstrated at 12 and 60 hours after the last caffeine dose. Since the A2a receptor is responsible for the anti platelet aggregatory properties of adenosine this effect may have clinical implications.

In summary, from the existing evidence it is apparent that the effects of acute and chronic treatment with caffeine are qualitatively different. Long term treatment with adenosine receptor antagonists can have effects that resemble those of acute administration of adenosine receptor agonists. Further research is needed to answer the question of what consequence chronic caffeine intake may have for the outcome of cerebral ischaemia. On the one hand caffeine is expected to aggravate damage by blocking adenosine receptors, on the other hand the upregulation of the adenosine receptors will tend to counteract this.

5.4 Implications for future research

This study has demonstrated that 250mg caffeine given after a 48-hour period of abstention leads to a significant reduction in cerebral blood flow in patients recovering from an acute ischaemic stroke. Further research is needed to address several important and interesting questions. Firstly, what are the clinical implications of this reduction in cerebral blood flow? Secondly, is the effect of caffeine dose related? Finally, what would be the effect of chronic caffeine consumption on cerebral blood flow?

5.4.1 Clinical relevance of the study

There have been several epidemiological trials, which have demonstrated that caffeine is not an independent risk factor in the incidence of coronary heart disease or stroke (99, 103,187). However, there have been no randomised controlled clinical trials investigating the effect of caffeine on patients recovering from an acute ischaemic stroke.

To address the question whether caffeine induced blood flow changes are clinically significant a randomised controlled clinical trial is needed. This is likely to be a large multi centre trial in which stroke patients would be randomised to a caffeine diet or a non-caffeine diet for a certain length of time. Primary outcome measures could be the proportion of patients alive and independent (i.e. Modified Rankin Score 0-2) six months later. Secondary outcome measures could include recurrent ischaemic stroke or a change in a neurological scale for stroke prognosis such as the National Institute of Health Stroke Scale (NIHSS). The power calculation would need to be based on expected likely outcomes after an ischaemic stroke.

During the present study a questionnaire was circulated to stroke physicians (members of the British Association of Stroke Physicians) around the country to assess whether they would be interested in participating in such a study. This was designed to assess their current knowledge of the effects of caffeine in stroke patients and to determine whether they would be interested in participating in a multi centre trial to further study this.

100 copies of the questionnaire were sent out to which we received 35 replies. Respondents were first asked if they were aware of any evidence of caffeine having an adverse effect after an acute ischaemic stroke. 88% were not aware of caffeine having an adverse effect while 12% did think caffeine had an adverse effect. The second question asked more specifically about the effect of caffeine on cerebral blood flow. 8% thought caffeine increased flow, 12% thought caffeine decreased flow and the majority (80%) was unaware of caffeine having an effect on blood flow. The third question asked about the availability of decaffeinated tea and coffee on their stroke unit. 3% stroke units served only decaffeinated tea and coffee, 70% served only caffeinated tea and coffee and 19% served both. Finally, respondents were asked if they would consider their patients taking part in a study where stroke patients are randomised to either a caffeine or caffeine free diet. The majority (85%) said they would be willing to participate in such a study.

5.4.3 Dose Effect of caffeine

Prior to setting up a multi centred trial as described above there are some preliminary questions that need answering. Is it feasible to withhold caffeine from patients, how commonly is caffeine consumed by stroke patients and is there a dose response to caffeine?

Several TCD studies have investigated the effects of different doses of caffeine on the cerebral circulation in normal healthy volunteers. The summary of these can be seen in Table 14. It can be seen from the table a dose related relationship between caffeine and reduction in Vmca is emerging.

Table 16. Effects of caffeine on Vmca

Dose of caffeine (mg)	Reduction in Vmca	Subjects	Reference
400	23%	Volunteers	Kerr
150	9%	Volunteers	Coutier
117	5.8%	Volunteers	Perod
45	Not significant	Volunteers	Perod
250	12%	Stroke patients	Hanrahan

Turning to the previous studies using Xenon clearance as the method of analysing effects of caffeine on cerebral blood flow in normal individuals, maximum CBF changes were demonstrated after 250mg caffeine (108,109). Further increments in dose did not produce more CBF reductions. Cameron (1990) used PET scanning to demonstrate that a dose of 250mg caffeine caused a 31% fall in cerebral blood flow in normal individuals (115)

On the present evidence, it seems likely that a dose related relationship does exist between the dose of caffeine and its effect on the cerebral circulation in healthy individuals. It would be fairly straightforward to set up a study confirming this dose related relationship in patients recovering from an acute ischaemic stroke.

5.4.4 Chronic Caffeine Consumption

Most patients with stroke are chronic caffeine consumers and the effect of caffeine in these subjects is of clinical relevance as previously discussed. In the present study subjects abstained from caffeine for 48 hours. In some cases patients with a diagnosis of acute stroke are kept nil by mouth for the first 24-48 hours, (for example 40% will have dysphagia acutely) and therefore this study protocol reflects the effects of acute ingestion of caffeine following this period.

A study protocol to assess the effects of caffeine on chronic caffeine consumers could assess changes in Vmca after 250mg caffeine in two groups. One group would be on a caffeine free diet and the other group would be taking regular caffeine supplements for 3-4 weeks prior to the study.

5.4 Conclusions

1. 250mg caffeine (equivalent to 2-3 cups of instant coffee) significantly reduces cerebral blood flow up to 2 hours after ingestion in patients recovering from an acute ischaemic stroke.
2. There is no difference between the effect of caffeine on cerebral blood flow in ischaemic stroke patients and normal healthy controls.
3. There is no difference in the effect of caffeine on cerebral blood flow between the affected and unaffected cerebral hemisphere in stroke patients.
4. Changes in cerebral blood velocity induced by caffeine reflect changes in cerebral blood flow.
5. Caffeine causes a bigger reduction in cerebral blood flow in the front of the brain compared to the rear in both stroke patients and controls.
6. Further research is needed to investigate whether this caffeine induced reduction in cerebral blood flow in patients recovering from an acute ischaemic stroke leads to adverse clinical consequences.

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