Pathophysiology of post stroke hyperglycaemia and brain arterial patency

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Abstract

The pathophysiology of acute post-stroke hyperglycaemia (PSH) is important as hyperglycaemia affects the majority of stroke patients, and is consistently associated with poorer outcome in terms of survival, disability and markers of brain injury such as infarct expansion. There appears to be an interaction between brain arterial patency and hyperglycaemia that has not been fully characterised.

This thesis initially reviews the literature on hyperglycaemia and stroke before focusing on animal models of PSH and clinical trials of insulin treatment for PSH in two systematic reviews with meta-analysis. The thesis then looks at the relationship between glucose profiles and clinical outcome in a historical population receiving IV thrombolysis for acute ischaemic stroke, specifically exploring alternative indices of glycaemic state to compare the optimal predictive index for functional outcome as measured by the modified Rankin scale.

The main body of the thesis details a prospective observational clinical study which recruited 108 patients within 6 hours of acute ischaemic stroke. These patients had careful monitoring of blood glucose levels over a 48 hour period and detail brain imaging including CT perfusion scanning to examine the ischaemic penumbra, CT angiography on admission and at 24-48 hours to document brain arterial patency with follow-up CT brain imaging to assess outcome infarct volume. The relationship between 48 hour blood glucose profiles, clinical outcome and imaging findings is then explored.

The main findings of the thesis are summarized below.

- Animal models of PSH have shown that hyperglycaemia exacerbates infarct volume in MCA occlusion models but studies are heterogeneous, and do not address the common clinical problem of PSH because they have used either the streptozotocin model of type I diabetes or extremely high glucose loads.

- Animal models show that insulin has a non-significant and significantly heterogeneous effect on infarct growth.

- Clinical trials of insulin for post stroke hyperglycaemia have shown no benefit in terms of improved functional outcomes or mortality. Insulin is associated with an increased risk of hypoglycaemia.

- In a historical cohort mean capillary blood glucose over 48 hours was more predictive of clinical outcome that admission blood glucose or two consecutive elevated glucose measurements.

- A high proportion of acute stroke patients have a blood glucose level above 7mmol/L within 6 hours of onset. Different patterns of blood glucose levels define different populations.

- Higher admission and mean glucose levels correlate with larger infarct volumes. Larger core perfusion lesion volumes are associated with a greater risk of mortality. Admission hyperglycaemia is more harmful than hyperglycaemia after 6 hours.

- In patients with angiographic evidence of an arterial occlusion infarct volume varies significantly with glycaemic status. In some populations late hyperglycaemia is associated with better imaging outcomes.

- Tandem occlusions are associated with bad outcomes after ischaemic stroke.

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Dedication

This work is dedicated to the patients and families who participated in this research. I hope that their misfortune will in some way help others.

It is also dedicated to the memory of Dr Margaret H Gladden, Reader Emeritus in Physiology at the University of Glasgow who supervised my first undergraduate research project and sadly died of a stroke during the course of this project.

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I am also grateful to my research fellow colleagues, Dr. Ferghal McVerry and Dr. Krishna Dani. They were here for most of this thesis and they gave me collegiate support and different perspectives on life as a stroke research fellow. We had some good fun.

The other research fellows who joined the department towards the end of my research time; Dr. Dheeraj Kalladka, Dr. Fiona Moreton and Dr. Xuya Huang, have kept the torch of stroke research burning and have tolerated me squatting in their offices on an ad hoc basis.

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Finally, I am always in debt to my parents who have always been there. They are very much appreciated.

Author’s declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature _____

Printed name _____

Details of work and collaboration

The work presented within this thesis was made possible through a clinical research fellow post, funded by The Stroke Association of the United Kingdom (TSA 2006/03). The research was undertaken in the South Glasgow Stroke Service. During the period of study, I was enrolled at the University of Glasgow as a post-graduate student studying towards a doctorate of medicine. In accordance with the rules of the University I attended the compulsory courses outlined in the curriculum for that degree. Additional training in research skills was supplied by the University.

The research position involved screening of patients admitted to the stroke unit of the Southern General Hospital, patient recruitment, clinical management and ensuring completion of study protocols.

Data collection was undertaken by me and the interpretation of this data, including the statistical analysis is my own work. Work from this thesis has been presented at local, national and international meetings. A list of presentations is included in subsequent pages.

There have been no external influences involved in the analysis and the interpretation of the data presented. For purposes of clarification the contribution of others to respective chapters is as follows.

Chapters 1: Literature review and interpretation of literature

Chapter 2: Xuya Huang acted as second observer for all scan reading. Rita Coinu prepared a set of test scans for assessment of inter-observer reliability. Keith Muir acted as adjudicator for any disagreement over scans.

Chapter 3: Systematic literature review, data extraction and meta-analysis undertaken by me. Elements of this chapter have been published as a paper in collaboration with Keith Muir.

Chapter 4: Systematic literature review, data extraction and meta-analysis undertaken by me. Elements of this chapter have been presented as an abstract in collaboration with Keith Muir

Chapter 5: Retrospective cohort study using data on patients treated with alteplase at the Southern General Hospital in Glasgow between May 2003 and November 2008, were identified from our local Safe Implementation of Thrombolysis in Stroke (SITS) database. Additional data on glucose profiles were extracted from the original clinical records by me. Statistical analysis was undertaken by me. Elements of this chapter have been presented as an abstract in collaboration with Keith Muir

Chapter 6-9: Patient recruitment was undertaken by me with help from the other clinical research fellows in the department (Ferghal McVerry, Krishna Dani, Dheeraj Kalladka, Fiona Moreton and Xuya Huang) and Keith Muir. I would often supervise contrast administration for study specific scans. Clinical follow-up was undertaken by me with help from Angela Welch the Stroke Research Nurse and the other research fellows if needed. Image analysis was performed as detailed for Chapter 2. Data collection and input to database was performed by me. Statistical analysis was undertaken by me. Xuya Huang helped with the programming of SAS for the Rankin shift analysis.

Chapter 10: Final discussion written and interpreted by me.

Definitions/abbreviations

Abbreviation	Definition
1.5T MRI	1.5 Tesla MRI
3T MRI	3 Tesla MRI
95% CI	95% Confidence Interval
ABG	Admission Blood Glucose
ADA	American Diabetes Association
ADL	Activities of daily living
AF	Atrial Fibrillation
AIF	Arterial Input Function
ANOVA	Analysis of variance
AOL	Arterial Occlusive Lesion
ASPECTS	Alberta Stroke Program Early CT score
ASTRAL	Acute Stroke Registry and Analysis in Lausanne
BMI	Body Mass Index
BP	Blood Pressure
CAMARADES	Collaborative Approach to Meta-Analysis and Review of Animal Data in Experimental Stroke
CAN	Cardiovascular autonomic neuropathy
CASES	Canadian Activase for Stroke Effectiveness Study
CASL	Continuous Arterial Spin Labelling
CBF	Cerebral Blood Flow
CBF	Cerebral Blood Flow
CBG	Capillary Blood Glucose
CBV	Cerebral Blood Volume
CCS	Causative Classification of Stroke
CGMS	Continuous Glucose Monitoring System
CLOTBUST	Combined Lysis Of Thrombus in Brain ischemia using transcranial Ultrasound and Systemic tPA
CNS	Central Nervous System

CSF	Cerebro-spinal fluid
CT	Computerised Tomography
CTA	CT angiography
CTP	CT perfusion
DEFUSE	Diffusion and Perfusion Imaging Evaluation for Understanding Stroke Evaluation
DIGAMI	Diabetes-Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction
DM	Diabetes Mellitus
DWI	Diffusion Weighted Imaging (MRI)
ECASS	European Cooperative Acute Stroke Study
ECG	Electrocardiogram
eGOS	Extended Glasgow Outcome Score
END	Early Neurological Deterioration
EPITHET	Echoplanar Imaging Thrombolytic Evaluation Trial
FBG	fasting blood glucose
GIST UK	United Kingdom Glucose Insulin in Stroke Trial
GKI regime	Intravenous delivery of a infusion containing insulin dextrose with potassium supplementation
GLIAS	Glycemia in Acute Stroke
GLUT-1	Glucose Transporter 1
GSP	Glycated serum proteins
H	Hours
HbA1C	Glycosylated Haemoglobin, Type A1C
HG	Hyperglycemia
HOMA	Homeostasis Model Assessment - a measure of insulin resistance
HR	Hazard ratio
ICA	Internal Carotid Artery
ICH	Intra-cerebral haemorrhage
ICU	Intensive Care Unit
IFG	Impaired Fasting Glucose
IGF	Insulin like growth factor

IGT	Impaired Glucose Tolerance
IIT	Intensive insulin therapy
IQR	Intra-Quartile Range
IRS-1	Insulin receptor substrate 1
IV	Intravenous
LACS	lacunar syndrome
L-NMMA	NG-monomethyl-L-arginine
LSD	Fisher's Least Significant Difference test
MAST-E	Multicentre Acute Stroke Trial
MCA	Middle Cerebral Artery
MCAO	Middle Cerebral Artery Occlusion
MCBG	Mean Capillary Blood Glucose
MI	Myocardial Infarction
MONICA	MONItoring of trends and determinants in CArdiovascular diseases
MRA	MRI angiography
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
mRS	modified Rankin scale
mRs-SI	modified Rankin scale Structured Interview
MTT	Mean Transit Time
N	Number
NBM	nil by mouth
NCCT	Non-Contrast CT scan
NHS	National Health Service
NICE-SUGAR	Normoglycaemia in Intensive Care Evaluation and Survival Using Glucose Algorithm Regulation
NIHSS	National Institute of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NOS	Nitric Oxide Synthase
OCSP	Oxfordshire Community Stroke Project

OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
OVID	a bibliographic database
OXVASC	Oxford Vascular Study
PACS	Partial Anterior Circulation Syndrome
PAI-1	Plasminogen Activator Inhibitor-1
PCA	Posterior Communicating Artery
PET	Positron Emission Tomography
PFO	Patent Foramen Ovale
POCS	Posterior Circulation Syndrome
POSH	Post Stroke Hyperglycaemia and Brain Arterial Patency study
PROACT II	PROlyse for Acute Cerebral Thromboembolism
PROGRESS	Perindopril Protection Against Recurrent Stroke Study
PSH	Post Stroke Hyperglycaemia
PWI	Perfusion Weighted Imaging (MRI)
RCT	Randomised Controlled Trial
REACH	Reduction of Atherothrombosis for Continued Health
ROS	Reactive oxygen species
r-proUK	recombinant pro-urokinase
RR	Relative Risk
rtPA	recombinant tissue –Plasminogen Activator
SD	Standard deviation
SELESTIAL	Spectroscopic Evaluation of Lesion Evolution in Stroke: Trial of Insulin for Acute Lactic acidosis
sICH	symptomatic Intra-Cerebral Haemorrhage
SITS	Safe Implementation of Thrombolysis in Stroke
SITSMOST	Safe Implementation of Thrombolysis in Stroke: Monitoring Study
SPSS	a statistical software package
STZ	Streptozotocin
TACS	Total Anterior Circulating Syndrome

TCD	Transcranial Doppler
THIS	Treatment of Hyperglycaemia in Ischaemic Stroke trial
TIA	Transient Ischaemic Attack
TIBI	Thrombolysis in brain ischaemia
TIMI	Thrombolysis in myocardial infarction
TOAST	Trial of ORG 10172 in Acute Stroke Treatment
TUCSON	Transcranial Ultrasound in Clinical SONothrombolysis
UK	United Kingdom
UKPDS	United Kingdom Prospective Diabetes Study
VISTA	Virtual International Stroke Trial Archive
VOF	Venous Output Function
VSMCs	Vascular Smooth Muscle Cells

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1 Introduction – sugar and stroke

1.1 Fundamental concepts in Stroke

1.1.1 Epidemiology – stroke is important

Stroke is the third leading cause of death worldwide after coronary disease and cancer. Every year an estimated 150.000 people in the UK have a stroke. It is also the most common cause of severe disability.[1] The Stoke Association (www.stroke.org.uk) estimate that more than 250,000 people in the UK live with disabilities due to stroke.

The UK economy is also drained by stroke. One recent study estimated that the societal cost of stroke in the UK amount to £8.9 billion a year. Stroke treatment accounts for approximately 5% of total NHS expenditure.[2]

1.1.2 Pathophysiology of stroke

An ischaemic stroke occurs when the blood supply to the brain is interrupted resulting in destruction of brain tissue. Stroke is a syndrome of end organ damage with many different causes. The most common causes are atherothromboembolism[3, 4], intracranial small vessel disease[5-7] and cardiac embolisation[8, 9]. Atrial fibrillation is the most common cause of cardiac embolisation in the developed world[10-13].

Different systems for stroke aetiological subtype classification have been used to categorise the probable cause of an ischaemic stroke in clinical reseach.[14, 15] Classification of stroke subtype may be useful in different scenarios. Subtyping may be necessary for a clinical trial or to clearly define populations in an epidemiological or genetic study. Stroke subtyping may also be important in clinical decision making.[16] The merits of different classification systems have been debated.

The TOAST system (Trial of Org 10172 in Acute Stroke Treatment) was widely used in many papers.[14, 16] TOAST uses five subtypes of ischaemic stroke (large-artery atherosclerosis, cardioembolism, small-vessel occlusion, stroke of other determined aetiology and stroke of undetermined aetiology) to classify strokes. There were several weaknesses in TOAST; the classification of lacunar stroke was inexact, patients with 2 co-existing definite causes of stroke would be classified as ‘stroke of undetermined aetiology’ and TOAST was accused of discouraging detailed diagnostic investigation.[16]

More recently the computerised Causative Classification of Stroke (CCS) system has been shown to allow reliable classification of stroke subtypes by using an evidence-based algorithm.[15] CCS is a revision of TOAST in which the 'undetermined cause' category was subdivided and recent advances in diagnostic techniques have been considered.[17]The computerised version of CCS is available for academic use online.[18]

The A-S-C-O system attempts to phenotype stroke.[19] A stands for atherosclerosis, S stands for small vessel disease, C for cardiac source and O for other. Each phenotype is scored for probability. The quality of evidence to support each diagnosis is also graded. A-S-C-O has been praised for it's comprehensive approach,[20] although it does not always reduce the incidence of 'undetermined' causes of stroke.[21]

One study comparing A-S-C-O, CCS and TOAST concluded that no classification system was clearly superior.[22]

Clinically, stroke syndromes can be classified by the arterial blood supply of the area of brain that has been injured to produce a neurological deficit.[23, 24]

1.1.3 Ischaemic Penumbra

The concept of the ischaemic penumbra is important in the development of modern acute stroke therapies. The penumbra was first defined in animal studies, initially using rats[25] and later primates[26] in experiments that identified areas of electrical inactivity after the onset of focal ischaemia implying that areas of tissue were dysfunctional but not dead. This tissue was distinct from the infarct core which had no activity and was dead. When blood flow was restored to these penumbral areas a degree of functional improvement was observed but the core tissue was unable to regain electrical activity.[25] Earlier human studies looked at the effect of systemic hypotension on cerebral blood flow (CBF) and noted that focal neurological signs appear when CBF falls below 31.5 ml/100g/min.[27] Further animal studies showed that cellular survival and functional recovery of neurons was related to both residual blood flow and duration of ischaemia.[28, 29] It was recognised that urgent revascularisation might be important therapeutically for stroke patients.[30] The concept of a therapeutic window in acute stroke treatment was established.[29]

Positron emission tomography studies looking at cerebral blood flow and oxygen consumption in humans within 18 hours of a first ischaemic stroke revealed 3 patterns of perfusion and oxygen consumption. The first pattern of greatly reduced perfusion and

oxygen consumption suggested tissue infarction and predicted a poor clinical outcome. The second pattern showed a moderate to large perfusion reduction and a moderate or large reduction in oxygen consumption and was felt to represent the ischaemic penumbra. This pattern was associated with variable outcomes. The third pattern showed an increase in perfusion associated with some areas of reduced perfusion with normal or slightly reduced oxygen consumption. This pattern probably represented tissue oligaemia and predicted a good outcome.[31] Later PET studies confirmed the existence of potentially viable areas of tissue up to 17 hours after an ischaemic stroke.[32]

1.1.4 Stroke thrombolysis

The concept of stroke thrombolysis has existed for some time although initial trials with urokinase and streptokinase were unsuccessful.[33, 34] Thrombolysis aims to re-open an occluded artery, restore blood flow and save the ischemic penumbra.

The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group was the first clinical trial to show that the use of tissue plasminogen activator in acute ischaemic stroke was associated with an improved clinical outcome.[35] Initially stroke thrombolysis was considered safe below 3 hours but more recent trials such as ECASS-III (European Cooperative Acute Stroke Study III) and observational studies have suggested that good outcomes are likely up to 4.5 hours.[36-38] The IST-3 trial suggested treatment up to 6 hours may be beneficial.[39]

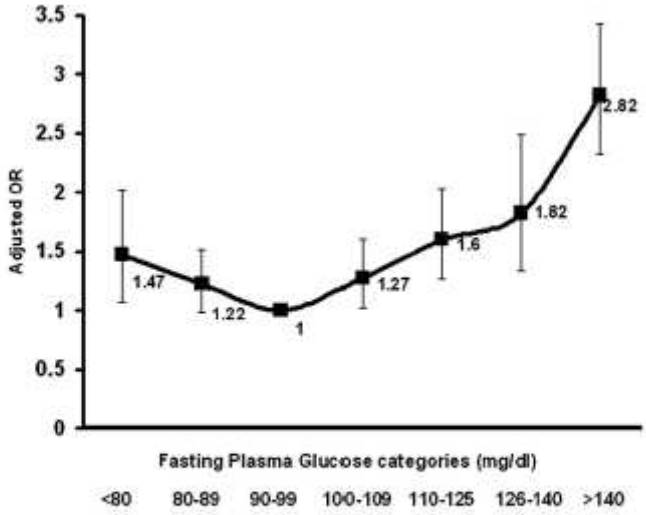
1.2 Pre-stroke hyperglycemia

The physiological state of a patient does not begin at the moment that they have a stroke. A patient may have had abnormal glucose metabolism for some time before they have a stroke. Post-stroke hyperglycaemia cannot be discussed without some consideration of pre-stroke hyperglycaemia.

1.2.1 Hyperglycemia as a risk factor for cardiovascular events

Abnormal blood glucose levels are associated with an increased risk of cardiovascular events such as myocardial infarction or stroke as evidenced in a large meta-analysis of 20 studies combined data from 95783 patients over 12.4 years which found a relationship between blood glucose levels and the risk of cardiovascular events.[40]

A 2004 paper by Tanne and colleagues explored the link between fasting plasma glucose levels and the risk of incident ischaemic stroke,[41] using data from 13999 patients who were screened for inclusion in a cardiovascular disease secondary prevention randomised control trial. All patients had a fasting blood glucose level checked at the baseline of the trial. From this prospectively recruited cohort they were able to identify 576 verified cases of ischaemic stroke or TIA. The median fasting glucose level was between 90 and 99mg/dl. When compared to this group patients with a lower blood glucose (80-89mg/dl) had a greater risk of stroke with an odds ratio of 1.27 (95% CI 1.02-1.6) while patients with higher glucose levels had an even higher risk of stroke. A patient with fasting blood glucose levels above 140mg/dl had an odds ratio for risk of ischaemic stroke of 2.82 (95% CI 2.32-3.43). There appears to be a J-shaped curve for the relationship between fasting blood glucose and risk of ischaemic stroke (see Figure 1 below).



Adjusted relative odds (95% CI) for ischemic cerebrovascular disease by categories of fasting glucose levels. Relative odds of 90 to 99 mg/dL, which constitutes the largest category, is defined as 1.

Figure 1 - The relationship between fasting blood glucose and ischaemic stroke risk (Tanne 2004)

A similar J-shaped curve was also seen in a meta-analysis of 102 studies that examined the relationship between diabetes mellitus, fasting plasma glucose and vascular outcomes such as ischaemic stroke,[42] (Figure 2 below) with lowest risk at 5.2mmol/L and increased risk at lower and higher levels of fasting plasma glucose. More studies are needed to elucidate the link between lower fasting glucose levels and vascular risk.

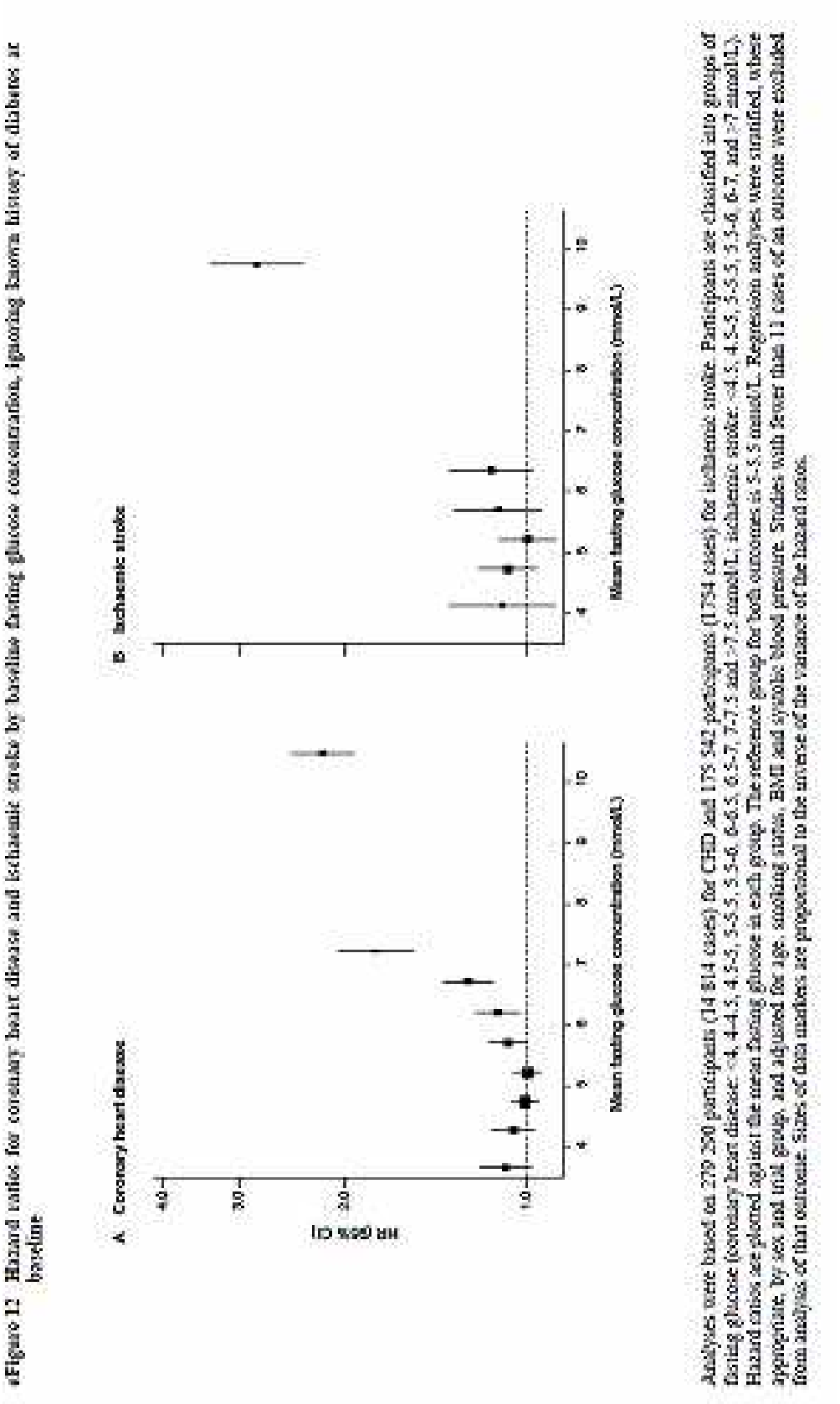


Figure 12 Hazard ratios for coronary heart disease and ischaemic stroke by baseline fasting glucose concentration, ignoring known history of diabetes at baseline

Analyses were based on 279 290 participants (14 814 cases) for CHD and 175 942 participants (1754 cases) for ischaemic stroke. Participants are classified into groups of fasting glucose (coronary heart disease: <4, 4–4.5, 4.5–5, 5–5.5, 5.5–6, 6–6.5, 6.5–7, 7–7.5 and >7.5 mmol/L; ischaemic stroke: <4.5, 4.5–5, 5–5.5, 5.5–6, 6–7, and >7 mmol/L). Hazard ratios are plotted against the mean fasting glucose in each group. The reference group for both outcomes is 5–5.5 mmol/L. Regression analyses were stratified, where appropriate, by sex and trial group, and adjusted for age, smoking status, BMI and systolic blood pressure. Studies with fewer than 10 cases of an outcome were excluded from analyses of that outcome. Sizes of data markers are proportional to the inverse of the variance of the hazard ratio.

Figure 2 - Meta-analysis of risk for ischaemic stroke by baseline fasting glucose concentration

A large Korean study[43] using data from 652,901 men identified 10954 strokes. A linear relationship between fasting blood glucose level and risk of stroke was observed for glucose levels above 5.6mmol/L. The p-value for this linear trend was <0.0001.

In an analysis of data from 13116 non-diabetic men in the Whitehall study there appears to be a relationship between increasing post oral glucose tolerance test glucose level and death from stroke.[44] Risk of death from stroke began to increase as post-challenge glucose levels increased above 4.6mmol/L. For every 1mmol/L increase in plasma glucose above this level there was an increase in the adjusted risk of stroke (Hazard ratio 1.17, 95% CI 1.04-1.31).

Hyvarinen and colleagues combined data from 21706 patients enrolled in 13 cohort studies to look at the relationship between fasting plasma glucose and plasma glucose level at 2 hours after an oral glucose load.[45] In men an elevated post-challenge glucose level more strongly predicted mortality than fasting plasma glucose. In women fasting plasma glucose level was a stronger predictor of death.

Hyperglycemia also appears to be a risk factor for peri-operative stroke during carotid endartectomy. One retrospective observational study examined the records of 1201 patients who underwent endartectomy.[46] All of these patients had a blood glucose level checked at least 3h hours before the start of the operation. When the blood glucose levels were dichotomised at 200mg/dl (11.1mmol/l) it was found that pre-operative glucose readings above this level were associated with an increased odd ratio of stroke (OR 2.78, 95% confidence interval 1.37-5.67, p=0.005). Hyperglycemia was also associated with an increased risk of MI or death.

1.2.2 Hyperglycemia and clinical outcomes in critical care

Hyperglycemia is also associated with poor outcomes in critically unwell patient groups. In a large retrospective cohort study Falcigila and colleagues looked at data from 259040 patients in 173 American intensive care units.[47] When compared with normoglycemic patients, hyperglycemic patients are at an increased risk of death with the risk increasing as the plasma glucose level increases. This effect varies with reason for admission to the intensive care unit. For stroke patients a blood glucose level of above 300mg/dl (16.6mmol/l) is associated with a risk of death with a hazard ratio of 8.49 (95% CI 4.46-16.2, P<0.0001).

1.2.3 Insulin resistance

Insulin resistance is an abnormal physiological state in which a normal amount of insulin produces a subnormal physiological response. The gold standard for the identification of insulin resistance is a euglycemic hyperinsulinaemic clamp. Most patients with Type II Diabetes Mellitus have insulin resistance. In one study of 72 non-diabetic patients with a recent TIA or stroke 36 patients had impaired insulin sensitivity.[48] In another study 52% of patients were found on investigation to have insulin resistance or previously unknown diabetes after a TIA or stroke.[49]

Homeostasis model assessment or HOMA is an accurate and less inconvenient method of assessing insulin sensitivity.[50]A model is used to predict the homeostatic concentrations of glucose and insulin based on fasting blood samples allowing researchers to estimate insulin sensitivity and β-cell function[51]. In a paper published in 2000 Hanson and colleagues established that HOMA results correlated with euglycemic hyperinsulinaemic clamp results to an acceptable degree.[52]

Pathophysiologically, insulin resistance appears to be determined by defective muscle glycogen synthesis.[53]

1.2.4 Insulin Resistance and risk of stroke

Insulin resistance has been posited as a risk factor atherothrombotic stroke.[54] As part of a cohort study looking at cardiovascular disease in northern Sweden 94 cases of first ever stroke in patients without diabetes were found to have elevated pre-stroke proinsulin levels.[55] In the Helsinki Policeman population study hyperinsulinaemia was associated with increased stroke risk (age-adjusted hazard ratio, 2.12; 95% CI, 1.28 to 3.49) although this was not independent of other risk factors such as obesity.[56]

In another Finnish study the patients with the higher insulin levels appeared to have a greater risk of stroke but when adjusted for lipid levels, blood pressure and obesity this effect was no longer statistically significant.[57]

In a 1999 paper, Folsom and colleagues[58] found that an elevated fasting plasma insulin level increased the risk of stroke after correction for other risk factors with a relative risk ratio of 1.14 (CI 1.01-1.3, p=0.03). When the highest quartile of insulin levels was compared with the lowest quartile the corrected relative risk increased to 2.11.

A 1999 paper by Wannamethee and colleagues looked at the association between non-fasting insulin concentrations and risk of stroke.[59]. Men with diabetes were at a significantly increased risk of stroke. There was also an apparent J-shaped relationship between non-fasting insulin levels and stroke risk with the lowest risk seen in patients with insulin levels of between 6.65 and 9.8 mU/L.

Several papers have suggested that insulin resistance as characterised by a homeostasis model assessment (HOMA) is associated with risk of incident ischaemic stroke [60] Patients with higher insulin levels may be at a greater risk of ischaemic stroke (HR 2.83, 95% CI 1.34-5.99).[61] Insulin resistance may be a modifiable risk factor for stroke that should trigger pharmacological intervention.[62] The evidence for this is not completely consistent as demonstrated in a 2009 paper by Tanne and colleagues,[63] insulin sensitivity status was assessed using HOMA. Incident strokes occurred in 137/2938 patients but there was no apparent increased risk of stroke with increased insulin resistance in this population.

Elevated pro-insulin levels may be associated with an increased risk of stroke.[55] In a prospective cohort study 94 patients with first ever ischaemic stroke and 178 randomly-selected controls were compared. The highest tertile of pro-insulin levels was associated with an increased risk of stroke after adjustment for other risk factors (OR 3.4, 95% CI 1.4 to 8.4). The risk was even greater in women with and odds ratio of 13.7 (95% CI 1.3 to 146).

1.2.5 Impaired Glucose Tolerance and Risk of Stroke

We cannot be sure that impaired glucose tolerance is a risk factor for stroke.

Impaired glucose tolerance as defined by an oral glucose tolerance test was not associated with the risk of stroke in a 1998 paper.[64] In this study 6547 adults had an oral glucose tolerance test to define if they had normal glucose metabolism, impaired glucose tolerance or diabetes. The prevalence of stroke was 1.82% in the normal group, 2.17% in the impaired glucose tolerance group and 4.96% in the diabetic group. In this study impaired glucose tolerance was associated with an adjusted risk ratio of 0.9 (95% CI 0.5-1.6) for non-fatal stroke while diabetes mellitus was associated with an adjusted risk ratio of 1.6 (95% CI 1 – 2.6). Similar results were seen in a 2006 Finnish study where stroke occurred more frequently in patients with impaired glucose tolerance although this was not statistically significant (RR 1.48, 95% CI 0.91-2.41, p=0.12).[65]

A secondary analysis of data from the Dutch TIA Trial suggests that impaired glucose tolerance may be associated with recurrent stroke in a population of patients who have experienced a minor stroke or TIA.[66] In this study 3127 patients were enrolled. Over 2.6 years 272 patients had a stroke. Impaired glucose tolerance (IGT) was diagnosed on a non-fasting glucose sample and defined as a glucose level of between 7.8 and 11mmol/L. IGT was associated with an increased risk of recurrent stroke (HR 1.8, 95% CI 1.1 to 3). Diabetes was associated with an even greater risk (HR 2.8, 95% CI 1.9-4.1). Interestingly patients with blood glucose levels below 4.6mmol/L also had an increased stroke risk (HR 1.5, 95% CI 1 to 2.2).

Impaired glucose tolerance has been identified as a risk factor for stroke in a Japanese population as described in a 2008 paper by Oizumi and colleagues.[67] Glucose tolerance at baseline was defined in a cohort of 2938. Impaired glucose tolerance was associated with an increased risk of stroke (OR 1.87, 95% CI 1.73-2.03) as was diabetes mellitus (OR 3.57, 95% CI 3.21-3.98).

It has also been reported that the 'metabolic syndrome' is associated with an increased risk of incident stroke or TIA.[68] The 'metabolic syndrome' is a controversial entity with debate over the existence and definition of the syndrome.[69, 70] In 2005 the American Diabetes Association and the European Association for the Study of Diabetes issued a joint statement to say that the metabolic syndrome was imprecisely defined and that it's pathogenesis was uncertain.[69] This statement concluded that there is not enough information available to clearly define this syndrome and that the term may not be useful.

1.2.6 Insulin resistance as a risk factor for carotid artery disease

An association between insulin resistance and carotid artery disease has been reported. A Swedish study from 1967 found a relationship between angiographically defined disease of the major cerebral blood vessels and impaired glucose tolerance as defined by an oral glucose tolerance test.[71] More severe disease was associated with a greater extent of disease on angiogram.

The Insulin Resistance and Atherosclerosis Study (IRAS) found a significant relationship between carotid intima thickness and insulin resistance in white or Hispanic people but not in black people.[72] Carotid intima thickness was generally greater than average in hyperglycemic or diabetic people.[73] Similar findings have been made in other studies.[74]

Diabetic patients have a high prevalence of carotid artery occlusive disease[75] with 20% found to have a degree of carotid artery occlusive disease in one study. Measures of hyperglycemia did not appear to be related to carotid disease although higher systolic blood pressure, higher cholesterol and reduced adiposity were. Another study found that while diabetes mellitus predicted increased carotid intima-media thickness plasma insulin levels did not.[76]

Insulin resistance may be an important pathophysiological factor in atherothrombotic stroke.[54] In a small angiography study patients with atherothrombotic stroke had severe insulin resistance and compensatory hyperinsulinaemia while the lacunar or cardioembolic patients did not. Increased insulin resistance defined by HOMA-IR is associated with intracrainial atherosclerosis diagnosed on MR or digital subtraction angiography.[77]

The Lausanne Stroke Registry found a significant relationship between large artery stroke and diabetes mellitus.[78] In this study 31% of non-diabetic patients had a large artery stroke compared to 42% of diabetic patients ($p<0.0001$). In this population diabetes mellitus is associated with an increased risk of large artery stroke with an odds ratio of 2.02 (95% CI 1.31-3.02, $p=0.002$).

A study of 415 926 ischemic stroke patients found that carotid stenosis was significantly more prevalent in diabetic patients (5.2% vs.4.2%, <0.0001).[79] It has also been suggested that insulin resistance is a risk factor for sub-clinical cerebral infarction associated with thickening of the carotid intimal media.[80]

1.2.7 Insulin resistance and lacunar stroke

A 1996 study by Zunker and colleagues suggests that insulin levels are higher in patients with ischaemic stroke.[81] In a prospective study insulin levels were checked in patients with lacunar disease, subcortical arteriosclerotic encephalopathy and stroke due to large vessel disease. Insulin levels were significantly higher in lacunar stroke patients than in other groups. The authors conclude that elevated insulin levels may represent a pathophysiological factor in the development of cerebral small vessel disease.

1.2.8 Diabetes and stroke

In a large meta-analysis recently published in *The Lancet* individual patient data from 698782 participants in 102 studies were combined.[42] In this cohort diabetes mellitus was associated with an increased hazard ratio of 2.27 for ischaemic stroke (95% CI 1.95-2.65)

An early description of this association was made in 1972 Paffenbarger published data from a cohort study involving 3991 Longshoremen who were followed up for 18.5 years.[82] At baseline these men were assessed for cardiovascular risk factors including diabetes mellitus and impaired glucose tolerance. Paffenbarger found that patients with impaired glucose tolerance had a risk ratio of 1.97 for death from stroke while patients with diabetes mellitus had an even greater risk ratio of 2.96.

The Rochester Community Study,[83] The Honolulu Heart Programme[84] and large British.[85] and American[86] studies have clearly established diabetes as a risk factor for stroke. A similar picture has been seen in Sweden,[87] Japan[88] and Hong Kong [89] In the Hong Kong study diagnosis of diabetes was previously unrecognised in 19% of patients who presented with an ischaemic stroke. Diabetes is associated with an increased risk of stroke after myocardial infarction. [90]

Recurrent stroke also appears to be more likely in patients with diabetes[91] as seen in the Leigh Valley Stroke Programme where diabetes was found to convey an increased relative risk of 5.6 for recurrent stroke ($p<0.0001$).

1.2.9 Diabetes and outcome after stroke

Diabetes appears to be a poor prognostic marker in stroke.[92] Diabetic patients have an increased risk of death and recurrent stroke or myocardial infarction.[93] In one study diabetes was associated with an increased incidence of limb weakness, dysarthria, ischaemic stroke and lacunar stroke.[94] Diabetic patients also appeared to have higher 3 month Rankin scores and more severe Barthel scores. This paper was a large population based study combining several European databases so it is possible that some diagnostic accuracy (for both lacunar stroke and diabetes) has been compromised.

Tuttolomondo and colleagues did not report significant outcome differences between diabetic and non-diabetic patients in their 2008 paper.[95] Diabetic patients in this cohort had better scores on the Scandinavian Stroke Scale than non-diabetics. The authors felt

that this may be due to the higher prevalence of lacunar stroke in the diabetic population. Lacunar strokes may result in lower levels of disability.

In a 2007 Spanish language paper Ortega-Casarrubios and colleagues looked at patient outcomes in a cohort of 2213 stroke patients, 661 (29.9%) of whom had a history of diabetes.[96] In this cohort patients with diabetes had significantly higher rates of in-hospital complications (OR: 1.377; CI 95%: 1.053-1.799) such as urinary tract infection, multi-organ failure, neurological deterioration and recurrent stroke. Despite these findings there did not seem to be any association between diabetes mellitus and severity of initial stroke, mortality, length of hospital stay or stroke outcome.

Another study suggests that diabetes does not seem to influence the motor or functional outcome of patients who enter rehabilitation after a first ischaemic stroke.[97] In a prospective observational study 395 patients were assessed for diabetes and entered into a rehabilitation programme. Both the diabetic and non-diabetic patients were observed to make a significant and progressive improvement in all outcome measures ($P<0.01$) with no differences between groups. The paper does not explicitly state that outcomes were assessed by research staff blinded to the diabetic status of the patients so there is a possibility that a degree of bias could have distorted these results. However these results suggest that a mechanism other then poor rehabilitation potential may be responsible for the poor clinical outcomes after stroke.

In another study using data from VISTA (the Virtual International Stroke Trial Archive) predictive models were developed for cardiac death and serious cardiac events after stroke.[98] Diabetes was found to predict a cardiac event with an odds ratio of 2.11 (95% CI 1.39-3.21, $p<0.0001$).

A very large American study assessed the quality of care and clinical outcomes amongst diabetic patients admitted to hospital after an ischaemic stroke.[79] In this study data were obtained from 415 926 ischemic stroke patients admitted to 1070 American hospitals between 2003 and 2008. In this cohort 130 817 (31%) had diabetes. Patients with diabetes who presented within two hours of stroke onset were less likely to receive treatment with alteplase than non-diabetics (adjusted odds ratio 0.83; 95% CI, 0.79-0.88, $p<0.0001$). Patients with diabetes also had an increased risk of death (adjusted OR, 1.12; 95% CI, 1.08-1.15).

In a prospective observational study involving 142 patients with diabetes who had suffered a stroke there was no apparent link between glycaemic control as assessed by HbA1c and risk of recurrent stroke.[99] These results may be misleading as the majority of patients in this cohort had well controlled diabetes and the sample size of 142 patients is small. Tight glycaemic control would need to have a very large effect on the risk of recurrent stroke for that effect to be detectable in a cohort of 142 patients.

Risk factors such as diabetes are often under-treated in patients who have experienced a stroke.[100] In one large study 4.5% of patients had no treatment for their diabetes. This study suggested that undertreatment of modifiable risk factors for stroke is a global problem.

Treatment of risk factors does appear to be beneficial for ischaemic stroke patients who have diabetes. In a subgroup analysis of the Perindopril Protection Against Recurrent Stroke Study (PROGRESS) outcomes for diabetic and non-diabetic patients were compared.[101] In patients with diabetes treatment with perindopril reduced the relative risk of recurrent stroke by 38% (95% CI 8-58) while in non-diabetic patients there was a relative risk reduction of 28% (95%CI 16-39). There was no evidence of heterogeneity between these results (P homogeneity = 0.5). The reasons for this benefit are uncertain.

1.2.10 The prevalence of diabetes in stroke patients

The prevalence of diabetes in stroke patients may well be underestimated. In a study population of stroke survivors at least 6 months after their stroke glycaemic status was categorised using both a fasting blood glucose sample and an oral glucose tolerance test.[102] The fasting plasma glucose level was found to have a sensitivity of only 49% for predicting abnormalities in the glucose tolerance test. In this population of stroke survivors 77% were found to have abnormal glucose metabolism.

1.2.11 Post mortem studies of stroke in diabetes

Post mortem studies have shown that diabetes is associated with an increase in the frequency and severity of cerebral atherosclerosis in all age groups.[103] Pathological studies from the first generation of Type 1 diabetics treated after the discovery of insulin in 1922 showed ‘softening’ in the brains of all the patients studied.[104] One paper reported 16 patients with a duration of diabetes of between 16 and 36 years who had died before the age of 40.[104] Fourteen of these sixteen patients had some neurological symptoms while 4

had clinical strokes. Evidence from post-mortems also suggests that stroke occurs much more frequently in diabetic patients than non-diabetic patients.[105] Non-diabetics over the age of 85 were less likely to have evidence of stroke than diabetic patients in younger age groups.

1.2.12 Type of stroke in diabetes

The characteristics of first ever stroke in diabetic patients were examined in a study of the Lausanne Stroke Registry.[78] This registry included 3690 ischaemic stroke patients, including 572 patients with diabetes mellitus. As I have discussed in an earlier paragraph large artery disease was significantly more common as a cause of stroke in the diabetic patients. Diabetes was also associated with a higher prevalence of subcortical infarction (OR 1.34, 95% CI 1.11-1.62, p=0.009) and a greater frequency of small vessel disease (OR 1.78, 95% CI 1.31-3.82, p=0.012). Cardioembolic strokes were not associated with diabetes. The registry also includes intra-cerebral haemorrhage patients and there was lower relative prevalence of ICH among patients with diabetes.

Tuttolomondo and colleagues analysed the prevalence of different stroke sub-types defined by the TOAST criteria in 102 diabetic and 204 non-diabetic stroke patients.[95] A higher incidence of lacunar stroke was observed amongst diabetic patients even after adjustment for hypertension (adjusted OR 3.37, 95% CI 1.9-5.99, p=0.0001). Differences in incidence of other TOAST subtypes were non-significant.

Patients with diabetes appear to be at an increased risk of recurrent lacunar stroke. In a prospective study of lacunar stroke quantified with MRI. A total of 175 patients presenting with a first ever lacunar stroke were included in the study. Diabetes mellitus was associated with the presence of multiple lacunar strokes (OR 2.43, 95% CI 1.09–5.4, P=0.026).

Diabetes also seems to be associated with subclinical cerebral infarctions.[106]In a Japanese prospective observational studies 360 hypertensive patients with no history of stroke underwent MRI scanning. In this population 247 silent cerebral infarcts were identified on MRI. Silent cerebral infarctions were found in 82% of the diabetic patients compared to 58% of the non-diabetic patients ($p<0.001$). Multiple silent cerebral infarctions were also more common in the diabetic population ($p<0.001$).

Diabetes may be associated with an increased prevalence of asymptomatic intracranial large vessel occlusive disease.[107] In a prospective study of 510 patients referred to a vascular imaging laboratory for carotid duplex and transcranial Doppler ultrasound 66 patients (12.9%) were found to have asymptomatic intracranial arterial occlusions. Diabetes was present in 28% of these patients (p=0.021).

1.2.13 Cerebral blood flow in diabetic stroke

In a 1978 study Dandona and colleagues compared cerebral blood flow in diabetics and non-diabetics.[108] A group of 59 patients with diabetes mellitus and no history of stroke was recruited. Twenty-eight non-diabetics with no history of stroke were used as a control group. Cerebral blood flow was measured using the 133-xenon gamma camera technique developed by David Wyper and colleagues at the Institute of Neurological Sciences in Glasgow.[109]

After challenge with carbon dioxide cerebral blood flow increased in all of the control patients but decreased in 26 of the diabetic patients and remained static in 10 more diabetic patients. The authors conclude that these results indicate that patients with diabetes have impaired cerebrovascular reserve and are at an increased risk of stroke.[108] A similar 2003 paper by Kadoi and colleagues found an inverse correlation between absolute carbon dioxide reactivity and glycosylated haemoglobin ($r = 0.69$, $p<0.001$).[110] Several subsequent papers have attempted to further characterise cerebrovascular reactivity in diabetic patients.[110-117] In patients with longstanding type 2 diabetes the vasodilatatory ability of cerebral arterioles is diminished.[116]

In a 1990 longitudinal study comparing cerebral blood flow in both diabetic and non-diabetic stroke patients cerebral blood flow was better maintained in treated diabetics than in non-diabetic subjects.[118] However there was a greater incidence of cognitive decline in the diabetic patients despite the maintenance of cerebral blood flow.

In a 2007 paper Last and colleagues aimed to evaluate the regional effects of type 2 diabetes on cerebral tissue volumes and cerebral blood flow (CBF) regulation.[111] Diabetic patients and normal controls had their regional brain volumes and vasoreactivity determined by anatomical imaging and continuous arterial spin labelling (CASL) of blood flow using 3 Tesla MRI. Diabetic patients were found to have smaller volumes of both gray and white matter. Baseline cerebral blood flow was reduced in diabetic patients.

Higher HbA1c levels were associated with lower cerebral blood flow and greater CSF volumes in the temporal region.

Mankovsky and colleagues observed impaired cerebral autoregulation in a cohort of diabetic patients with known cardiovascular autonomic neuropathy or orthostatic hypotension.[113] Cerebral blood flow was assessed with transcranial Doppler ultrasound in both diabetic patients and normal controls. Cerebral blood flow velocity was markedly reduced in patients with diabetes after one minute of standing suggesting that cerebral autoregulation was impaired in this group. This could be a mechanism explaining the worse outcome often seen in patients with diabetes after ischaemic stroke. If vascular autoregulation is impaired collateral blood supplies may be slowly recruited worsening the anatomical hypoperfusion and resulting in accelerated penumbral loss. Several small prospective studies have suggested that impaired cerebral vasomotor reactivity was a predictive factor for stroke in patients with asymptomatic carotid stenosis.[113, 119, 120]

Another study compared vascular reactivity in diabetic patients with normal controls.[112] In an attempt to investigate the effect of diabetes on basal cerebrovascular endothelial function 14 patients with type 2 diabetes and 15 normal controls had their cerebral blood flow monitored during exposure to the nitric oxide synthase (NOS) inhibitor NG-monomethyl-L-arginine (L-NMMA). In the non-diabetic group cerebrovascular blood flow there was an appropriate response to the L-NMMA while cerebrovascular reactivity was markedly impaired in the diabetic group.

Cardiovascular autonomic neuropathy (CAN) is associated with an increased risk of incident ischaemic stroke.[121] Patients with known Type II diabetes mellitus were assessed for cardiovascular autonomic neuropathy and given a CAN score. Patients were followed up over a 7 year period and data on subsequent ischaemic stroke were collected. Patients with higher CAN scores were found to be at a greater risk of incident ischaemic stroke after Cox proportional hazard regression analysis (adjusted HR 2.7, 95% CI 1.3-5.5, p=0.006). The presence of cardiovascular autonomic neuropathy may be associated with cerebrovascular autonomic neuropathy. Some animal studies support this hypothesis.[122, 123]

1.2.14 Special risk factors for stroke in Type 2 Diabetes Mellitus

Some studies have tried to define clinical markers that may predict stroke risk in patients with diabetes mellitus. In a small prospective observational study 133 patients with non-

insulin dependent diabetes mellitus were followed up for 10 years.[124] In this cohort 19 patients had an ischaemic stroke. Toyry and colleagues found that pre-existing parasympathetic neuropathy was a strong independent predictor for later ischaemic stroke with an odds ratio of 6.7 (95% 1.5-29.9, p=0.012) while sympathetic autonomic neuropathy was associated with a slightly lower risk (OR 1.2, 95% CI 1.01-1.2, p=0.042). Interestingly the use of beta-blockers was also strongly predictive although this was probably confounded by underlying cardiac disease (OR, 6.7; 95% CI, 2.1 to 21.5).

Diabetic autonomic neuropathy also emerged as a risk factor for stroke in a prospective study that included 950 patients with known type 2 diabetes mellitus.[125] In this population diabetic autonomic neuropathy conveyed an increased risk of stroke with an OR of 2.2 after multivariate analysis (95% CI 1.1-4.44).

Asymmetrical retinopathy may be a marker of an increased risk of stroke in diabetic patients.[126] Theoretically, diabetic retinopathy should be symmetrical and asymmetry may represent localised ischaemic retinopathy due to a problem with the extraoccular blood supply representing an increased risk of ischaemic stroke. Lino and colleagues performed a prospective study of 142 patients with diabetic mellitus, monitoring them for retinopathy and incident ischaemic stroke over a period of 8 years. Ischaemic stroke occurred in 41.7% of patients with asymmetrical retinopathy compared to only 7.7% of patients with symmetrical retinopathy and 7.7% of those with no retinopathy (p<0.005%).

1.2.15 Glycosylated haemoglobin

Glycosylated haemoglobin (HbA1c) levels indicate the exposure of a red blood cell to glucose over approximately 120 days.[127] More recently HbA1c has suggested as a means of diagnosing diabetes.[128] For many years it has been known that stroke or TIA is associated with an elevated HbA1c level even in patients with no clinical history of diabetes mellitus.[129] In their 1982 study Riddle and Hart found that 62% of stroke/TIA patients had an elevated HbA1c level and when compared with various control groups the prevalence of an elevated HbA1c level was greater than in a healthy non-stroke community and similar to populations that attended diabetes clinics.

1.2.16 Glycosylated haemoglobin and prognosis in stroke

It has been suggested that patients with an elevated level of glycosylated haemoglobin (HbA1c) have a worse prognosis after a stroke although this has not been a universal finding.

In a letter to the BMJ in 1985 Oppenheimer and colleagues suggested that the relationship between post-stroke hyperglycaemia and poor outcome in stroke could be explained by the elevated HbA1c of many patients[130] Oppenheimer suggested that the negative effects of PSH may just represent the natural history of stroke in diabetes. The next year Cox and colleagues published a similar study which suggested that patients with a raised HbA1c and post stroke hyperglycaemia had better outcomes that those who had a normal HbA1c and hyperglycaemia.[131]

Topic and colleagues prospectively studied the glycaemic status of 148 patients in a 1989 paper.[132] They evaluated long term glycaemic status with HbA1c levels. Patients with an HbA1c of above 8.6% were assumed to be diabetic. On this basis 16% of patients had unrecognised diabetes in addition to 13% who had known diabetes. Patients with an elevated HbA1c had worse clinical outcomes. Patients with normoglycemia had better outcomes. Outcomes in patients with transient hyperglycemia had poor outcomes although these were not as bad as the diabetic subgroup.

In the 1987 study by Gray and colleagues 86 acute stroke patients had their glycaemic status prospectively assessed with blood glucose and HbA1c levels.[133] No significant correlation between HbA1c level and outcome was found in this group.

In a 1992 prospective observational study Murros and colleagues collected data from 99 stroke patients.[134] These patients had HbA1c levels checked to establish glycaemic status before they had a stroke. In this group 76 patients were classified as having a normal glycaemic status before the stroke (HbA1c<7) while 23 had abnormal glycaemic metabolism (HbA1c above 7). There did not appear to be any relationship between HbA1c and mortality, severity of hemiparesis, functional outcome or infarct size. However in non-diabetic patients the fasting blood glucose level correlated strongly with the severity of hemiparesis and predicted stroke outcome.

In a small prospective study carried out in 1988 by Power and colleagues there was no significant correlation between mortality and HbA1c.[135] 58 patients were enrolled in this

study, 28 of whom died. While there was a tendency for dead patients to have higher HbA1c values the confidence intervals of the two groups overlapped and the study was too small to produce a definitive answer.

A cohort study published in 1994 by Moss and colleagues looked at the association between glycaemia and cause specific mortality in a diabetic population.[136] HbA1c levels were checked at baseline in a population of 1780 diabetic patients who were followed up for a median period of 8.3 years. In this population HbA1c was significantly associated with risk of mortality. Higher HbA1c levels were associated with a hazard ratio of 1.17 for mortality related to stroke (95% CI 1.05 – 1.30).

Data from the UK Prospective Diabetes Study (UKPDS) suggest that patients with a higher HbA1c are at a greater risk of having a fatal stroke.[137] UKPDS was a prospective study which recruited 5102 patients with a new diagnosis of Type II diabetes mellitus. Patients were followed up for a median of 7 years. During this time 234 patients had an ischaemic stroke with enough available covariate data for analysis (34 further strokes occurred but insufficient data were available). Forty-eight patients had a fatal stroke. The risk of fatal stroke increased as HbA1c levels increased with a hazard ratio of 1.37 for every 1% increase in HbA1c (95% CI 1.09-1.72, P=0.0071).

More recently in an abstract presented at the 2010 European Stroke conference Juttler and colleagues have suggested that HbA1c levels strongly predict the risk of haemorrhagic transformation after thrombolytic treatment.[138] In a prospective observational study 800 consecutive patients had various measures of glycaemic status tested. HbA1c levels appeared to have the strongest predictive value for haemorrhagic transformation and symptomatic intracerebral haemorrhage independent of a history of diabetes or acute blood glucose levels.

1.2.17 Glycosylated haemoglobin and risk of stroke

In a large prospective observational study involving 10489 patients Myint and colleagues examined the relationship between HbA1c levels and risk of stroke.[139] They did not find a linear relationship such as that between blood glucose and coronary heart disease. Instead they found a threshold relationship. In patients with an HbA1c of between 5.5% and 6.9% had an adjusted relative stroke risk of 0.83 (95% CI 0.54-1.27) while patients with an HbA1c above 7% had a adjusted relative risk of 2.83 (95% CI 1.4 – 5.74).

A 2004 meta-analysis conducted by Selvin and colleagues looked at the relationship between an increase in HbA1c level of 1% and risk of stroke.[140] Data from 3 studies involving 5962 patients were pooled and the relative risk of stroke in a diabetic patient was found to increase by 1.17 for every 1% increase in HbA1c level (95% CI 1.09-1.25).

Selvin and colleagues subsequently tried to assess the relationship between HbA1c and risk of stroke in diabetic and non-diabetic patients in an analysis of data from the Atherosclerosis Risk in Communities (ARIC) study.[141] ARIC was a large cohort study including 15792 patients. At the second stage of the study 10866 patients without diabetes and 1635 patients with diabetes had HbA1c levels checked. After follow-up of approximately 10 years 89 of the diabetic patients and 167 of the non-diabetic patients had ischaemic strokes. In non-diabetic patients a HbA1c level in the highest tertile conveyed a relative risk of stroke of 1.58 (95% CI 0.94-2.66) when compared with patients with HbA1c levels in the lowest tertile. In diabetic patients a HbA1c level in the highest tertile conveyed a relative risk of stroke of 4.71 (95% CI 2.69-8.25) when compared with patients with HbA1c levels in the lowest tertile. The paper concludes that an elevated HbA1c may be an independent risk factor for stroke in both diabetic and non-diabetic individuals.

In a 2008 paper a prospectively recruited cohort of 6445 Hong Kong Chinese patients with type 2 diabetes mellitus were followed for a mean of 5.37 years to further establish risk factors for ischaemic stroke.[142] In this study there appeared to be an additive risk of stroke in patients with both microalbuminuria and an elevated HbA1c level of above 6.2%.

1.3 Post-stroke Hyperglycaemia

The phenomenon of post-stroke hyperglycaemia (PSH) is common with up to 68% of acute stroke patients having a plasma glucose concentration >6.0mmol/L.[143-147]

1.3.1 Early observations of post-stroke hyperglycemia

Observations of abnormal glucose metabolism in stroke patients have been made for many years. In a 1967 paper Jakobson performed glucose tolerance tests in a series of 52 stroke patients, 47 of whom had stroke disease confirmed angiographically.[71] He found that 42% of patients with cerebrovascular disease had abnormal glucose metabolism compared to 17% of a control group who had no evidence of stroke.

Another early observational study on post-stroke hyperglycemia was published by Melamed in 1976.[148] Melamed knew that in experimental models of stroke induction of acute cerebral ischemia would often be followed by transient generalised metabolic abnormalities including marked hyperglycemia.[149, 150] Melamed examined the notes of stroke patients (including those with intracerebral haemorrhage) admitted to a neurology unit over a six year period. In a cohort of 392 patients he identified 79 (20%) who had known diabetes mellitus and 108 (28%) without known diabetes who had what he described as 'reactive hyperglycemia'. He also noted a 54% mortality rate in the 'reactive hyperglycemia' group, compared to 35% mortality in the diabetic group and only 17% mortality in the non-hyperglycemic group.

In a 1978 paper Abu-Zeid and colleagues studied prognostic factors in 1484 stroke patients including 952 patients with ischaemic stroke.[151] In this cohort admission blood glucose level was correlated with survival. Patients with a blood glucose level of less than 5.55mmol/L were more likely to survive in both the short term and the long term ($p<0.005$).

1.3.2 The relationship between blood glucose level after stroke and outcome

Woo and colleagues performed a prospective observational study correlating admission blood glucose levels with neurological outcome in 252 acute stroke patients.[152] This 1988 paper included patients with intracerebral haemorrhage as well as ischaemic strokes. Patients with admission hyperglycemia were more likely to be dead at 6 weeks irrespective of stroke subtype and diabetic status ($p<0.05$). If only non-diabetic patients are considered mortality is significantly higher at both 1 week ($p<0.05$) and at 6 weeks ($p<0.05$). If only intracerebral haemorrhage is considered the effect appears to be even more marked irrespective of prior diabetic status (significance levels; 1 week $p<0.01$, 6 weeks $0<0.005$, 3 months $p<0.005$).

In a follow on study Woo and colleagues recruited a further 216 acute stroke patients (again including intracerebral haemorrhage).[153] In this study HbA1c levels were measured in all patients and an oral glucose tolerance test was performed in survivors where possible. Plasma glucose levels did not correlate with HbA1c levels. There was a non-significant trend towards surviving patients having higher HbA1c levels if known diabetic patients were included in the analysis. If diabetic patients were excluded from analysis there was a non-significant trend towards lower HbA1c levels in survivors. HbA1c levels

did not correlate with mortality. A relationship between admission hyperglycemia and mortality was only seen in intracerebral haemorrhage patients. The authors suggest that hyperglycemia in the presence of a normal HbA1c may represent a 'stress' response and suggest that attempts at lowering plasma blood glucose may be unnecessary.

A further prospective study included 304 patients, 174 of whom had an ischaemic stroke or lacunar infarction.[154] In this study 74% of patients with stress hyperglycemia died compared to 23% of patients with diabetes mellitus and 24% of patients with normal glucose metabolism. Patients who died had the highest mean glucose levels. In this study the relationship between stress hyperglycemia and death was only significant when the ICH patient data were analysed with the ischaemic stroke patient data.

In a 1992 prospective observational study Toni and colleagues recruited 327 ischaemic stroke patients within 12 hours of onset of first ever ischaemic stroke.[155] Glucose levels were checked as soon as patients were admitted to hospital and before any treatments were started. Patients were split into three groups; diabetic patients, non-diabetic hyperglycaemic patients and normoglycaemic patients. Diabetic patients included patients who had persistently elevated glucose levels during admission and required anti-diabetic treatment. No glycosylated haemoglobin levels were checked so it is possible that the diabetic group included patients with persistent post-stroke hyperglycemia but no previous glycaemic abnormality.

A plain CT brain scan was performed within 12 hours of stroke onset and repeated at 21 days. Admission glucose levels and initial stroke severity were compared with clinical outcome and final stroke lesion seen on CT. 21.5% of the patients were diabetic and all of these patients were hyperglycaemic at admission. A further 28.5% were hyperglycaemic at admission with no history of diabetes. There was no correlation between admission glucose level and final infarct size. Initial severity of neurological deficit was predictive of final infarct size. Mortality at 30 days was highest in the diabetic group at 38.6% compared to 22.6% mortality in the hyperglycaemic non-diabetic group and 9.2% in the normoglycaemic group ($p<0.001$).[155]

In 1993 De Falco and colleagues published a study of 104 patients with ischaemic stroke where they found a correlation between blood glucose level within 12 hours of stroke onset and cerebral infarct size.[156] The correlation between infarct size and blood glucose level was highly significant. Interestingly the relationship between glucose level and infarct size

was more significant in the non-diabetic patients (p=0.0373) than in the diabetic patients (p=0.056). The authors felt that the effect of hyperglycemia on infarct size may be mediated by 'vascular insufficiency'.

In a paper from the North Manhattan Stroke Study published in 1994 glucose level predicted stroke mortality and recurrence of ischaemic stroke.[157] In this prospective cohort study 323 patients were followed for a mean of 3.3 years. Hyperglycemia (defined as a blood glucose level above 140mg/dl) was observed in 38% of patients. An elevated blood glucose level in the first 48 hours after onset was an independent predictor of recurrent ischaemic stroke with a relative risk ratio of 1.2 per 50mg/dl (2.7mmol/L). Hyperglycemia was also a significant predictor of mortality after adjustment for age with a risk ratio of 1.7 (95% CI 1.4-2.8).

Hyperglycaemia in acute stroke predicts a poor outcome.[92, 144, 146, 147, 157-166] In an Australian retrospective cohort study 416 patients admitted to a tertiary referral centre were identified.[164] Patients were divided into 4 groups; normoglycemic, normoglycemic diabetics, hyperglycemic diabetics and hyperglycemic non-diabetics. Hyperglycemia was defined as a blood glucose of above 8mmol/L. Hyperglycemia without a history of diabetes was an independent predictor of in-hospital mortality in this cohort with an odds ratio of 3 (95% CI 1.1-8.3, p=0.035). The risk of in-hospital mortality was lowest in normoglycemic patients. It is interesting to note that hyperglycemic diabetic patients had a better outcome than hyperglycemic patients without known diabetes.

Another Australian prospective study looked at factors which predicted prolonged hospital admissions and disability.[166] Diabetes was found to be a significant predictor of both disability and prolonged admission in this population of 257 ischaemic stroke patients. Unfortunately data on hyperglycemia were only available for 173 patients. Hyperglycemia did appear to predict severe disability with p=0.026.

A retrospective study published by Stead and colleagues in 2009 correlated glucose levels checked in the emergency department with patient outcomes.[165] Data from 447 patients were analysed. In this cohort hyperglycemia, defined as an admission blood glucose of >7.2mmol/L was associated with greater stroke severity (P=0.002) and greater functional impairment. As seen in several other studies hyperglycemia without a prior history of diabetes was associated with the worst stroke severity (P<0.0001) and functional impairment (P<0.001). There was no relationship between hyperglycemia and infarct

volume. It is interesting to note that the intra-quartile range for HbA1c levels in the 'hyperglycemia without diabetes' group in this paper is 5.7-6.8 suggesting that potentially more than a quarter of the patients in this cohort may have undiagnosed diabetes based on recent discussions on the use of glycosylated haemoglobin to diagnose diabetes.[128]

Some studies suggest that the effect of hyperglycemia on outcome is less significant when other factors are considered. In a prospective cohort of 185 acute stroke patients the unadjusted risk of death was significant with an odds ratio of 5 (95% CI 2.3-10.7, $p<0.001$). However when other factors such as age, atrial fibrillation and stroke severity were taken into account the effect of hyperglycemia was not significant (OR 1.2, 95% CI 0.4-3.5).[167]

Post-stroke hyperglycemia may also be associated with early recurrence of ischaemic stroke. In an 1989 paper Sacco and colleagues used prospectively collected data from the NINDS Stroke Data Bank to look for factors that may predict early recurrence of ischaemic stroke.[168] Data from 1273 patients were used. Hyperglycemia defined as a glucose level above 140mg/dl (6.66mmol/L) was present in 36% of patients and was associated with a 6.36% risk of recurrent stroke compared to a risk of 2.07% in patients with lower blood glucose levels ($p=0.001$). Multivariate logistic regression suggested that the risk was even greater if a patient was both hypertensive and hyperglycemic.

In a retrospective cohort of 811 patients admitted to an acute stroke unit, admission glucose levels were correlated with clinical outcome at 3 months.[147] This cohort included 105 patients with haemorrhagic stroke. Sixty-one patients with known diabetes mellitus were excluded from outcome analysis. As HbA1c was not checked it is not possible to be sure if any patients had previous dysglycaemia. After adjusting for other variables hyperglycemia (defined as a random glucose on admission of >8mmol/L) was associated with a relative hazard ratio for death at 3 months of 1.87 (95% CI 1.43 to 2.45, $p<0.0001$).

Another retrospective study was published by Williams and colleagues in 2002.[169]A cohort of 656 acute stroke patients was identified. Post stoke hyperglycemia, defined as a serum blood glucose level above 130mg/dl, was present in 40% of patients. The mean blood glucose level in the hyperglycemic group was significantly higher than the mean glucose level of the normoglycemic group (207mg/dl vs. 105mg/dl, $p<0.0001$). Mortality was significantly higher in hyperglycemic patients both at 30 days and at 1 year. The mean length of hospital admission was 7.2 days in the hyperglycemic group and 6 days in the

normoglycemic group (P=0.015). There was also a significant difference in the cost of hospital admissions for the two groups, with the median cost of an admission for a normoglycemic patient being $5262 compared to a median cost of $6611 for hyperglycemic patients (p<0.001). Hyperglycemia was significantly associated with mortality at 1 year with a hazard ratio of 1.75 (95% CI 1.14-2.67, p=0.01).

A large Chinese prospective study published in 2009 by Wang and colleagues fits in with this general trend.[170] In this study glucose data were collected on 2178 patients with ischaemic stroke and 1760 patients with haemorrhagic stroke. The study outcomes were in-hospital death or dependency. Dependency was defined as a modified Rankin score above 2.[171, 172] Patients were categorised by glucose levels; <6.1mmol/L, 6.1-6.9mmol/L, 7-7.7mmol/L, 7.8-11mmol/L and >11.1mmol/L. Normoglycemia was defined as a glucose level below 6.1mmol/L and multivariate adjusted odds ratios for the risk of death or dependency were calculated for each glycaemic category.

In the ischaemic stroke cohort a blood glucose of 6.1-6.9mmol/L was associated with an odds ratio of 0.53 (95% CI 0.23-1.27, p=0.154) for unfavourable outcomes. However a blood glucose level of 7-7.7mmol/L was associated with a higher risk of mortality or morbidity (OR 2.22, 95% CI 1.21-4.11, p=0.010).[170]

Ntaios and colleagues examined the relationship between blood glucose and clinical outcome in a cohort of 1446 patients enrolled in the Acute Stroke Registry and Analysis in Lausanne (ASTRAL).[173] In this cohort some patients with a plasma glucose level of above 10mmol/L were treated with insulin. Glucose levels were correlated with Rankin score at 12 months and NIHSS score at 24 hours. For both endpoints glucose appeared to have a J-shaped relationship. The relationship between glucose and 12 month Rankin was J-shaped with best outcomes related to a glucose level of approximately 5mmol/L and poorer outcomes observed at higher and lower glucose levels. A similar relationship existed for 24 hour NIHSS and glucose. This study does not contain precise data on which patients were treated with insulin and individual clinical outcomes so the possibility that insulin may have been related to poor outcomes in some patients cannot be discounted.

Hyperglycemia may be particularly associated with a negative outcome in elderly stroke patients with an altered level of conciousness.[174] In a retrospective study examining data from 469 patients with a median age of 80 were grouped according to admission blood glucose level. Mortality was greatest in the highest tertile of blood glucose (levels above

7.2mmol/L) with the greatest odds ratio for mortality seen in patients with hyperglycemia and decreased consciousness (OR 9.6, 95% CI 1.65-52.5).

This negative association appears to continue up to 5 years.[175] A correlation has also been noted between acute hyperglycaemia and reduced survival of MRI perfusion-diffusion mismatch tissue (the ischaemic penumbra), greater final infarct size and poorer final outcome.[176]

Hyperglycemia may be associated with a reduced risk of intra-cerebral haemorrhage in a stroke population that has not been treated with alteplase.[177] In a combined analysis of data from 12648 patients originally enrolled in eight original studies blood glucose levels above 7mmol/L were associated with a reduced risk of subsequent ICH (HR 0.33, 95% CI 0.14-0.74).

In Table 1.1 there is a summary of studies that reported an odds ratio for increased mortality with PSH.

Table 1-1 Odds ratio for mortality with post stroke hyperglycaemia

Study	Number of patients	Glucose level (mmol/L)	Odds ratio for increased mortality	Confidence interval	p-value
Zuliani[174]	469	7.2	9.6	1.65 to 52.5	n/a
Wang[170]	2178	7 to 7.7	2.22	1.21 to 4.11	0.010
Ahmed[178]	15336	6.66	1.24	1.07 to 1.44	0.004
Williams[169]	656	7.2	1.75	1.14 to2.67	0.01
Weir[147]	811	8	1.87	1.43 to 2.45	<0.001
Wong[167]	185	7	5	2.3 to 10.7	<0.001
Wang[164]	416	8	3	1.1 to 8.3	0.035
Sacco[157]	323	6.66	1.7	1.4 to 2.8	n/a

1.3.3 Post-Stroke Hyperglycemia as a risk factor for a bad outcome after thrombolytic treatment

In stroke thrombolysis hyperglycaemia is a risk factor for a negative outcome.[144, 179-185] Diabetes was found to be a risk factor haemorrhagic transformation after thrombolysis in early trials with streptokinase.[34]

This effect is not always clear-cut. In one series of 268 consecutive acute stroke patients treated with alteplase elevated glucose levels on arrival at hospital were not significantly associated with adverse clinical outcomes.[186] There was a trend towards increased mortality in hyperglycaemic patients but this was non-significant (OR 1.71 per 100mg/dl increase, 95% CI 0.92 to 3.13, P=0.06). This trend did not correlate with intra-cerebral haemorrhage or disability at discharge. The authors of this paper acknowledge that the sample size in this paper was relatively small and that more significant results may be seen in a larger population.

In one prospective observational cohort study Saposnik and colleagues looked at 216 acute stroke patients treated with alteplase.[181] They looked for factors related to a lack of clinical improvement at 24 hours. Admission glucose levels, onset to treatment time and cortical involvement on imaging were associated with a poor outcome. A blood glucose level of

8mmol/L or greater was associated with a increased odds ratio for a poor 24 hour outcome of 2.89 (95% CI 1.4-5.99) after adjusting for age, sex and stroke severity. Lack of improvement at 24 hours predicted a poor 3 month outcome and death.

In an analysis of 16049 patients treated with alteplase and entered into the SITS database admission glucose level was recorded in 15336 patients.[178] In this prospectively recruited cohort hyperglycemia considered as a continuous variable was independently associated with higher mortality ($p<0.001$), worse functional outcome ($p<0.001$) and increased risk of symptomatic intracerebral haemorrhage ($p=0.005$). When a threshold of 120mg/dl was applied to use glucose as a categorical variable hyperglycemia was associated with an increased odds ratio for mortality (OR 1.24, 95% CI 1.07-1.44, $p=0.004$) and a decreased odds ratio for good functional outcome (OR 0.58, 95% CI 0.48-0.70, $p<0.001$). Symptomatic intracerebral haemorrhage was more likely at glucose levels of above 180mg/dl (OR 2.86, 95% CI 1.69-4.83, $p<0.001$).

In a small Norwegian study involving 127 patients treated with alteplase hyperglycemia after treatment was associated with an increased risk of a poor outcome (OR 1.33, 95% CI 1.02 – 1.74, $p=0.03$)compared to hyperglycemia before treatment (OR 1.04, 95% CI 0.75-1.2, $p=0.8$).[187]

In a Swiss cohort of 325 patients treated with alteplase, hyperglycemia was again found to be associated with an increased risk of a poor outcome at 3 months with an odds ratio of 1.29 (95% CI 1.07-1.55, $p=0.002$).[184]

Prior diabetes may not be a valid reason to withhold thrombolytic therapy in acute stroke.[188] Mishra and colleagues combined data from the SITS database with data from the Virtual International Stroke Trials Archive (VISTA). Diabetic patients treated with alteplase were more likely to have a favourable outcome than those not treated although this was not statistically significant (OR 1.3, 95% CI 1.05-1.6, $p=0.1$).

In the Third International Stroke Trial (IST-3) there was no significant relationship between glucose levels and the chances of being alive and independent at follow-up.[39]

1.3.4 Post-Stroke Hyperglycemia causing increased rates of intracerebral hemorrhage after thrombolysis

Jaillard and co-authors produced a post-hoc analysis on data from the MAST-E (Multicentre Acute Stroke Trial) streptokinase for stroke thrombolysis study in 1999.[34] In this analysis diabetes was predictive of haemorrhagic transformation after streptokinase but they did not present data on hyperglycemia.

Hyperglycaemia increases the risk of intracerebral haemorrhage after thrombolysis.[182, 189] Some of these studies are summarised in Table 1.2. In an Italian prospective observational study 1125 ischemic stroke patients were admitted to a stroke unit and 67 of these patients were treated with rt-PA.[189] Their admission blood glucose levels were divided into three groups. Patients with the highest blood glucose levels (above 8.3mmol/l) had a parenchymal haemorrhage rate of 6.4% compared to a rate of 2.1% in patients with glucose levels below 6.1mmol/L ($p<0.05$). In this group the relationship between blood glucose level and risk of haemorrhagic transformation appeared to be linear.

In secondary analysis of data from 748 patients treated with alteplase in the second European Cooperative Acute Stroke Study (ECASS II) persistent hyperglycemia at 24 hours was associated with poor neurological recovery, poor functional outcome, increased 90 day mortality and parenchymal haemorrhage.[144] The odds ratio for mortality at 90 days with persistent hyperglycemia was 7.61 (95% CI 3.23 – 17.9) while the odds ratio for parenchymal haemorrhage was 6.64 (95% CI 2.51 – 14.1). The authors concluded that in addition to isolated glucose level at time of hospital admission the pattern of glucose change should be considered when predicting stroke outcome.[144] Persistent hyperglycemia was more deleterious in patients who were not known to be diabetic. Another paper has suggested that fluctuations in blood glucose levels post stroke may be due to regression to the mean, a statistical artefact.[190]

In a 2002 paper Tanne and colleagues looked at data from 1205 patients treated with rt-PA.[191] In this group 72 patients had symptomatic intracranial haemorrhage while a further 86 had asymptomatic ICH. Glucose was associated with an increased risk of any intracerebral haemorrhage with the risk increasing by an odds ratio of 1.36 (95%CI 1.11-1.67) for every increase in plasma glucose level of 2.78mmol/L. A pre-existing diagnosis of diabetes mellitus was also associated with symptomatic intracranial haemorrhage.

Table 1-2 Risk of ICH with increasing blood glucose levels

Study	Number of patients	Odds ratio for ICH	Confidence interval	P value
Tanne[191]	1205	1.36	1.11 to 1.67	0.005
Poppe[183]	1098	1.69	0.95 to 3	0.003
Ahmed[178]	15336	2.86 (with glucose above 18mg/dl)	1.69 to 4.83	<0.001
Yong[144]	748	6.64	2.51 to 14.1	<0.0001
MacDougall[192]	312	1.23 (per mmol/L)	1.03 to 1.48	0.024

Similar results were seen in a Canadian paper published in 2009 by Poppe and colleagues.[183] In a prospective, observational study data from 1098 patients were collected. Hyperglycemia was defined as a blood glucose level above 8mmol/L. After adjusted multivariate regression hyperglycemia was associated with an increased risk of symptomatic intra-cranial haemorrhage (OR 1.69, 95% CI 0.95-3, p=0.03), a reduced chance of a good outcome (OR 0.7, 95% CI 0.5-0.9, p<0.001) and an increased risk of death (OR 1.5, 95% CI 1.2-1.9, p<0.001). This study also analysed the relationship between glucose as a continuous measurement and outcome finding that increasing plasma glucose levels inversely correlated with the likelihood of a favourable outcome.

In an abstract presented at the European Stroke Conference in 2010 Jüttler and colleagues looked for glycaemic parameters that predict intra-cerebral haemorrhage after thrombolysis.[138] In a series of 800 consecutive patients treated with alteplase predictors of haemorrhage were identified with multivariate regression. HbA1c levels were most strongly predictive of subsequent haemorrhagic transformation and symptomatic intracerebral haemorrhage. These findings may indicate that long term glycaemic status may be the greatest predictor of outcome.

1.3.5 Effect of post stroke hyperglycemia in intra-arterial treatment for acute stroke

In the PROACT II (PROlyse for Acute Cerebral Thromboembolism) randomised control trial hyperglycemia was associated with an increased risk of symptomatic intracerebral haemorrhage (sICH).[193] In this study 36% of patients with a baseline blood glucose of above 200mg/dl had sICH compared to only 9% of those with lower blood glucose levels. The relative risk of sICH with hyperglycemia was 4.2 (95% CI 1.04 - 11.7, p=0.022).

Hyperglycemia is also associated with a poor outcome in patients treated with multimodal reperfusion therapy including endovascular recanalization of acute stroke.[194] In a retrospective observational study published by Vora and colleagues in 2007 data from 185 patients were collected. A heterogeneous selection of interventions was used in this cohort including stenting devices, angioplasty devices, snaring devices and intra-arterial administration of both urokinase and alteplase. Some patients were also pre-treated with intravenous alteplase. The outcome measures in this study were haemorrhagic infarction and parenchymal haemorrhage as defined in another paper.[195] In this cohort haemorrhagic infarction was associated with the extent of visible changes seen on the initial plain CT scan while parenchymal haemorrhage was associated with associated with alteplase or urokinase treatment and tandem occlusions as well as hyperglycemia. Hyperglycemia conveyed an increased odd ratio of 2.8 for parenchymal haemorrhage (95% CI 1.1-7.7, p=0.043).

Another retrospective observational study looking at risk factors for haemorrhagic transformation after intra-arterial thrombolysis was published by Kidwell *et al* in 2002.[196] In this study mean glucose levels were higher in patients who suffered from haemorrhagic transformation after treatment (p=0.04) and higher still in those with symptomatic intracerebral haemorrhage (p=0.02). In a multivariate model there was no significant association between hyperglycemia and haemorrhagic transformation.

1.3.6 Effect of post-stroke hyperglycemia on recanalization rates after treatment with alteplase

Hyperglycaemia appears to be more harmful if present before blood vessel recanalization post-thrombolysis.[180, 197-199] In a Spanish study recanalization rates after thrombolysis were evaluated in a prospectively recruited series of 139 acute stroke patients.[200] Complete Recanalization was observed in 32% of patients. Patients who recanalized had lower

admission glucose levels (127 vs146 mg/dl, p=0.039) although HbA1c levels were the same. An admission glucose level of above 158 mg/dl (8.7mmol/L) was an independent predictor of no recanalization with an odds ratio of 7.3 (95% CI 1.3 to 42.3, p=0.027). Other less powerful independent predictors included platelet count above 219 000/ml and proximal MCA occlusion. The authors suggest that acute hyperglycemia has more of an effect that chronic hyperglycemia on recanalization rates although causality cannot really be established in this study. They also state that there was no apparent graded response to glucose level but an apparent threshold effect for reduced recanalization at levels of approximately 160mg/dl (8.9mmol/L). Ribo and colleagues hypothesise that at this level an anti-fibrinolytic effect may be triggered by glycation of key proteins in the fibrinolysis chain.

Similar findings were seen in another small prospective observational study that recruited 27 patients.[201] In this cohort of patients treated with alteplase trans-cranial Doppler ultrasound was used to assess recanalization at 2 hours and 24 hours. The group of patients that did not recanalize had higher glucose levels (mean glucose 8.16mmol/L vs. 6.25mmol/L, P=0.5).

1.3.7 Relationship between post-stroke hyperglycemia and arterial patency

In a later study the Barcelona group recruited 47 patients in an attempt to evaluate the effect of glucose burden on MRI DWI lesion growth in relation to the duration of ischemia. [197] All of the patients were treated with t-PA and had serial TCD recordings to monitor for recanalization and define total occlusion time. They had continuous glucose monitoring via a subcutaneous probe which recorded a glucose level every 5 minutes. It is important to note that these patients were subjected to a fast-acting insulin sliding scale which aimed to maintain blood glucose below 140mg/dl (7.7mmol/L). Patients had MRI on admission (MR angiography, perfusion weighted imaging and diffusion weighted imaging) followed with a second MRI-DWI scan at between 24 and 36 hours. Lesion growth was the difference in size between the first and second DWI lesions.

In this study a poor clinical outcome was defined as an improvement of less than 50% in NIHSS. Hyperglycemia during arterial occlusion was found to be the only independent predictor of a poor outcome with an odds ratio of 20.3 (95% CI 3.77 to 108.8, $P < 0.001$). DWI lesion growth correlated with total occlusion time (p=0.007) and hyperglycemia (p=0.01).

The discussion in this paper notes that:

'…after adjusting for total time of ischemia, those patients with suboptimal glucose control despite tight insulin treatment experienced a worse clinical outcome…'.

It is impossible to firmly conclude that hyperglycemia and not insulin exposure is responsible for the poor clinical outcome in these patients. Despite the clear problems with causality in this paper the authors then state;

'A tight subcutaneous insulin sliding scale showed to be insufficient to maintain normoglycemia in as high as 51% of all patients, therefore more intense glucose control measures, such as intravenous insulin, should be considered in future studies.'

This paper does suggest that hyperglycemia during arterial occlusion is associated with poor clinical outcomes and DWI lesion growth but the use of insulin reduces the validity of the authors' conclusions.

1.3.8 Hyperglycemia may attenuate the effects of alteplase on reducing infarct growth

The EPITHET (Echoplanar Imaging Thrombolytic Evaluation Trial) authors suggest that diabetes and hyperglycemia may attenuate the effects of rt-PA on infarct evolution.[202, 203] EPITHET was a small randomised control trial which allocated patients to alteplase or placebo between 3-6 hours after stroke onset. A total of 101 patients were enrolled in the trial, 22 of whom were known to be diabetic. There were no significant differences in baseline DWI and PWI lesion size between diabetic and non-diabetic patients.

Fifty-two patients were randomised to alteplase treatment while 48 received placebo. Eleven diabetics were allocated to each group. In the active treatment group there was evidence of infarct attenuation in the non-diabetics who had a median relative infarct growth of 0.96 (IQR 0.58-1.74) compared to diabetics who exhibited greater median infarct growth (1.27, IQR 0.51-6.78). The difference in infarct growth between these groups was significant ($p=0.007$). In the placebo group there was no significant difference in infarct growth between diabetics and non-diabetics. Alteplase significantly attenuated infarct growth in non-diabetics ($p=0.012$) but had no significant effect in diabetics ($p=1$).

EPITHET was a small study and is only powered to produce statistically significant results when large differences between groups exist. No differences in reperfusion or recanalization rates were apparent between diabetics and non-diabetics. The authors hypothesise that recanalization and reperfusion may occur at a slower rate in diabetics explaining the greater relative mean infarct growth.

The interaction between hyperglycemia and poor outcomes from thrombolysis may be mediated by impaired collateral circulation in diabetics.

The EPITHET authors hypothesise that alteplase failed to attenuate infarct growth due to a lack of collateral circulation in diabetic patients.[203] They suggest that diabetes may impair the development of collateral circulation. These ideas echo the earlier work of Toni *et al* that I will discuss in a later section.[204]

1.3.9 Could blood glucose be controlled before administering thrombolytic therapy?

In one prospective observational study two hyperglycaemic patients were treated with insulin by their family doctor before being given t-PA. These patients had better clinical and radiological outcomes than similar hyperglycaemic patients.[205] While these observational data are interesting we do not know enough about the underlying pathophysiological reasons for the poor alteplase response seen in diabetic and hyperglycemic patients. There is currently no clinical evidence to support the routine use of insulin in acute ischaemic stroke as I discuss in my systematic review of the evidence in Chapter 4. The animal evidence for the use of insulin is also of limited value and cannot justify clinical trials at present (see Chapter 3).[206]

1.3.10 Different effects of hyperglycemia in stroke and TIA

Two papers have suggested that hyperglycemia may have less of a deleterious effect on patients who suffer a transient ischaemic attack.[175, 207]

Both studies were fairly small and may not have enough statistical power to conclusively state that hyperglycemia has no effect on mortality after TIA. Previous studies have suggested that patients with known diabetes who present to an emergency department with symptoms of TIA are at an increased risk of subsequent stroke within 90 days (OR 2, 95% CI 1.4-2.9, $p<0.001$).[208]

In a 2010 paper Thuy and colleagues retrospectively looked at 194 patients who had presented with TIA.[207] In this cohort 27.8% of patients were hyperglycemic (defined as blood glucose levels above 7mmol/L) and 22% were diabetic. In this cohort acute hyperglycemia was not associated with mortality after TIA. Thuy and colleagues acknowledge that there was a low death rate in this group which may make the result less statistically valid.

In another retrospective cohort study Kostulas and colleagues examined data from 509 patients presenting to Karolinska University Hospital in Sweden.[175] This cohort included 114 (22%) patients with TIA. Hyperglycemia was observed in 28% of patients with ischaemic stroke and 18% of patients with TIA. Mean admission blood glucose level was lower in patients with TIA (P=0.0002). Admission hyperglycemia did not appear to have any effect on mortality rates in TIA patients.

It has also been suggested that stroke may be more common than TIA in diabetic patients.[209, 210] This observation was reported by Weinberger and colleagues in 1983 when they studied factors contributing to stroke in patients with carotid atherosclerosis.[209] In a cohort of patients attending a vascular laboratory for arterial assessment they noted that the incidence of stroke as opposed to TIA was twice as high in patients with diabetes.

In a secondary analysis of data from the Dutch TIA study diabetes was found to be an independent predictor of major stroke after TIA or minor stroke.[211] In these data hyperglycemia did not predict stroke in multivariate analysis although diabetes continued to be predictive even after CT and ECG findings were taken into account. After an initial TIA diabetes is associated with a hazard ratio of 2.1 for subsequent stroke (95% CI 1.5-2.9).

In a 1988 paper published by Lithner and colleagues 428 consecutive unselected patients presenting to a stroke unit were prospectively studied.[210] These patients were divided into a diabetic group of 75 and a non-diabetic group of 353. Transient cerebral ischemia was observed in 4% of the diabetics compared to 14% of the non-diabetics ($p<0.01$). Cerebral embolism was observed in 32% of the diabetics compared to 22% of non-diabetics ($p<0.05$). Stroke was more common in diabetic patients than non-diabetic patients while TIA was more common in non-diabetic patients than in diabetic patients. Several possibilities could explain this difference. Patients with diabetes may be more susceptible to cerebral ischaemia and may therefore be more likely to develop a stroke when cerebral

blood flow is compromised. It is also possible that TIA symptoms are misdiagnosed in diabetic patients (for example as the effects of transient hypoglycaemia).

This issue was also considered by Fritz and colleagues in a 1987 paper.[212] This paper examined the hypothesis that diabetes mellitus predisposes the brain to irreversible ischaemic damage as opposed to reversible transient ischaemic symptoms. In a prospectively collected observational series of 525 patients presenting with TIA or minor stroke, 54 had diabetes. In this group 388 patients had a TIA but only 6.7% of TIA patients had diabetes. However 28/54 patients with diabetes had strokes compared to only 109/471 patients without diabetes. Patients with diabetes were more likely to present with a stroke ($p<0.0001$)

Interestingly in a prospective observational study published by Matz and colleagues in 2006 it appears that diabetic patients have more severe NIHSS scores than non-diabetic patients.[158] The Matz study was a prospective observational study that carefully defined the dysglycaemia of stroke patients. Patients were classified as having diabetes, impaired glucose tolerance, transient hyperglycemia and normoglycemia. The median NIHSS on admission in the diabetic group was 7.2+/-6.6 compared to 4.6 +/- 3.1 for patients with impaired glucose tolerance, 4.2 +/- 4.4 for patients with transient hyperglycemia and 3.7 +/- 3.6 for patients who were always normoglycemic ($p<0.001$).

1.3.11 Measuring blood glucose after stroke

The accurate assessment of glucose levels and glycaemic status after a stroke can be difficult. Plasma glucose is a constantly changing physiological variable that will alter with many internal and external factors. The majority of papers that discuss post-stroke hyperglycemia deal with admission blood glucose level and can only provide a point estimate of glucose exposure.

Several studies use repeated capillary blood glucose measurements and this may give a better estimate of average glucose exposure.[161, 213] Other studies have used constant glucose monitoring which gives an accurate glucose profile but is invasive and costly.[214, 215] Glycosylated haemoglobin levels can give a historical picture of glycaemic status but they are insensitive to acute changes.

One paper, published in 2002 by Bhalla and colleagues evaluated Glycated Serum Proteins for predicting outcome after acute stroke.[216] Glycated Serum Proteins (GSP) reflect plasma

glucose levels over the preceding two weeks. Albumin is one protein that may be used as a GSP. In a prospective study 167 patients had their GSP levels checked within 24 hours of admission. GSP levels were subsequently rechecked 14 days later. For patients whose GSP levels increased, every 1% increase in GSP levels increased the odds ratio of death by 3 months by 1.28 (95% CI 1.1 to 1.62, p=0.04). This ratio was obtained after multiple logistic regression analysis and was independent of admission plasma glucose, age, stroke severity, stroke subtype and serum albumin change

1.4 Hypotheses on the pathophysiology of post-stroke hyperglycaemia

While post-stroke hyperglycaemia has been recognised for some time its pathological basis has been unclear.[148] It is also possible that an individual patients' glucose metabolism may alter over time after a stroke.[217]

1.4.1 Post-Stroke Hyperglycemia as a stress response

Several hypotheses on the origin of PSH have been advanced. PSH may be a stress response which reflects a severe stroke.[218, 219] This idea was put forward by Woo and colleagues in a 1988 paper that noted a lack of correlation between HbA1c levels and plasma glucose levels after an acute stroke.[153] This disparity was also observed by Gray and colleagues in their 1987 paper.[133]

In a small 1991 study of 23 patients, only 15 of whom had complete data, O'Neill and colleagues prospectively collected a series of blood samples for glucose, insulin, c-peptide, catecholamine, cortisol, glucagons and lactate levels.[218] In this small sample of 15 patients glucose levels varied with insulin, cortisol and glucagon levels leading the authors to conclude that post stroke hyperglycemia probably reflects the intensity of a stress hormone response. The authors admit that their population is too small to establish any significant statistical associations between hormonal levels, glucose and stroke outcomes. The conclusions of the O'Neill paper on a link between stress and glucose levels are probably premature as PSH is seen in all types of stroke and does not appear to correlate with stroke severity.[147]

Tracey and colleagues carried out a similar study in 1993.[220] They analysed data on blood glucose, HbA1c and cortisol levels from 66 prospectively recruited acute stroke patients. Patients were followed up at 3 months with a plain CT scan. Plasma glucose levels were

higher in patients who died during the study (p=0.025). Mean cortisol levels were also higher in patients who died. After multivariate analysis only age and cortisol levels were significantly associated with mortality. Infarct size correlated more strongly with cortisol levels than with glucose levels or insulin levels although all of these relationships were significant.

Another study published by van Kooten and colleagues in 1993 found that catecholamine levels were not associated with hyperglycaemia. This study prospectively recruited 91 stroke patients presenting within 24 hours of stroke onset. All patients had catecholamine levels checked. Norepinephrine levels were associated with severity of stroke and hypertension.[221] No significant association was found between catecholamine levels and blood glucose levels or glycosylated haemoglobin levels. In a 2001 study by Sander and colleagues that included 112 acute stroke patients there was no significant difference in average plasma glucose levels of patients who had Norepinephrine levels below 300pg/mL compared with those who had levels above 300pg/mL.[222] In their 1997 paper Weir and colleagues argue that post-stroke hyperglycemia cannot be a pure stress response as it still predicts final outcome in a statistical model that takes other prognostic factors into account.[147]

More recently copeptin has been proposed as a prognostic biomarker in acute illness.[223] Copeptin has recently been shown to predict recurrent ischaemic events after TIA while cortisol does not.[224] It may be interesting to look for a relationship between copeptin and post stroke hyperglycemia in the future.

It has also been noted that pre-operative hyperglycemia is associated with an elevated risk of stroke in patients undergoing carotid endartectomy as described in a paper by McGirt and colleagues that I have already discussed.[46] As this group of patients had pre-existent hyperglycemia it suggests that in at least some cases post-stroke hyperglycemia is not related to a stress response. This relationship between pre-operative hyperglycemia and stroke was also independent of the presence of diabetes.

1.4.2 Post-stroke hyperglycemia due to a specific neuroanatomical lesion

Another possibility is that specific neuroanatomical lesions cause acute stress hyperglycaemia although this has not been confirmed by later studies.[225, 226] This concept was partly based on earlier observations on the effects of specific neuroanatomical lesions

on the glucose metabolism of animals.[227, 228] In a classical experiment in 1854 Claude Bernard noticed that damage to the floor of the fourth ventricle would produce glycosuria.[227] In a study including 31 patients who had acute MRI diffusion weighted imaging within 24 hours of stroke onset Allport and colleagues found that patients with insular cortex ischemia had a higher mean glucose that patients who had no insular cortex damage.[225] Moreton and colleagues tried to correlate insular cortex hypoperfusion on CT perfusion imaging in a retrospective study looking at 35 patients.[226] They found no relationship between hyperglycemia and insular cortex hypoperfusion.

A retrospective analysis of data from 966 patients in the Canadian Activase for Stroke Effectiveness Study (CASES) found that insular cortex damage was not an independent predictor of hyperglycemia or hypertension.[229]

Post-stroke hyperglycemia may reflect a hyperglycaemic state immediately before stroke

1.4.3 Post-Stroke Hyperglycemia as undiagnosed Type 2 Diabetes Mellitus

Post-stroke hyperglycaemia may represent undiagnosed type 2 diabetes mellitus.[230] In 1985 Oppenheimer and colleagues carried out a prospective observational study comparing glycosylated haemoglobin levels with outcome and plasma glucose.[130] They found that a highly significant correlation existed between plasma glucose levels and HbA1c levels. They also observed a significant relationship between diabetes mellitus and early death after stroke. They concluded that pre-stroke abnormal glucose metabolism may be a major determinant of post-stroke hyperglycemia. They hypothesised that the poor outcomes for hyperglycemic stroke patients may reflect the clinical course of stroke in diabetes mellitus.

Studies suggest that up to one third of acute stroke patients have diabetes mellitus but that in patients with post stroke hyperglycaemia only two-thirds of survivors have impaired glucose tolerance or diabetes mellitus diagnosed at 12 weeks.[231] Certainly in non-stroke populations the prevalence of undetected type 2 diabetes mellitus is significant.[232] Retrospective studies have suggested that many clinicians do not screen acute stroke patients for unrecognised Type II DM.[233]

A 1989 study by Topic and colleagues which prospectively observed glucose metabolism in acute stroke patients found that while 41% of patients were hyperglycemic after an acute stroke only 29% had diabetes as defined by an elevated glycosylated haemoglobin level.[132]

Insulin resistance may be a mechanism for post-stroke hyperglycemia in cases where patients do not have clinically overt diabetes mellitus. A 2002 review paper found six methodologically sound papers that supported the hypothesis that insulin resistance may be a prevalent risk factor for stroke.[234] This review was followed by a prospective observational study aiming to establish the prevalence of insulin resistance in a cohort of non-diabetic patients presenting with TIA or non-disabling stroke.[48] Of the 72 participants in this study 36 (50%, 95% C.I 38% to 62%) had evidence of insulin resistance. However subgroup analysis of an 18 year follow up study in Japan suggests that diabetes is a risk factor for stroke while insulin resistance is not.[235]

In a prospective observational study published in 2005 Vancheri and colleagues formally assessed glucose metabolism in a cohort of 106 acute stroke patients.[236] Patients with known diabetes were excluded as were patients on medications such as beta-blockers or diuretics which may interfere with glucose metabolism. Patients had a glucose tolerance test at discharge from their acute admission. Insulin and HbA1c levels were also measured and insulin resistance was assessed using HOMA. These tests were repeated at 3 months after discharge.

At discharge based upon the results of the oral glucose tolerance test 45.8% of patients had diabetes mellitus, 38.5% had impaired glucose tolerance and 15.6% had normal metabolism. In the same group of patients at 3 months 36% had diabetes mellitus, 26% had impaired glucose tolerance and 35% had normal metabolism. Among the patients with abnormal glucose metabolism at 3 month follow up 43.5% had a normal HbA1c (below 5.7%) at baseline. These results suggest that there is a high prevalence of unrecognised diabetes mellitus in acute stroke populations and that the glycaemic status of patients may change over time.

A Japanese study assessed glucose metabolism in 427 ischaemic stroke patients. An existing diagnosis of diabetes mellitus was present in 155 patients (36%). Oral glucose tolerance testing was performed in 113 patients. The remaining patients were unable to undertake a glucose tolerance test. Previously undiagnosed diabetes was discovered in 28% of patients suggesting that the overall prevalence of diabetes mellitus in this cohort is at least 42%. A further 39 patients had impaired glucose tolerance. The prevalence of both diabetes and impaired glucose tolerance are likely to be higher in this cohort if patients who were unable to be tested were included. Patients with previously undiagnosed

diabetes were more likely to have had an atherothrombotic stroke (64.3%) than a lacunar stroke (32.1%) or a cardioembolic stroke (3.6%).

In a similar study published by Dave and colleagues in 2010 a prospective observational study attempted to evaluate the prevalence and predictors of persistent hyperglycemia in non-diabetic patients with stroke.[237] Patients were prospectively assessed with an oral glucose tolerance test within 5 days of admission. In this study 107 patients were initially recruited. Abnormal glucose function was apparent in 65 patients (61%), 24% of whom had diabetes mellitus while 37% had impaired glucose tolerance. After 3 months 44 of the dysglycaemic patients were re-investigated with either an OGTT or with HbA1c if on hypoglycaemic treatment. At this point 26 patients (59%) had normal glucose tolerance, 6 (14%) had diabetes and 12 (27%) had impaired glucose tolerance.

Interestingly 23% of the patients who had diabetes based on the acute OGTT had normal glucose tolerance at follow-up. One patient who had impaired glucose tolerance acutely developed clinical diabetes. These results reflect the possibility that the glycaemic status of a stroke patient may change between the acute period and convalescence.

A causal relationship between glycaemic status and stroke outcome has been considered. It has been observed that hyperglycaemic patients without a history of diabetes mellitus appear to have larger infarcts on CT imaging and that there is a correlation between admission glucose concentration and poor stroke outcome.[238] MRI studies support this observation.[176]

1.5 The definition of post-stroke hyperglycaemia

It is not known if there is a clear cut-off level at which blood glucose becomes pathogenic and we do not know the best way to measure blood glucose level to accurately predict prognosis. Many different levels of blood glucose have been suggested for hyperglycaemia as can be seen in Table 1.3.

Table 1-3 Blood Glucose levels used to Define Post-Stroke Hyperglycaemia

Author	Year	PSH level mmol/L	PSH level mg/dl	Number of patients	Percentage with PSH	Percentage with DM
Abu-Zeid[151]	1978	5.55	100	1484	51	n/a
Scott[239]	1999	6	108	303	50	n/a
Gray[240]	2004	6	108	452	n/a	15.3
Gray[231]	2004	6	108	582	n/a	14
Lindsberg[241]	2004	6	108	n/a	n/a	n/a
Gray[242]	2007	6	108	933		16.5
Wong[190]	2008	6	108	124	n/a	0
Mazighi[243]	2009	6	108	477	n/a	88
Cazzato[244]	1991	6.1	110	76	71	22.4
Capes [146]	2001	6.1	110	n/a	n/a	n/a
Bang[245]	2005	6.1	110	512	40	n/a
Johnston [246]	2009	6.1	110	74	n/a	59
Paciaron[189]	2009	6.1	110	1125	42.4	20.7
Wang[170]	2009	6.1	110	2178	40.1	11.3
Dziedzic[247]	2010	6.1	110	302	36.4	0
Benedetti[248]	1993	6.4	115	94	50.5	17.2
Jorgensen[249]	2001	6.5	117	396	n/a	n/a
Pulsinelli[92]	1983	6.6	120	107 + 31	n/a	n/a
Levy[250]	1985	6.6	120	214	56	n/a
Toni[155]	1992	6.6	120	327	28.5	21.5
Toni[204]	1994	6.6	120	82	n/a	n/a
Lavy[251]	1973	6.66	120	1522	n/a	20
Chalela[252]	2004	6.66	120	27	66.6	n/a
Melamed[148]	1976	6.7	120	392	48%	20
Matcher[253]	1992	6.7	120	146	n/a	23
de Falco[156, 254]	1993	6.7	120	104	51.9	33.6
Bell[255]	1994	6.7	120	n/a	n/a	n/a
Ahmed[178]	2010	6.7	120	16049	44	17.3
Topic[132]	1989	7	126	148	59	29
Dutch TIA trial study group[211]	1993	7	126	302	n/a	n/a

Sulter[256]	1998	7	126	41	36.5	n/a
Scott[143]	1999	7	126	53	100	15.1
Milionis[257]	2005	7	126	163	7	28
Stollberger[258]	2005	7	126	992	n/a	30
Wong[167]	2005	7	126	186	37.8	19
Allport[214]	2006	7	126	59	81	36
Walters[259]	2006	7	126	25	100	n/a
Moreton[226]	2007	7	126	35	51.4	11.4
Ortega-Casarrubios[96]	2007	7	126	2213	n/a	29.9
Uyttenboogaart[260]	2007	7	126	1375	51.1	20.9
Kruyt[261]	2008	7	126	113	38	30
Dziedzic[262]	2009	7	126	689	13.6	22.2
Gunarathne[263]	2009	7	126	60	n/a	46.5
Kruyt[264]	2009	7	126	10	100	20
Scott[265]	2010	7	126	224	45	25
Staszewski[266]	2010	7	126	50	100	0
Thuy[207]	2010	7	126	194	27.8	22
Muir[267]	2011	7	126	2649	53.7	14
Lindegard[268]	1987	7.2	130	1379	78.5	32
Williams[169]	2002	7.2	130	656	40	52
Gentile[269]	2006	7.2	130	960	38.9	36.4
Zuliani[174]	2006	7.2	130	469	n/a	25.8
Stead[174]	2009	7.2	130	447	34.2	25.7
Folsom[58]	1999	6.655		191		
Sacco[168]	1989	7.7	140	1273	36	26
Hier[270]	1991	7.7	140	1273	27.9	26.1
Sacco[157]	1994	7.7	140	323	38.1	28.5
Alvarez-Sabín[180]	2003	7.7	140	73	42.5	23
Bravata[233]	2003	7.7	140	90	33	
Alvarez-Sabin[271]	2004	7.7	140	138	37.3	20.3
Dora[272]	2004	7.7	140	46	43.4	15.2
Yong[144]	2008	7.7	140	748	32.8	21.5
Kim[273]	2009	7.7	139	115	50	40.9
Mankovsky[274]	1996	7.77	140	41	0	53.7

Martini [275]	2006	7.77	140	117	27.7	22.2
Ribo[197]	2007	7.77	140	47	51	19
Woo[154]	1990	7.8	140	174	31.6	27
Kiers [238]	1992	7.8	140	176	34	28.4
Milia[276]	2010	7.9	143		n/a	n/a
Cox[131]	1986	8	144	81	16	
Gray[133]	1987	8	144	86	22	8
Gray[277]	1989	8	144	200	22.8	8.5
Van Kooten[221]	1993	8	144	91	43	24.2
Counsell[278]	1997	8	144		n/a	0
Weir[147]	1997	8	144	645	25.1	8.8
Wang[164]	2001	8	144	416	26	20
Wang[279]	2001	8	144	440	n/a	n/a
Parsons[176]	2002	8	144	63	34.9	28.6
Spratt[166]	2003	8	144	257	43	27
Saposnik[181]	2004	8	144	216	n/a	23.1
Diener[280]	2008	8	144	4946	23.6	22.1
Kostulas[175]	2009	8	144	509	26	18
Poppe[183]	2009	8	144	1098	27	15.1
Putaala[281]	2010	8	144	851	43.6	13.3
Berger [282]	1986	8.3	150	39	30.7	35.9
Leigh[198]	2004	8.3	150	201	n/a	23
Bruno[283]	2008	8.3	150	46	100	91
Fuentes[161]	2009	8.6	155	476	37.2	25
Fuentes[213]	2010	8.6	155	476	n/a	n/a
Ribo[200]	2005	8.7	158	139	n/a	38
Bruno[284]	2004	9.4	170	24	n/a	100
Els[205]	2002	9.9	178.2	31	45.2	0
Woo[152]	1988	11	198	252	7.9	12.3
Di Bontio[285]	2003	11	198	286	n/a	49.6
Kase[193]	2001	11.1	200	110	10	14.5
Mcgirt[46]	2006	11.1	200	1201	10.7	27
Al-Himyar[286]	2007	11.1	200	50	12	n/a
Vora[194]	2007	11.1	200	185	23.2	22.7

As the table demonstrates, plasma glucose values ranging from 5.55 to 11.1mmol/L have been used to define post stroke hyperglycaemia. This variation in definition leads to the differing incidences that are proposed for PSH.

More methodical attempts have been made to define PSH. Fuentes and colleagues took an alternative approach in another prospective observational study involving 476 patients.[161] In an attempt to establish the capillary glucose level that best predicts poor outcome capillary blood glucose was measured 3 times daily for 48 hours. They calculated that a glucose value of 155 mg/dl (8.6mmol/l) or above at any time during the first 48 hours was associated with a 2.7 times increase in the risk of a poor outcome (95% CI 1.79 – 8.1).

In a similar study presented as an abstract at the European Stroke Conference Milia and colleagues attempted to establish a threshold value that best predicts short term mortality after stroke.[276] Data from 851 patients were included and an admission blood glucose level of 143mg/dl was found to be most predictive with a sensitivity and specificity of 72%.

A consensus statement on the definition of post stroke hyperglycaemia may be valuable. Future research would be more cohesive if researchers were using the same definition.

1.6 Uncertainties regarding post stroke hyperglycaemia

There are several interesting issues around post stroke hyperglycaemia that may merit further investigation in the future.

1.6.1 Could the effects of post-stroke hyperglycemia be mediated by collateral blood supply?

Toni and colleagues attempted to look for a relationship between glycaemic status, collateral cerebral blood flow and clinical outcome.[204] They prospectively recruited 82 patients all of whom underwent cerebral angiography within 4 hours of stroke onset. The patients were divided into 3 groups by glycaemic status; diabetics, patients with transient hyperglycemia and patients who were normoglycemic at all times. The ‘diabetic’ group had slightly ambiguous inclusion criteria as previously seen in the 1992 paper by this group.[155]

Using the cerebral angiogram the site of the arterial occlusion was defined and collateral blood flow was assessed. Patients with no arterial occlusion, carotid disease only or

complete carotid occlusion were excluded from the study. Patients were followed up with a repeat CT scan and clinical assessment at day 30.

Nine patients were classified as diabetic, 40 were classified as hyperglycemic and 33 were classified as normoglycemic. In the diabetic group the main serum blood glucose was 15mmol/L, in the hyperglycemic group the mean glucose was 7.84 and in the normoglycemic group the mean was 5.5. At day 30 89% of the diabetic patients were dead, 72% of the hyperglycemic patients were dead and 54% of the normoglycemic patients had died ($P<0.05$). Collateral blood supply was present in 33% of diabetic patients, 55% of hyperglycemic patients and 66% of normoglycemic patients. Patients with good collateral blood supply had smaller than expected infarcts at 30 day follow-up. However smaller infarcts were more likely to be seen in normoglycemic patients (82%) than hyperglycemic patients (64%). No diabetic patients had smaller than expected infarcts. In patients without a collateral blood supply infarcts were usually of the expected size and no significant differences were observed between glycaemic groups.

The authors hypothesise that diabetes may reduce the effectiveness of the collateral blood supply. This could be due to a functional problem, with reduced autonomic recruitment of collateral blood vessels, or due to an anatomical shortage of blood vessels due to diabetic arteriopathy. This hypothesis is similar to the stated hypothesis of De Silva and the EPITHET investigators that I have discussed in an earlier section.[203]

1.6.2 Gender Differences in relation to Post-Stroke Hyperglycemia

One study suggested that admission hyperglycemia may only be associated with brain infarction and higher 5 year mortality in females.[243] This study is part of a larger French stroke genetics study which excluded anyone with non-Caucasian parents. Patients could be enrolled in the study within one week of stroke. The quantification of hyperglycemia was based on one fasting blood glucose sample. Glycosylated haemoglobin values were not checked in this study.

1.6.3 Post-Stroke Hyperglycemia as a protective mechanism

It has also been suggested that post-stroke hyperglycaemia may be a protective mechanism although the current weight of evidence does not support this idea.[287] In a 2007 letter to *Brain* Metso and Murros highlighted the argument that the association between hyperglycemia and poor outcome does prove causality. They highlight that our knowledge

of the pre-stroke glycaemic status of a patient is often incomplete and that it may be dangerous to assume that hyperglycemia in itself is detrimental. They cite the results of the GIST-UK study, where patients with the greatest reduction in serum glucose levels during the acute phase of stroke died, as evidence for this hypothesis. They also express concern at contemporary guidelines that advocated treatment of hyperglycemia and espouse the principle of *primum non nocere.* First do no harm.

1.6.4 Post-Stroke hyperglycemia may have different effects on different stroke sub-types

In a secondary analysis of data collected prospectively for two clinical trials of luceluzole in acute stroke Uyttenboogaart and colleagues found a dichotomous relationship between hyperglycemia and clinical outcome when lacunar strokes were compared to non-lacunar strokes.[260] Data from 168 lacunar stroke patients and 1207 non-lacunar patients were available. Hyperglycemia defined as a blood glucose above 8mmol/L was associated with a lower chance of a good outcome (mRs<2) at 3 months in non-lacunar stroke (OR for good outcome 0.60; 95% CI 0.41–0.88, P=0.009). However in lacunar patients hyperglycemia was associated with a good outcome (OR 2.70, 95% CI 1.01- 7.13, P=0.048).

1.6.5 Associations between post-stroke hyperglycemia and cerebral oedema

In a 1986 paper Berger and Hakim retrospectively reviewed the cases of 39 patients to study the effect of serum glucose levels on clinical and radiological progression after stroke.[282] All patients had at least two CT scans during their admission and an average glucose level over the first 30 days of the admission was calculated. Patients were divided into tertiles of mean glucose level. In this cohort there was a significant trend in the association between rising mean glucose levels and the proportion of scans showing midline shift or ventricular compression. When the diabetic and non-diabetic patients were separated the trend for worse clinical outcome and death was not significant in the diabetic patients but continued to be significant in the non-diabetic patients ($p<0.005$). Younger patients were also more likely to have bad outcomes with higher average glucose levels ($p<0.001$).

1.6.6 Markers of neuronal damage and PSH

Neuron specific enolase (NSE) is a marker of brain injury that is released into the peripheral blood and the cerebro-spinal fluid. It has been suggested that elevated NSE levels may be a biomarker for on-going neuronal damage in stroke patients. One small prospective observational study recruited 41 patients who had their NSE levels checked within 24 hours of stroke onset.[256] There appeared to be a correlation between higher NSE levels and higher blood glucose levels. The authors suggest that this correlation lends further weight to the hypothesis that hyperglycemia promotes neuronal necrosis during the acute stage of ischemic stroke.

1.6.7 Could post-stroke hyperglycaemia be a hypercatabolic state associated with acute starvation?

Acute stroke can be associated with acute starvation if swallowing is impaired. When food or other exogenous energy sources are not available the body has to mobilise endogenous stores via gluconeogenesis and glucose sparing to maintain blood glucose at an adequate level to nourish the brain. Synthesis of protein and fat is curtailed during fasting.

In a 1985 paper Bjorkman and Eriksson demonstrated that insulin resistance had increased after 60 hours of fasting in normal subjects.[288] Euglycemic 1-mU insulin and hyperglycemic glucose clamp studies were undertaken on subjects after overnight fasting and prolonged (60 hour) fasting. A greater degree of insulin resistance was apparent after a longer period of fasting.

A study similar to this would be difficult to perform in unstable acute stroke patients. The subjects enrolled in the Bjorkman and Eriksson study were healthy and non-obese making them not directly comparable to the average stroke patient. However it is possible that acute insulin resistance will develop in some stroke patients if they are undernourished for the first few days of their hospital admission.

Acute stroke is a hypercatabolic state. In a 2004 paper Chalela and colleagues reported a prospective observational study that aimed to assess nitrogen balance in acute stroke patients.[252] In patients with an acute illness who require tube feeding the Harris-Benedict Equation is used to measure Resting Energy Expenditure. Adjustments for clinical conditions that elevate energy expenditure are made with ‘stress factors’. There is no ‘stress factor’ available for acute stroke. Chalela and colleagues wanted to validate the

Harris-Benedict Equation in stroke patients who required enteral nutrition by analysing their nitrogen balance. Twenty seven patients were prospectively recruited. Post stroke hyperglycemia was seen in 66% of these patients. A negative nitrogen balance was seen in 44% of patients suggesting that they were catabolic. No evidence of catabolism or anabolism was seen in 16% of patients while the remaining 40% showed evidence of anabolism.

Enteral feeding determined by the Harris-Benedict Equation underestimates the requirements of acute stroke patients in a significant proportion of patients as anabolism was only achieved in 40% of patients. Stroke patients requiring enteral support were being underfed. This was a small group and there was no significant correlation between glycaemic status and catabolism.

Johanssen and colleagues attempted to assess the relationships between circulating levels of pro-inflammatory cytokines, adrenocortical hormones and leptin in the acute stroke period.[289] Multiple blood samples collected over the first 7 days post-stroke were collected in 12 patients and compared with 10 healthy controls. A significant correlation was observed between IL-6 and cortisol in the first two days after stroke ($P<0.05$). The diurnal rhythm of leptin levels was abnormal in half of the patients by the end of the week. Leptin is thought to be a key factor in neuroendocrine balance with reciprocal connections to several hormone systems. Leptin is also thought to be involved in the neuroendocrine response to fasting.[290]

Catabolism is associated with loss of lean body mass. Carlotti and colleagues have suggested that an increase in the rate of creatinine excretion may be a marker of the onset of catabolism.[291]

1.6.8 'Glucose Kinetics' - which pattern of post-stroke hyperglycemia is most harmful?

As the majority of patients are hyperglycemic at some point after a stroke it may be important to consider 'glucose kinetics' or the pattern of blood glucose levels that is most harmful.

In a prospective observational study Dziedzic and colleagues looked at the pattern of blood glucose levels in a cohort of non-diabetic patients who had experienced their first ever stroke.[247] A total of 302 patients had fasting blood glucose levels checked on days 1, 2, 3, 5

and 7 after admission. The patients were then divided into 4 groups – patients that were normoglycaemic at all times, patients who were initially normoglycaemic and then became hyperglycemic, patients who were initially hyperglycemic before becoming normoglycemic and patients who were hyperglycemic at all times. After multivariable adjustment post-admission hyperglycemia was associated with a higher risk of death regardless of admission glucose level (HR 1.80, 95% CI 1.39-2.86, P<0.01). Hyperglycemia at all times was associated with the greatest risk of death (HR 4.83, 95% CI 1.93-12.06, P<0.01) and late onset hyperglycemia after admission normoglycemia was also associated with an increased risk of death (HR 1.64, 95% CI 1.06- 2.54, p=0.04).

Patients who became normoglycemic after admission hyperglycemia had a lower risk of death than those who were persistently hyperglycemic (HR 0.21 95%CI 0.08-0.52). Spontaneous normalisation of blood glucose appears to be associated with better clinical outcomes. The authors conclude that hyperglycemia has a causative role in the poor outcomes associated with post stroke hyperglycemia and that patients should be given glucose lowering treatment for extended periods in future clinical trials.

Wong and colleagues put forward a different hypothesis on glucose kinetics in a 2008 paper published in *Neurology*.[190] In a prospectively recruited cohort of 124 non-diabetic patients they checked capillary blood glucose levels at a frequency of at least every 4 hours for the first 48 hours after admission. No thrombolytic therapy was given to any patient in the study. After statistical analysis the authors concluded that observed variation in capillary blood glucose levels in non-diabetic stroke patients was due to regression to the mean. Regression to the mean is a statistical phenomenon where random values above and below a mean value will gradually drift back to an average value over time. Regression to the mean occurs when repeated measurements are made of the same subject or unit of observation.[292] Observed values have a random error which produces non-systematic fluctuations in observed values of a measurement (such as glucose levels) which vary around a true mean. Mean glucose levels of individual patients appeared to be static for the duration of the study.

Regression to the mean may give the impression that glucose levels are normalising when all that is being witnessed is actually just a statistical phenomenon. The authors believe that the mean glucose level is more important than actual glucose levels at any one time. They also noted that serial glucose measurements are higher in patients with more severe stroke.

The hypothesis put forward by Wong *et al* is interesting but it is important to note that they excluded patients with known diabetes from their study and they did not attempt to define glycaemic status in study participants. The study size of 124 is also relatively small.

1.6.9 How should we treat post-stroke hyperglycemia?

There is no consensus on the treatment of post-stroke hyperglycemia. The academic community are not really sure that any treatment is indicated. Clinical guidelines make various suggestions for the initiation of hypoglycaemic treatment but these guidelines are not evidence based.[293, 294]

In a 2008 paper Casaubon and colleagues surveyed 278 physicians involved in the clinical care of stroke patients.[295] The results of the survey reflected the therapeutic uncertainty of clinicians with a great deal of variability in clinical practice. Intensive care physicians treat hyperglycemia in stroke patients most aggressively while emergency care physicians were the most conservative group.

Insulin treatment has inherent risks. In a letter to the journal Critical Care Medicine in 2005 Strong and colleagues suggest that lowering blood glucose with insulin may be associated with an increased risk of brain injury. They observed the 'complete disappearance' of glucose in the brain dialsyate in a traumatic brain injury patient treated with insulin. Strong et al suggest that this evidence, in conjunction with evidence that spontaneous depolarization events are related to low extracellular glucose levels and poor outcomes, should encourage clinicians to avoid hypoglycaemia in acute stroke and brain injury patients.[296, 297] A safe 'lower limit' for blood glucose in acute stroke patients may exist.

One retrospective observational study of 960 patients with thromboembolic stroke suggested that post stroke hyperglycemia (glucose >7.2mmol/L) was associated with a higher mortality rate than normoglycemia (OR 3.15, 95%CI 1.45 to 6.85; $p = 0.004$).[269] In this cohort persistent PSH over 48 hours had an even higher risk of death (OR = 6.54; 95% CI = 2.41 to 17.87; $p < 0.001$). Patients with initial hyperglycemia that was later normalised had lower mortality than those with persistent PSH (OR = 0.22; 95% CI = 0.05 to 0.96; $p < 0.05$). These results suggest that there may be some benefit in normalising blood glucose levels after stroke.

A recent randomised control study on the use of glucose, potassium and insulin infusions to control post-stroke hyperglycaemia was negative. This may be because the mean onset of therapy was 12 hours post stroke.[144] Further pilot studies of the use of intensive insulin therapy for post-stroke hyperglycemia have been completed and larger randomised control trials may be carried out in the future.[259, 283, 298]

In a post-hoc analysis of the GLIAS observational study Fuentes and colleagues assessed the treatment of persistent post-stroke hyperglycemia.[213] In this cohort of 476 patients, 291 received treatment for hyperglycemia while 117 were identified as having persistent post stroke hyperglycemia defined as 2 or more blood glucose readings above 155mg/dl. In 114 patients hyperglycemia persisted despite treatment.

Persistent hyperglycemia was an independent predictor of poor outcome in this study but given the high level of treatment for hyperglycemia it is possible that the intervention may have had some negative effects on the patients. In this study up to 97.5% of patients with persistent post-stroke hyperglycemia were treated with no clear benefit. The authors conclude that better protocols are required for the management of PSH but as yet there is no good evidence that lowering blood glucose is beneficial. Indeed if the data from the ASTRAL study are correct hypoglycaemia may be as harmful as hyperglycemia.[173]

Data from the retrospective study by Gentile and colleagues did suggest that treatment for PSH resulted in lower rates of mortality but evidence from published clinical trials does not currently support this.[246, 269, 299]

Intensive insulin protocols have been used in several clinical environments with variable results.[300-304] Patients on these protocols appear to be more likely to become hypoglycaemic according to the results of meta-analyses.[305, 306] At least one trial has been stopped early due to safety concerns about hypoglycaemia.[307]

Other, less acute studies such as the Insulin Resistance Intervention After Stroke (IRIS) trial are ongoing. IRIS is a randomised control trial treating patients with pioglitazone or placebo if they have biochemical evidence of insulin resistance. Pioglitazone is a thiaziolidnedione or ‘insulin sensitizer’ which is used in the treatment of Type II Diabetes Mellitus. Pioglitazone reduces blood glucose and insulin levels while increasing HDL-cholesterol. The PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) study identified an apparent reduction in recurrent strokes in patients with Type II

DM who were treated with pioglitazone.[308, 309] The IRIS trial hopes to replicate this beneficial effect in patients with insulin resistance many of whom may demonstrate post-stroke hyperglycaemia. As I have discussed earlier, insulin resistance has been reported in up to 50% of patients in a preliminary observational study by some of the IRIS investigators.[48] Further information on the IRIS trial can be found at the study website (http://iristrial.org/index.html).

Several animal models of post-stroke hyperglycemia have used insulin to reduce the size of experimentally induced infarcts. Unfortunately these studies are highly heterogeneous with varying results.[310-312] I discuss the limitations of the available evidence in chapter 3.

1.7 Pre-clinical science and post-stroke hyperglycaemia

1.7.1 Animal models of post-stroke hyperglycemia

Many of our theories about the pathophysiology of post-stroke hyperglycemia and potential treatments for PSH are based on animal models. Animal models of ischaemic stroke are useful because smaller observational human studies cannot easily control for the severity of the initial stroke.

While many studies suggest that hyperglycemia increases infarct growth in animal models of stroke others suggest that high blood glucose may be beneficial.[313-319]

Heterogeneity is a major problem in the use of animal models for PSH. These studies involve different strains and species of animal model. The method of induction of hyperglycemia may also be problematic as many experiments use streptozotocin which produces a model of type 1 diabetes mellitus that may no be relevant to most human cases of PSH.

I have conducted a systematic review of models of focal cerebral ischemia and hyperglycemia and this is included in Chapter 3.

1.7.2 Glucose metabolism in the normal brain

In a healthy individual the brain consumes 20-25% of total body glucose. The brain relies on a constant supply of blood glucose to function normally. Brain tissue requires 5mg of glucose per 100mg per minute to function normally. This works out as a requirement of approximately 140g of glucose per day. Glucose requirements are higher in more

metabolically active areas of the brain such as the grey matter.[320] In hypoglycaemic conditions the brain can function with glycogen.[321] Glycogen is converted into lactate which is then used as energy by the brain.

1.7.3 'The 'glucose paradox' of cerebral ischaemia' - Why does hyperglycemia worsen cerebral ischemia?

Glucose is thought to be the main source of energy for the brain. It had been suggested that no other substrate could enter the brain in large enough levels to meet cerebral energy requirements.[322] More recently it has been observed that the healthy brain can also use lactate for energy.[323]

The 'glucose paradox' of cerebral ischaemia refers to the observed phenomenon that pre-ischaemic hyperglycaemia aggravates post-ischaemic outcome.[324] This was initially described in 1977 by Myers and Yamaguchi who noticed that administration of glucose immediately before cardiac arrest results in more widespread neurological injury.[325]

The mechanism of this glucose related neurological injury is uncertain. Lactic acidosis has been suggested as one probable mechanism. Other hypotheses include an increased release of glucocorticoids, beta-amyloid precursor proteins or increased superoxide and nitric oxide production.[326]

1.7.4 Lactic acidosis

The lactic acidosis hypothesis is that pre-ischaemic hyperglycaemia results in elevated lactic acid levels during ischaemia and increases ischaemic brain damage. This situation is described as a paradox because the brain requires glucose to function normally. Why should glucose, the normal fuel of brain cells, cause damage under ischaemic circumstances?

Ischaemic or anaerobic energy metabolism is less energy efficient and produces lactate and acidic free hydrogen ions. Under anaerobic conditions, such as cerebral ischemia, glycolysis is the only process capable of producing enough adenosine triphosphate (ATP) to meet the energy requirements of the brain. Unless glucose is available in large quantities glycolysis could not last for more than a few minutes. As glucose is the sole substrate of

the glycolytic pathway, higher glucose levels mean that glycolytic ATP is produced for a longer period. Lactate is the main product of glycolysis. Persistent anaerobic metabolism results in intracellular acidosis.

At the end of an ischemic episode, and upon reperfusion/re-oxygenation, aerobic conditions (and often a shortage of glucose) exist. Under these circumstances lactate can easily enter the tricarboxylic acid cycle via pyruvate to maintain ATP production as efficiently as glucose. The `Pasteur Effect' phenomenon, the significant increase in glucose consumption that occurs upon the exhaustion of oxygen supplies is the regulatory mechanism responsible for lactate production. This process, which supplies ATP to oxygen-deprived tissue, has been thought to cause the demise of that very tissue under ischemic conditions.

It has been assumed that lactic acidosis is harmful to neurons although this had not been conclusively proven.[327] Schurr and colleagues noted that there is some discordance between in vivo and in vitro models of hyperglycemic cerebral ischemia.[324, 327]

For some time researchers using in vitro models were unable to reproduce the aggravation of cerebral ischemia by pre-ischaemic hyperglycemia (hyperglycemia actually appears to be neuroprotective in some models)[328-330]. These in vitro studies model global cerebral ischemia (i.e. cardiac arrest) suggesting that the neurotoxic effects of lactic acidosis may be mediated by the cerebrovascular blood supply

However one in vitro study suggests that glucose in combination with acidosis is harmful to neuronal cells while lactate does not have the same effect.[331] Cronberg and colleagues used a murine hippocampal slice model of in vitro ischemia. The synergistic mechanism by which glucose and acidosis combine to cause neuronal damage is still unknown.

In a 2009 paper Berthet and colleagues suggested that lactate may actually be neuroprotective after focal cerebral ischemia.[332] In an in vitro experiment they found that lactate protected against neuronal death when cells were deprived of oxygen and glucose. A subsequent in vivo experiment found that an injection of lactate into the cerebral ventricles at the time of reperfusion after middle cerebral artery occlusion resulted in a significant decrease in infarct size. When lactate was given 1 hour after reperfusion it did not reduce infarct size but neurological outcomes improved.

In animal experiments it has been observed that during focal cerebral ischaemia that the ischaemic penumbra exhibits heterogeneous development of intracellular cortical acidosis. In a 1999 paper Anderson and colleagues attempted to test two hypotheses; firstly they wanted to confirm that this acidosis was due to glucose utilization and secondly they wanted to confirm that intracellular acidosis leads to infarction of potentially salvageable tissue.[333] Experimental ischaemia was induced in rabbits using a 3-vessel occlusion model and hyperglycemia was produced using an intravenous dextrose solution. In vivo fluorescent imaging was used to assess brain pH, regional cortical blood flow and NADH. Final infarct volume was measured pathologically.

Anderson et al observed that pre-ischaemic hyperglycemia resulted in more pronounced intracellular acidosis and reduced NADH regeneration. They conclude that hyperglycemia worsens intracellular brain acidosis and mitochondrial function in the ischaemic penumbra supporting the hypothesis that the evolution of acidosis in the penumbra is related to glucose metabolism.

1.7.5 Other mechanisms by which hyperglycemia may exacerbate ischaemic brain damage

If lactate metabolism is beneficial it has been suggested that the harmful effects of hyperglycaemia may be mediated by steroids.[324] It has been hypothesised that hyperglycemia (or glucose loading) results in a transient increase in the release of glucocorticoids resulting in a worse outcome. In a rat model of global cerebral ischemia Schurr and colleagues used metyrapone, a corticosterone synthesis inhibitor, to reduce steroid levels. A significantly lower level of delayed neuronal damage was seen in these animals.

In a global ischemia model hyperglycemia has been seen to induce early intraneuronal expression of β-amyloid precursor protein. Rats that were exposed to dextrose at the time of ischemia had very widespread β-amyloid precursor immunoreactivity while normoglycemic rats only had very weak staining. It has been suggested that β-amyloid precursor protein or its metabolites may be involved in the process of ischaemic brain injury.[334]

In a rat model of focal cerebral ischemia Ste-Marie and colleagues found that hyperglycemia at the time of ischemia resulted in an early rise in superoxide and nitric

oxide production. This may in turn lead to an increase in production of hydroxyl radical formation leading to neuronal cell damage.[335]

1.7.6 Imaging biomarkers for brain metabolism

While data from animal models may suggest possible mechanisms by which hyperglycemia may exacerbate the ischemic brain damage of human stroke such studies will never give us definitive answers. Human studies will never give us tissue for histological data so imaging biomarkers of brain metabolism have been used in an attempt to elucidate the situation.

The 2003 paper by Smith et al examined cerebral metabolism using FDG-PET (18fluorodeoxyglucose positron emission tomography) scanning and suggested that lactate may be a preferred substrate in the healthy human brain.[323] In healthy human subjects who were infused with lactate whilst undergoing the FDG-PET scan. Whole brain glucose uptake was significantly reduced by approximately 17% during lactate infusion suggesting that the euglycaemic brain may preferentially metabolise lactate if available.

In a 2002 paper Parsons and colleagues used magnetic resonance spectroscopy to explore the relationship between hyperglycemia, lactic acidosis and clinical outcome in human acute stroke patients.[176] MR spectroscopy was used in 33 patients to assess the relationship between acute blood glucose levels and lactate production in ischaemic areas of brain. All of the patients initially had MRI assessment of perfusion-diffusion mismatch to estimate penumbral volume. Higher acute blood glucose levels in patients with perfusion-diffusion mismatch were associated with greater sub-acute lactate production and reduced salvage of mismatch tissue. In patients with no evidence of mismatch there was no correlation of blood glucose with outcome measures and no evidence of lactate production. The authors concluded that acute hyperglycemia increases brain lactate production and facilitates the infarction of penumbral tissue.

McCormick and colleagues subsequently undertook a randomised placebo controlled trial of a GKI (glucose, potassium and insulin) infusion to control blood glucose levels in acute stroke patients.[336] Infarct growth was assessed between admission and the seventh day after the stroke. MR spectroscopy was used to assess lactate production. The use of the GKI infusion was found to reduce blood glucose levels and brain lactate levels but had no effect on infarct growth.

The work of McCormick and colleagues suggests that while hyperglycemia may be related to lactate levels, increased lactate levels are not the only factor that contributes to infarct growth. These results may correlate with the results of Cronbergs' in vitro work.[331]

It may be interesting to use MR spectroscopy to monitor pH changes in relation to hyperglycemia in acute stroke patients although this would be a complex imaging process.

There are limitations to MR spectroscopy techniques that should be considered. MR spectroscopy does not directly quantify lactate levels and instead expresses a lactate:creatine or lactate:choline ratio.[337] It is of course possible that all of the brain metabolites used for MR spectroscopy are altered during stroke possibly skewing results. For practical reasons the MR spectroscopy voxel was placed in the DWI ('core') lesion as opposed to the mismatch ('penumbral') region in both studies. It is possible that lactate may have behaved differently in the mismatch region.

At present there is no conclusive solution to the 'Glucose paradox'.

1.7.7 Positron Emission Tomography studies on PSH

In a 1990 paper Kushner and colleagues used positron emission tomography (PET) scanning to look at the relationship between hyperglycemia within 12 hours of stroke onset, structural brain damage assessed using CT and brain metabolic disruption.[338] They were able to study 39 patients who had an appropriate blood glucose measurement. Patients had PET imaging within 7 days of stroke onset. In this study hyperglycemia was defined as a blood glucose level of above 6.7mmol. For the PET scanning 18F-fluorodeoxyglucose (FDG) was used to delineate cerebral metabolism.

In patients with low initial blood glucose levels PET imaging was normal or only slightly abnormal. In patients with high blood glucose levels a greater degree of metabolic abnormality was observed. In both qualitative and quantitative analysis of PET images hyperglycemia was associated with either lobar or multi-lobar hypometabolism. There was no statistically significant relationship between diabetes and abnormal metabolism.

In a 1993 study Heiss and colleagues looked the relationship between cerebral glucose metabolism as assessed by PET scanning and functional outcome.[339] Patients with diabetes were excluded from this study as diabetes may have a confounding effect on cerebral glucose transport and metabolism. Sixty-six patients were prospectively recruited

and scanned at an average of 9.2 days after stroke. Patients were followed up for an average of 50.5 months to assess final functional outcome. Good global, ipsilateral and contralateral glucose metabolism were significantly related to better final functional outcomes (P= 0.001). Multiple regression analysis revealed that cerebral glucose metabolism correlated more with functional outcome in hypertensive patients (P=0.016).

1.7.8 The effect of hyperglycemia on haemostasis

It has been suggested that hyperglycemia may have a pathological effect on haemostasis. Fibrinogen is associated with primary haemostasis and increased fibrinogen levels are associated with atherosclerosis and incident vascular events.[340-343] Permeability of the fibrin clot may be reduced in hyperglycaemic or diabetic patients.[344-346] Fibrin clots formed by diabetic patients have a denser, less porous structure than those of control subjects. Similar changes are seen in vitro and may explain the reduced efficacy of tissue plasminogen activator in hyperglycaemic patients.[347, 348] These structural changes may be related to poor glycaemic control and exposure to high levels of blood glucose.[349] Improving glycaemic control may reduce the cardiovascular risk associated with fibrinogen.[350]

Ozkul and colleagues looked at the interaction between insulin resistance and coagulation in the acute phase of ischaemic stroke.[351] Patients were prospectively identified after first ischaemic stroke. Patients with diabetes or other pre-existing conditions that may interfere with coagulation were excluded. They found that Protein C and Protein S levels were significantly lower in insulin resistant patients and that levels of these anticoagulants correlated with HOMA levels. A negative correlation was observed between NIHSS and Protein S levels. The authors suggest that the significant associations between insulin resistance and haemostatic markers may worsen stroke severity by creating a procoagulant state.

Lindahl et al reported an association between insulin resistance and low fibrinolytic activity in a sub-study of patients enrolled in the Swedish MONICA (MONItoring of trends and determinants in CArdiovascular diseases) study.[352] They investigated 756 patients by checking fasting insulin and glucose levels as well as PAI-1 activity and tPA activity. Subjects within the highest tertile of insulin resistance had higher PAI-1 activity and lower tPA activity.

Evidence of a relationship between insulin resistance and impaired haemostasis has also been seen in the Framingham Offspring Cohort.[353] Insulin levels and haemostatic factors were measured in 2962 study participants. Mean levels of haemostatic factors were observed to increase with insulin levels. Levels of both PAI-1 and tPA antigens were significantly higher in patients with highest insulin levels. The authors suggest that patients with insulin resistance may be at an increased risk of acute thrombotic disease.

1.7.9 Vascular effects of hyperglycemia

Martini and Kent reviewed the vascular effects of hyperglycemia in 2007 and concluded that hyperglycemia results in a pro-vasoconstrictive, pro-thrombotic and pro-inflammatory phenotype which may make the cerebral vasculature vulnerable to reperfusion injury.[354] Martini and Kent believe that the vascular injury that results from hyperglycemia is one of the main factors that limits the utility of other interventions that aim to provide neuroprotection

Hyperglycemia is known to induce various biochemical changes within endothelial cells.[355] The GLUT-1 transporter moves glucose from the blood stream into cells.[356] In vitro studies suggest that the GLUT-1 transporter in vascular endothelial cells is not insulin sensitive so hyperglycemia in the blood stream results in intracellular hyperglycemia.[357] Intracellular hyperglycemia is thought to result in many of the biological abnormalities seen in diabetes. Several biochemical pathways are activated by intracellular hyperglycemia.[358] Hyperglycemia seems to produce an increase in reactive oxygen species (ROS). Reactive oxygen species cause DNA damage resulting in poly(ADP-ribose) polymerase (PARP) activation which in turn decreases the activity of the key glycolytic enzyme glyceraldehyde-3 phosphate dehydrogenase (GAPDH). Inhibition of GAPDH activates 4 pathways that damage cells.[355, 358]

Hyperglycemia does not appear to be the major determinant of macrovascular disease in diabetes mellitus.[358] Macrovascular disease may be more linked to other features of diabetes such as insulin resistance and subsequent free fatty acid deposition in endothelial cells.

1.7.10 Effect of blood glucose on fibrinolysis

Type 2 diabetes mellitus is thought to be associated with abnormalities of haemostatic factors in the circulation. The fibrinolysis inhibitor plasminogen activator inhibitor-1

(PAI-1) appears to be closely related to insulin resistance.[359-361] Mansfield and colleagues attempted to investigate the relationship between PAI-1 levels and stroke in diabetic and non-diabetic patients.[362] Mansfield *et al* performed a case control study where 4 cohorts of patients were recruited: 40 diabetic patients with stroke, 80 diabetic patients without stroke, 80 stroke patients without diabetes and 40 controls without stroke or diabetes. In this population PAI-1 levels were highest in diabetic patients without stroke. In this subgroup levels were significantly higher than in either stroke patients without diabetes or normal controls ($P<0.0005$). Circulation tissue plasminogen antigen activator levels were also checked. Levels of t-PA antigen were lower in patients with diabetes who had experienced a stroke when compared with diabetic controls without a stroke. The authors conclude that these results do not support a hypothesised relationship between impaired fibrinolytic function and incident ischaemic stroke in patients with type 2 diabetes mellitus. This conclusion may be wrong if antigen levels change acutely after stroke although at least one study suggests this is not the case.[363] The authors admit that patients with the highest PAI-1 levels may be under-represented in this study if they die soon after stroke.

In 1993 Nordt and colleagues reported in vitro studies where they attempted to determine if PAI-1 secretion is dependent on glucose levels.[364] A significant increase in the secretion of PAI-1 was observed as plasma glucose levels increased. This effect of glucose on the synthesis of PAI-1 by endothelial cells may contribute to reduced local fibrinolysis.

In human studies patients with type 2 diabetes have been found to have significantly higher t-PA antigen and PAI-1 levels than normal controls.[365] Correlations have been found between PAI-1 levels, insulin levels, body mass index and apolipoprotein B although only the correlation between PAI-1 and insulin remained after adjustment.[361] Similar correlations have been observed in obese patients.[359] A small study compared tissue specimens from the mammary arteries of diabetic and non-diabetic patients who were undergoing coronary artery bypass grafting.[366] PAI-1 immunofluoresence was increased in the arterial wall of diabetic patients. An animal study published by the same group suggests that acute hyperglycemia and acute hyperinsulinaemia decrease plasma fibrinolytic activity and increase PAI-1 activity.[367]

Historically reduced reperfusion rates have been seen in diabetic patients who have been thrombolysed for myocardial infarction.[368] Abnormal platelet aggregation in diabetes may also partially explain poorer outcomes from thrombolysis in these patients.[369-371]

1.8 Imaging techniques in acute stroke

Brain imaging is very important in the clinical diagnosis and management of patients with stroke. Brain imaging is also very important in stroke research. There has been some debate about the best form of imaging in acute stroke patients.[372] Clinical scoring systems to predict the cause of a stroke are unreliable.[373]

1.8.1 Plain CT

Non-contrast computerised tomography (NCCT/'plain' CT) was first used by neuroradiologists over 35 years ago.[374] CT was soon used in the diagnosis of stroke.[375] CT imaging was correlated with post-mortem data.[376]

All suspected stroke patients should have a CT brain as soon as possible after admission.[377] Haemorrhage is instantly visible on CT.[378]

Early changes can be seen on CT in ischaemic stroke very soon after presentation.[379-382]

There has been some debate about the value of these changes but they do appear to be clinically useful.[383] Scoring systems such as the ASPECTS score are available to help clinicians predict clinical outcome based on early CT appearances.[384] It is also possible in some cases to see a 'hyperdense' middle cerebral artery on a plain CT scan which can represent arterial occlusion.[385, 386]

As time progresses an infarct becomes more clearly demarcated and hypoattenuated on CT after a few days.[387] Visible infarction on CT is associated with a poor functional outcome.[388]

1.8.2 CT angiography

CT angiography (CTA) has been used to evaluate intracranial arterial anatomy and has been compared with conventional angiography and MR angiography.[389] CTA can add useful information to non-contrast CT in acute ischaemic stroke. It can reveal the location and size of an occluded arterial segment as well as allowing evaluation of collateral blood flow.[390]

In the era of cerebral revascularisation it is important to be able to clearly define the degree of revascularisation in research studies. There are two distinct aspects to revascularisation

– recanalization and reperfusion. Recanalization implies that the primary arterial occlusive lesion (AOL) has been destroyed opening the original artery. In recanalization it is still possible that clot fragments have embolised to distal arteries causing further occlusion. Reperfusion implies that distal arteries are patent. It is possible for distal arteries to be reperfused while the proximal occlusive lesion has only been partially removed.

Various scoring methods have been developed to define the degree of recanalization and reperfusion after revascularization. The AOL recanalization score has been developed in interventional neuroradiology stroke trials.[391] The AOL score is detailed in table 1.4. This score was initially defined using conventional cerebral angiography but it can be applied to CT cerebral angiography.

Table 1-4 The AOL recanalization score

Score	AOL Recanalization
0	No recanalization of the primary occlusive lesion
I	Incomplete or partial recanalization of the primary occlusive lesion with no distal flow
II	Incomplete or partial recanalization of the primary occlusive lesion with any distal flow
III	Complete recanalization of the primary occlusion with any distal flow

Reperfusion has been described in interventional stroke trials using the TIMI (thrombolysis in myocardial infarction) score.[392] The TIMI score is detailed in Table 1.5. The TIMI score was developed using conventional angiography and is not intended for use with CT angiography.

Table 1-5 The TIMI (Thrombolysis in Myocardial Infarction) score

Score	TIMI reperfusion
0	No perfusion
1	Perfusion past the initial occlusion but no distal branch filling
2	Perfusion with incomplete or slow distal branch filling
3	Full perfusion with filling of all distal branches including M3, 4

CT angiography requires administration of intravenous contrast which has been associated with worsening renal failure. Current Royal College of Radiologists guidelines advise caution in the use of IV contrast although there has been some debate as to the real level of risk involved.[393] This relative contraindication can limit use of CT angiography and perfusion CT in some patients.

There has been some concern that iodinated contrast may inhibit fibrinolysis in acute stroke however a recent systematic review does not suggest that this is a serious problem.[394]

1.8.3 Perfusion CT

Imaging techniques that allow us to quantify cerebral blood flow may be valuable clinically. Such techniques could define the infarcted core tissue of a stroke and the ischaemic penumbra. This knowledge may make thrombolysis decisions easier.

Imaging methods such as positron emission tomography (PET), single photon emission computed tomography (SPECT), magnetic resonance (MR) imaging and stable xenon CT have been used to measure cerebral blood flow (CBF) and cerebral blood volume (CBV) for research purposes. These techniques are expensive and often not practical for many clinical uses.

Previous research has shown that stable xenon CT gives a quantitative and accurate map of cerebral blood flow.[395] Perfusion CT studies and xenon CT studies have been shown to provide similar estimates of regional cerebral blood flow allowing some validation of perfusion CT techniques.[396] Earlier research had validated the use of CT perfusion for the assessment of cerebral blood flow in animals.[397] Perfusion CT has also been found to have the potential to provide similar perfusion maps to PET in some studies.[398]

Perfusion CT uses contrast dye as an intravascular tracer with the CT scanner detecting brain blood flow. Tracking the contrast bolus allows measurement of CBF and CBV as well as meant transit time (MTT) and time to peak (TTP) of contrast. The change in signal intensity in Hounsfield units is proportional to the concentration of the dye in the pixel imaged. Repeated scanning allows the creation of pixel concentration against time curves which can be used to generate the different perfusion maps.[396, 399] It is thought that a prolonged MTT suggests penumbral tissue while a reduced CBV represents core infarcted tissue.[400]

Clinically, CT perfusion has been found to be more accurate at predicting final outcome than plain CT when using the ASPECTS score.[401] An example of CT perfusion imaging can be seen in Figure 3

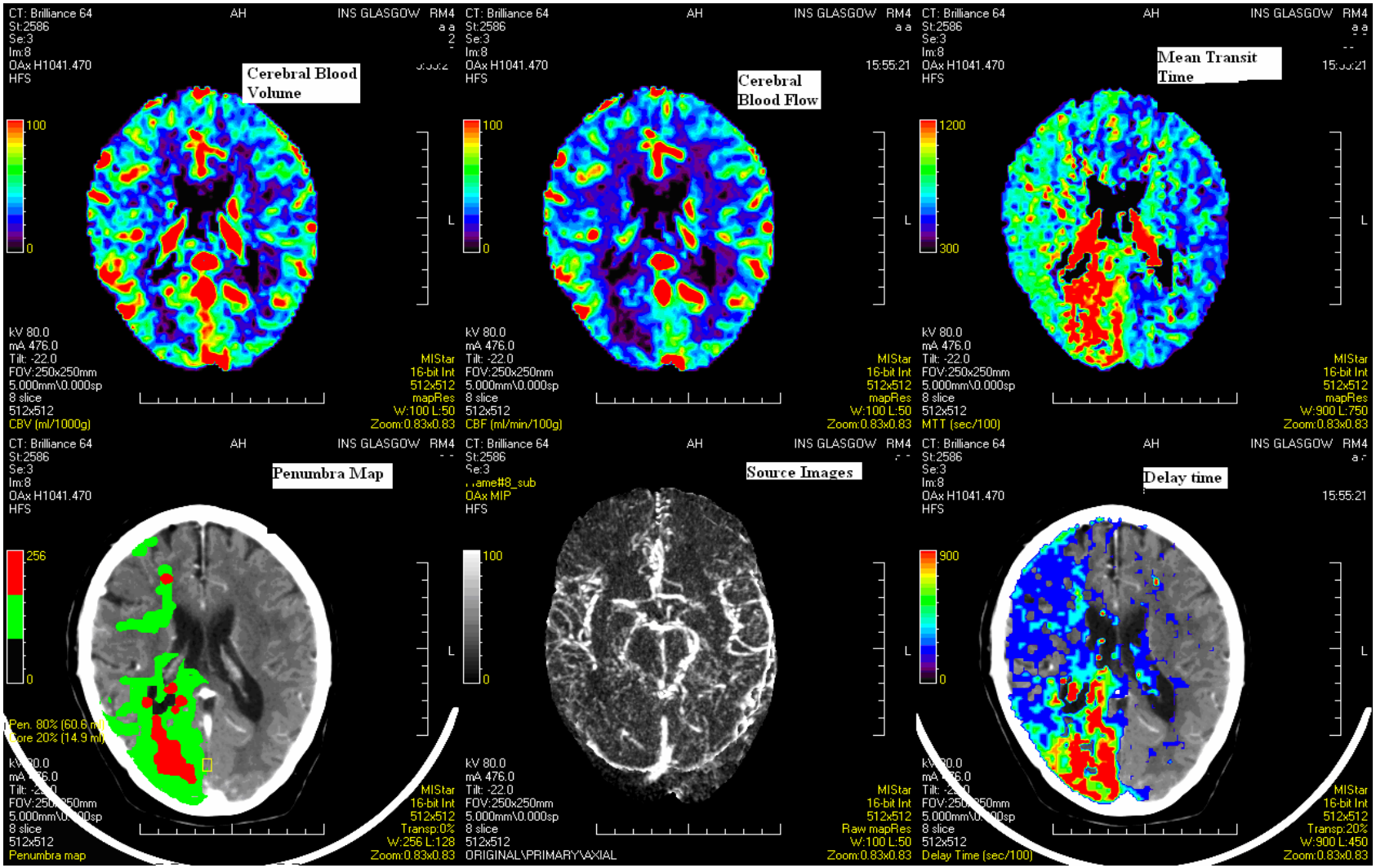


Figure 3 - CT perfusion images from the POSH study

1.8.4 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is another commonly used imaging modality for stroke. An MRI machine consists of a magnet, magnetic gradient coils, a radio frequency transmitter and receiver, and a computer that controls the acquisition of signals and computes the MR images.

MRI produces images by utilizing the properties of atomic nuclei, principally those of hydrogen in water molecules. A high intensity magnetic field forces the nuclei to align before a radio-frequency pulse displaces the nuclei from their position. When the pulse stops the nuclei return to their original state, releasing energy in the form of a radio frequency signal. A computer then analysis this 'resonance' signal and converts it into a grey scale image.[402] Imaging can be weighted in different ways to better visualise different tissue types.

MRI lets us examine the response of magnetised tissue to a radio frequency pulse and abnormal tissue will return abnormal signals.

MRI will produce good quality brain imaging but scanning times are longer than for CT and many patients cannot enter the scanner due to metal implants or pacemakers. Monitoring critically ill patients can be difficult in an MRI scanner and CT is often quicker and safer.[1]

1.8.5 MRI Diffusion Weighted Imaging

Acute ischaemic stroke results in cytotoxic oedema. Diffusion weighted imaging looks at the rate if diffusion of water through tissue Healthy cells will only let water diffuse in certain ways. Cytotoxic oedema will alter the diffusion of water through the cell. This will result in a high diffusion weighted signal that will make even very small strokes easy today in the first 5-10 days after onset.[403]

1.8.6 Transcranial Doppler Ultrasound

Transcranial Doppler (TCD) ultrasound is a non-invasive, portable tool that can be used to evaluate blood flow in cerebral blood vessels. Studies have shown that transcranial Doppler findings predict angiographic findings.[404] The TIBI score has been developed to grade flow in cerebral blood vessels.[405] The technique is operator dependant and requires training and experience to perform and interpret results.

TCD has been validated against CT angiography with a sensitivity of 79.1% and a specificity of 94.3%. TCD can also give useful information about embolisation and arterial blood flow that is unavailable from CT anfiography.[406] Power Motion Doppler (PMD) TCD was also compared to CT angiography in another prospective observational study of 100 patients within 24 hours of acute stroke.[407] PMD-TCD had 95.6% sensitivity and 94.5% specificity for middle cerebral artery occlusion when compared with CT angiography as a gold standard.

TCD is commonly used to screen children with sickle cell disease for stroke risk. It is also used after spontaneous subarachnoid haemorrhage to monitor for angiographic vasospasm. TCD can be used for perioperative monitoring during carotid endartectomy and coronary artery bypass grafting.[408] TCD is also very reliable when used in the detection of patent foramen ovale (PFO).[409]

Some studies have looked at the use of TCD for sonothrombolysis to augment alteplase[410, 411]. The initial CLOTBUST (Combined Lysis of Thrombus in Brain Ischemia Using Transcranial Ultrasound and Systemic t-PA) trial suggested a non-significant trend towards increased arterial recanalization with continuous TCD exposure. There was also a non-significant trend towards an increased rate of recovery with TCD compared with placebo.

The subsequent TUCSON (Transcranial Ultrasound in Clinical SONothrombolysis) trial looked at the use of intravenous microspheres to augment TCD sonothrombolysis and improve recanalization rates. The trial was a randomised, single blinded dose escalation study aiming to find a safe dose of microspheres. Unfortunately the study was stopped prematurely due to an excess of intracerebral haemorrhage with the first microsphere dose increase.[412] The trial was stopped at a very early stage by the sponsor and the numbers recruited were very small. Three of 11 patients in the higher dose group had symptomatic intracerebral haemorrhage but in such a small cohort such numbers could be entirely due to chance.[413]

1.8.7 Arterial patency in acute stroke and early neurological deterioration after thrombolysis

As described above arterial patency can be assessed in acute stroke using CTA and TCD. There is interest in investigating the patency of blood vessels after stroke thrombolysis as it is possible that some arteries may re-occlude.

In the initial NINDS stroke thrombolysis trial 13% of patients showed an early clinical deterioration after initial improvement.[414] This group of patients has a poorer long term prognosis.

A proportion of the patients who had early neurological deterioration (END) would have had intracerebral haemorrhage. Some patients may have malignant cerebral infarction which can be treated with decompressive craniotomy.[415-417] Other patients may have early neurological deterioration due to haemodynamic factors.[418] Some patients appear to have a further ischaemic stroke in a different arterial territory due to atrial fibrillation.[419] .

Arterial reocclusion after initial recanalization has been observed using TCD.[420] In one observational study of a consecutive series of 60 patients treated with t-PA were monitored using TCD. Forty-seven patients were seen to recanalize after thrombolytic therapy. Sixteen patients (34%) were seen to reocclude after initial recanalization. Mortality was higher in the group of patients with reocclusion (33%) than in the group with stable recanalization (8%). This difference was significant with a p value of <0.05.

As mentioned previously, there appears to be a relationship between early recanalization in the presence of hyperglycaemia and a poor clinical outcome.[180, 197, 198, 271]

In one prospective observational study looking at acute deterioration after recanalization (with IV t-PA or an intra-arterial procedure) there was a strong correlation between hyperglycaemia and a poor outcome.[198] Acute deterioration was defined as an increase in NIHSS of 4 or more points at 24 hours. Of 201 patients treated within 6 hours of symptom onset 13% worsened, 39% improved and 48% were unchanged at 24 hours. Hyperglycaemia, defined as blood glucose above 150mg/dl (8.3mmol/l), was more severe in patients who deteriorated even if an occluded vessel recanalized. Hyperglycaemia was associated with an odds ratio of 6.47 for a poor outcome ($P= 0.004$).

Similar results were seen in a series of papers from a Spanish centre.[180, 197, 271]

1.9 Clinical assessment scales and stroke research

In both clinical practice and research clinical assessment scales are useful to record neurological functional deficits. Such scales allow clinicians to communicate stroke severity and help guide clinical decision making. In research these scales can be used to gauge homogeneity of a study population and to assess patient outcomes. It is important that scales should be valid, reliable and easy to use[421]. A valid test accurately describes a phenomenon or disease. A reliable scale is reproducible between users and consistent for scale items.[422] A good scale should be reliable on an interobserver and intraobserver basis.

There are three main types of stroke assessment scale. Global outcome scales assign patients to broad categories. They are simple and easy to use but often not reproducible. They may be insensitive.[422] The modified Rankin Scale is an example[171, 423]. Physical deficit scales describe stroke related deficit based on neurological examination. The National Institute of Health Stroke Scale is a physical deficit scale.[422, 424] Activities of daily living (ADL) scales measure function needed for independent living. They score basic biological functions such as continence and more complex tasks like food preparation.[422] The Barthel Index is an ADL scale.[425]

1.9.1 The National Institute of Health Stroke Scale

The National Institute of Health Stroke Scale (NIHSS) is used both clinically and for research purposes. It is used to guide stroke thrombolysis decisions.[35] It was initially published in 1989 to clinically measure the severity of an acute stroke for therapeutic trials. It was based on three earlier scales[424] The NIHSS initially had a good positive predictive value for imaging outcomes and final outcomes.[426] When independent observers compared it with other neurological scoring scales it was found to have the best predictive value.[427] The initial scale had moderate to substantial interobserver agreement.[428]

The NIHSS scale was later modified to simplify its use while improving reliability and sensitivity.[429] Training in the use of the NIHSS is straightforward and can be done using various audiovisual materials, some of which are available on the internet.[430] The NIHSS scale is now used in most acute stroke trials.

2 Materials and methods

2.1 Introduction

This chapter provides a detailed description of the patients and general methods used in the clinical studies presented in this thesis. Methodology and endpoints that are only relevant to a single chapter are described in that chapter.

The main study was entitled Post Stroke Hyperglycemia and Brain Arterial Patency. The study was referred to by the acronym POSH which I will use later in the text.

2.2 Patient recruitment

Study participants were prospectively recruited from patients referred to the Acute Stroke Service at the Southern General Hospital in Glasgow between the 1st of January 2009 and the 31st of December 2011. All patients who presented to the unit within six hours of an acute stroke were considered for the study. While the majority of patients included in the study were domiciled in the immediate Glasgow area some patients were referred from the wider West of Scotland region.

The study was designed to prospectively recruit 100 acute stroke patients within 6 hours of stroke onset. We aimed to define the interaction of early and delayed hyperglycaemia with arterial patency and brain perfusion in these patients.

2.3 Exclusion Criteria

Exclusion criteria included a non-stroke diagnosis (e.g. primary intracerebral haemorrhage, tumour, subarachnoid haemorrhage or epilepsy), known sensitivity to iodinated contrast materials (previous contrast reactions or severe asthma), an inability to lie flat for the duration of additional imaging (e.g. severe cardiac failure, desaturation on lying flat, high risk of aspiration) and any intercurrent illness that was likely to limit survival to less than 30 days. Patients with severe renal failure were excluded as they could not have iodinated contrast material. Pregnant women were not approached for the study to avoid radiation exposure.

Patients enrolled in the study were treated with alteplase if appropriate. In some cases patients were enrolled in a concurrent clinical trial of thrombolytic therapy and randomised

for treatment with desmotoplase or placebo. Age, stroke severity and past history of dysglycaemia are not exclusion criteria for this study.

Informed consent for the study was sought where possible. If a patient was unable to consent due to their neurological condition informed assent was obtained from the next-of-kin.

Patient data were collected on admission using a specifically designed case report form. This form was designed to record information regarding the past medical history of the patient and to prospectively record clinical data during the study period.

2.4 Ethical approval

Ethical approval was granted for this study by the appropriate NHS Research Ethics Committee.

2.5 Timetable of Study Procedures

Before the trial began a timetable of the study protocol was established. Many of the data recorded were generated by routine clinical practice. The additional procedures that were study specific are detailed in Table 2.1. A flowchart of the study is included in Figure 4.

Table 2-1 Timetable of POSH study procedures

Procedure	Baseline (<6h)	6-24h	24-48h	72h	Day 7	Day 30
Informed consent or assent from next of kin	✓					
Non-contrast CT brain	×		*			
CT Perfusion	*					
CT Angiography	×		✓			
NIHSS	×		*	✓	✓	✓
Blood Sample – biomarkers	✓					
Blood sample – HbA1c, glucose	×					
Blood sample – U&Es	×		*			
Capillary Blood Glucose	×	×	×			
Modified Rankin Scale						✓
Oral glucose tolerance test						✓

✓ denotes study-specific procedure

× denotes clinically routine procedure, data captured for study

* denotes procedure clinically routine in some patients

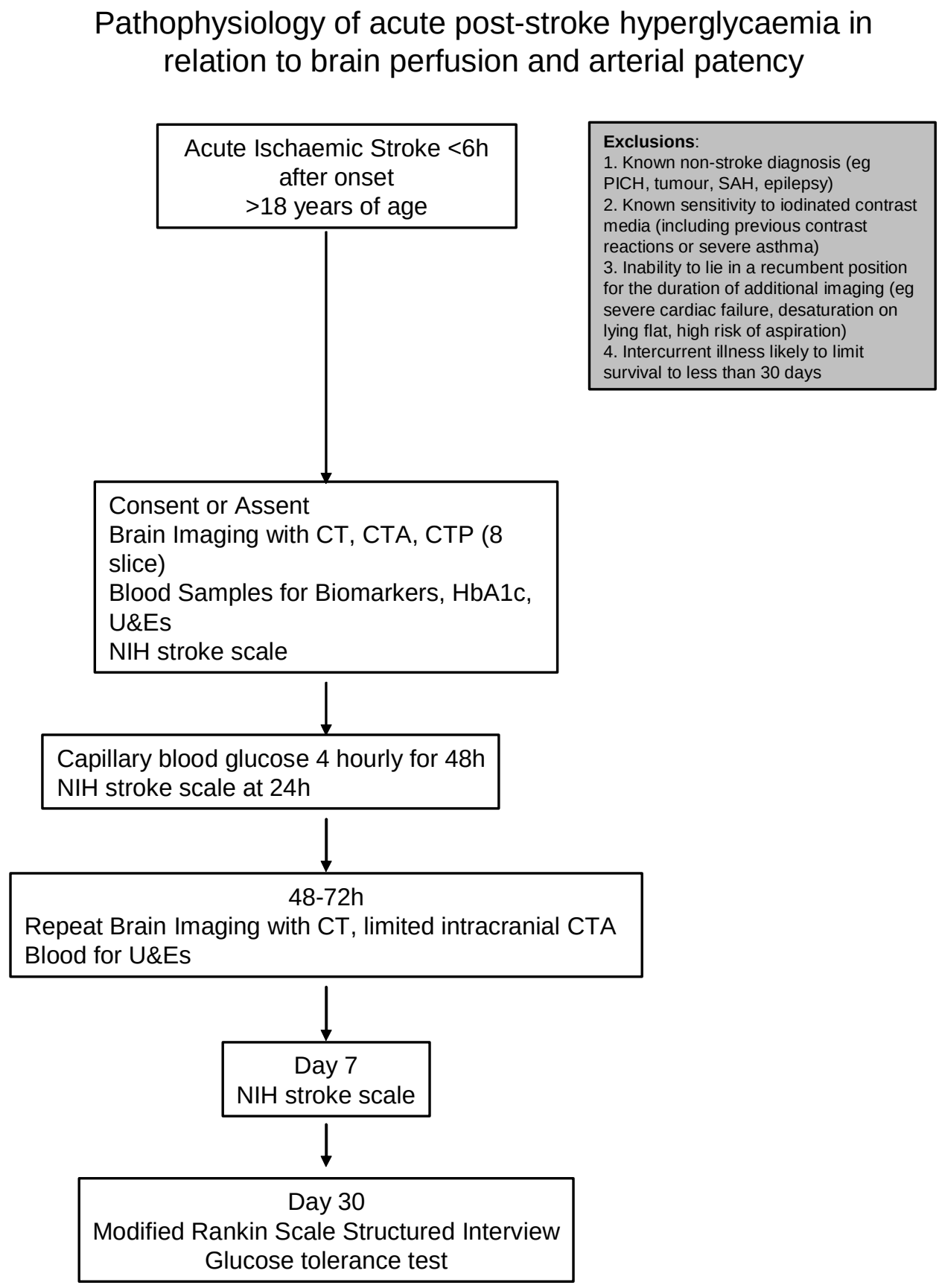


Figure 4 - POSH study procedures flowchart

2.6 Clinical assessment

All patients were clinically assessed by the clinical medical team. Severity of stroke was graded using the National Institute of Health Stroke Scale (NIHSS).[424, 429] The NIHSS was repeated at 24 hours in all patients and if possible 72 hours and 7 days to assess clinical change in stroke severity. At day 30 patients were invited to return to the hospital for clinical assessment and an oral glucose tolerance test (if not known to be diabetic). Clinical outcome was assessed using the modified Rankin Scale and the NIHSS. If a patient was unable to return to the hospital on day 30 their clinical condition was assessed by a research nurse (AW) using the modified Rankin Scale structured interview (mRS-SI) during a telephone call.[172, 431] If a patient was uncontactable their clinical status was established from doctors involved in their care or medical notes.

2.7 Blood samples

Blood samples were taken on admission for glucose, glycosylated haemoglobin (HbA1c) and renal function. Additional blood was stored for later analysis.

2.8 Blood capillary glucose monitoring

Capillary blood glucose levels are checked as a matter of routine every four hours in the Acute Stroke Unit. These data were recorded in the study proforma. Where possible, the initial ambulance capillary glucose level was recorded.

Serial capillary blood glucose concentrations were used to define groups: admission hyperglycaemia (blood glucose >7mmol/l within 6h of stroke onset), delayed hyperglycaemia (blood glucose >7mmol/l 6-48h after stroke onset), and normoglycaemia (blood glucose always below 7mmol/l).

2.9 Imaging protocol

Multimodal CT examination was obtained using a Multidetector Scanner (Philips Brilliance 64 Slice). Whole brain non-contrast CT was acquired first (5 mm slice thickness FOV 218 x 218 mm,120kv, 171 mA or 0.9 mm slice thickness, FOV 250x250mm, 120 kV, 404 mA).

This was followed by CT perfusion with 40mm slab coverage (8x5mm slices, FOV 25cm, 80kVp, 476 mAs, 2 second cycle time, 30 cycles) using a 50 ml contrast bolus

administered at 5mls/second (350 Xenetix) via a large-gauge venous cannula, usually placed in the antecubital fossa.

Finally a CT angiogram was performed from aortic arch to the top of the lateral ventricles (0.67 mm slice thickness, 120 kV, 475 mA) using bolus tracking to enable correct timing of image acquisition.

Follow up imaging consisted of whole brain non-contrast CT followed by intracranial CTA from base of skull to the top of the lateral ventricles. If the initial occlusion was extracranial, the subacute CTA was extended using the same protocol as the admission CTA. Follow-up CTA was only performed in patients with an arterial occlusion on initial CTA.

2.10 Image Analysis

CTP was processed using commercially available software (MIStar, Apollo Medical Imaging Technology, Melbourne, Australia) providing 4 perfusion parameters for analysis, Cerebral Blood Flow (CBF), Cerebral Blood Volume (CBV), Mean transit time (MTT) and Delay (DT). Motion correction was automatically applied after loading the CTP dataset. Arterial input function (AIF) and venous output functions (VOF) were selected semi-automatically after placing a region of interest (ROI) in the anterior cerebral artery and superior saggital sinus respectively.

Core volumes and penumbra volumes were measured on the 8 processed perfusion slices. This measurement was partially automated. The CT perfusion thresholds used came from the Parsons group in Australia. Core tissue was defined as having a delay time of > or = to 2 seconds and a cerebral blood flow (CBF) of <40%. Penumbral tissue was defined by a delay time of > or = to 2 seconds with a CBF of more than 40%.[432]

Final infarct volume was measured on non-contrast CT images. The non-contrast CT images were co-registered with the CTP images using the 3D fusion tool in the MIStar software. The infarct volume was initially measured in the co-registered slices. The total infarct volume visible in all CT slices was then measured.

All scan volumes were measured by two observers (NM and XH). For final analysis mean volumes were used.

Inter-rater and intra-rater reliability was assessed using an anonymised set of CTP scans. This set included scans from the POSH cohort as well as scans from two other prospective studies. Two observers (NM and XH) blinded to the preparation of this set measured core and penumbra volumes on these scans. Inter-rater reliability was assessed using both intra-class correlation coefficients and weighted Cohen's Kappa in Stats Direct.

2.10.1 Reliability Results in test population

For perfusion core the intra-class correlation co-efficient was 0.89 with 95% limits of agreement being -6.6 to 19.8. These results suggest that one observer thought core volume was larger than the other in several cases.

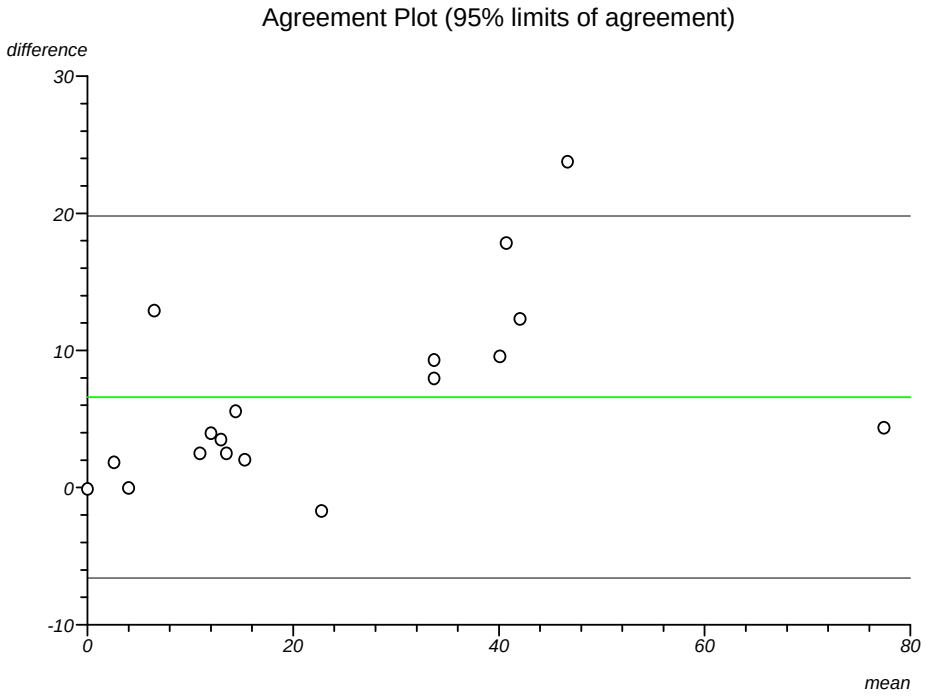


Figure 5 Agreement plot of inter-observer perfusion core volume

For penumbra the intra-class correlation co-efficient was 0.89 with 95% limits of agreement -24.3 to 15.7.

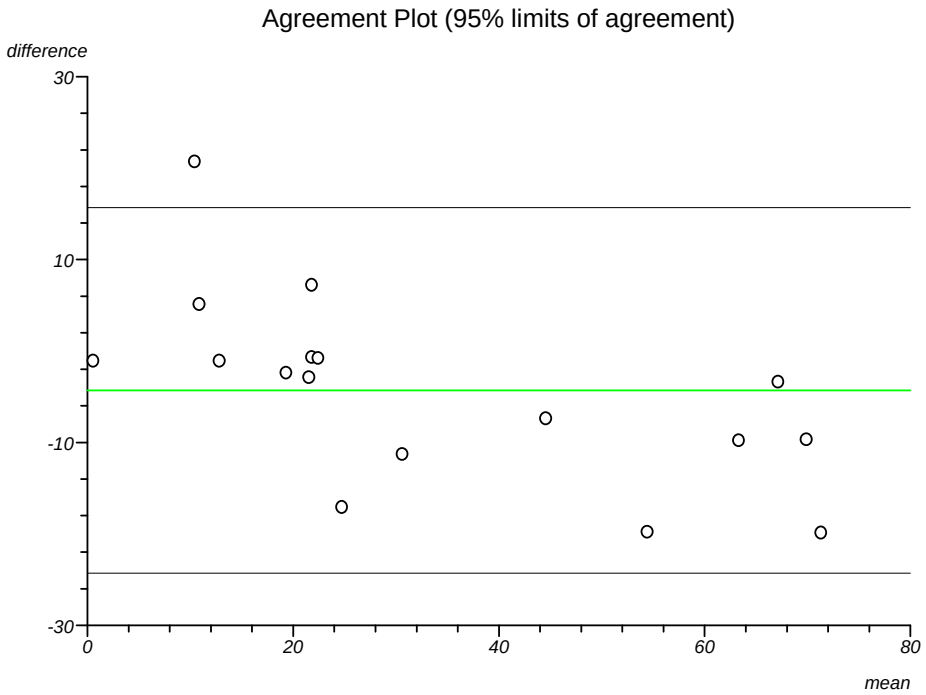


Figure 6 Agreement plot for inter-observer perfusion penumbra volume

For co-registered 24-48h infarct volume the intra-class correlation co-efficient was 0.67 with 95% limits of agreement being -88.3 to 99.4.

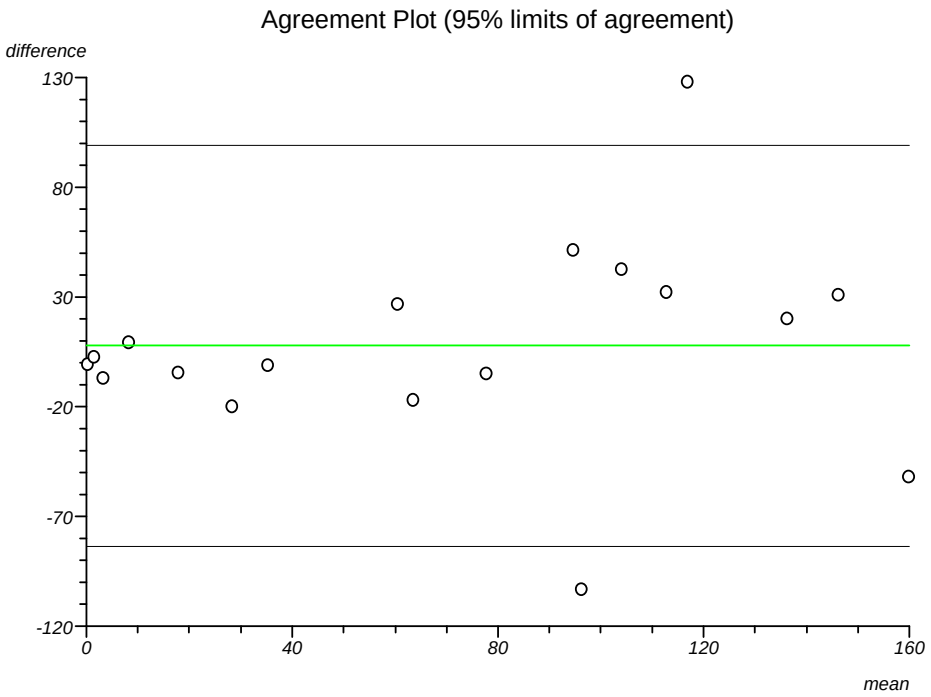


Figure 7 Agreement plot for inter-observer co-registered 24-48h infarct volume

For final infarct volume looking at all slices the intra-class correlation co-efficient was 0.92 with 95% limits of agreement being -80.8 to 69.6.

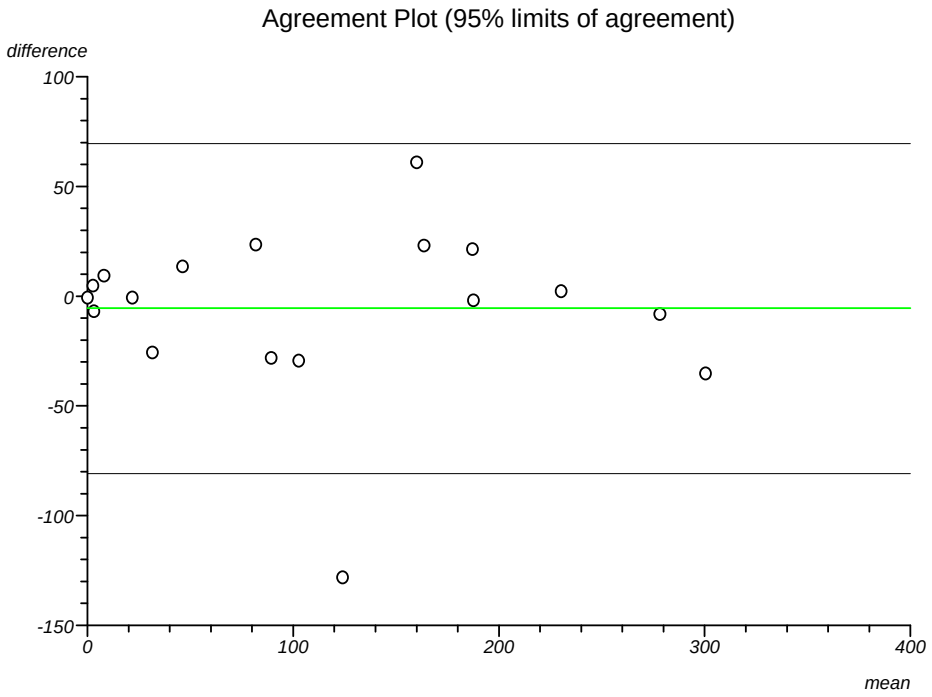


Figure 8 Agreement plot for inter-observer total 24-48h infarct volume

For arterial patency weighted Kappa was 0.689 (95% CI 0.52 to 0.859).

2.10.2 Inter-observer agreement for POSH scans

Inter-observer agreement was reassessed in the POSH cohort. For perfusion core the intra-class correlation co-efficient was 0.93 with 95% limits of agreement -20.5 to 13.8.

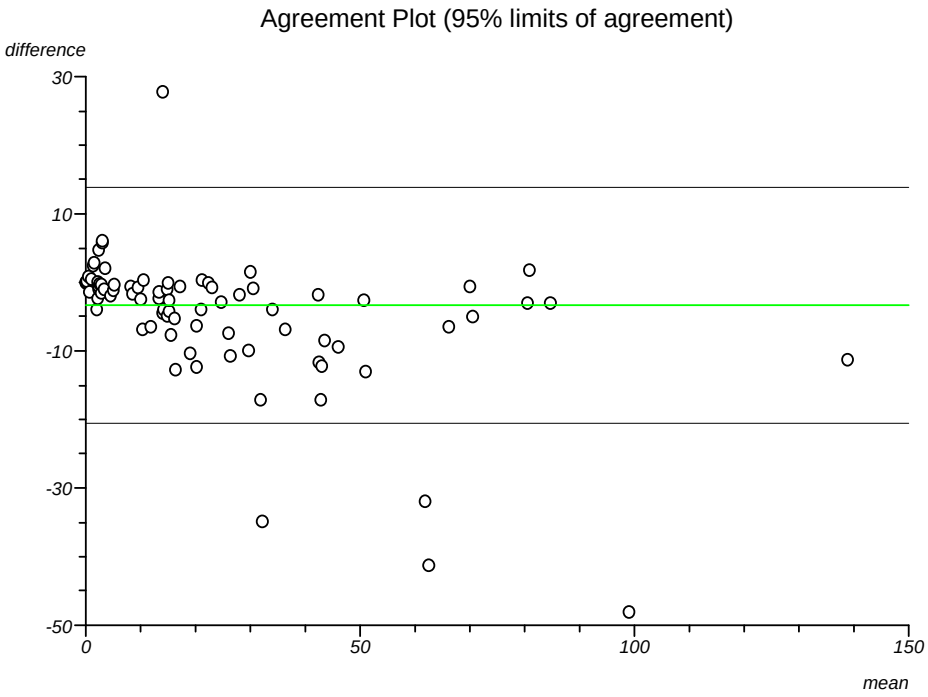


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Figure 9 – Agreement plot of inter-observer perfusion core volume in POSH cohort

For penumbra volume on perfusion imaging the intra-class correlation co-efficient was 0.84 with 95% limits of agreement -23.7 to 30.1.

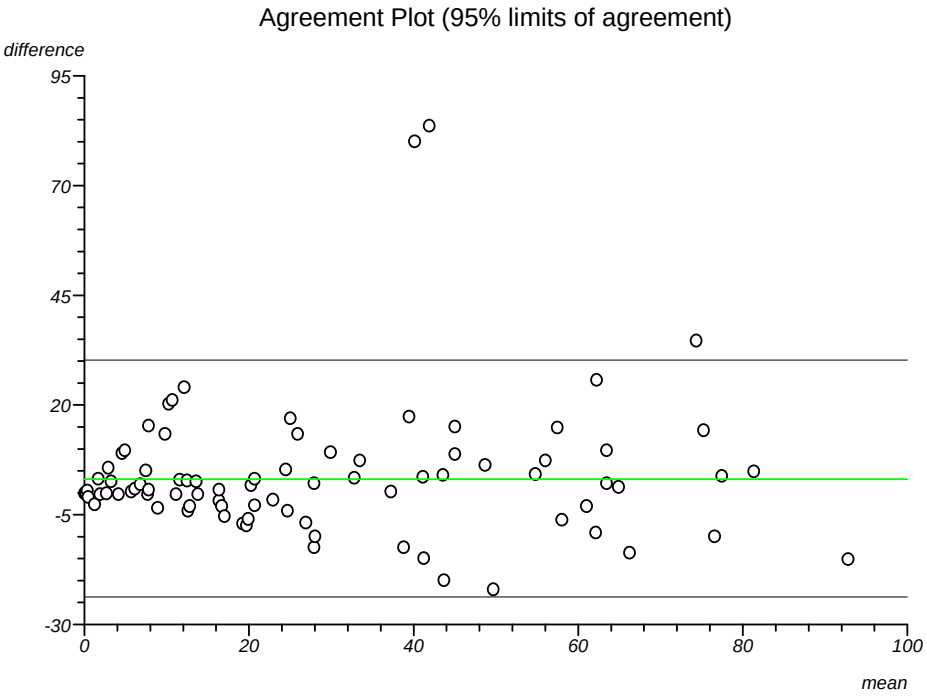

Figure 10 Agreement plot of inter-observer perfusion penumbra volume in POSH cohort

For co-registered 24-48h infarct volume the intra-class correlation co-efficient was 0.91 with 95% limits of agreement being -49.3 to 45.9.

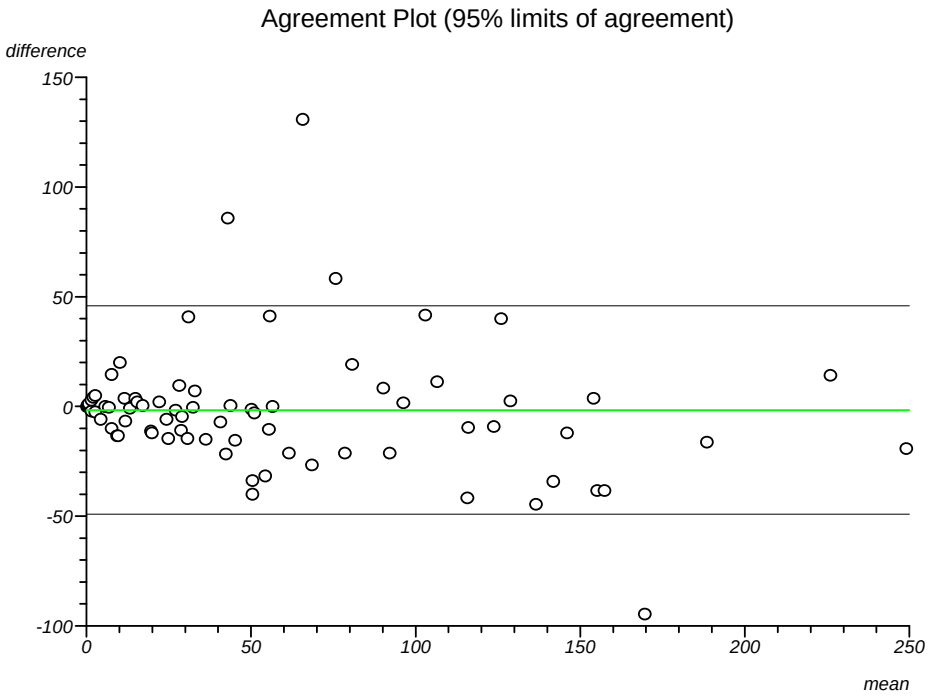

Figure 11 - Agreement plot of inter-observer co-registered 24-48h infarct volume in POSH cohort

For final infarct volume looking at all slices the intra-class correlation co-efficient was 0.94 with 95% limits of agreement being -70.8 to 48.9

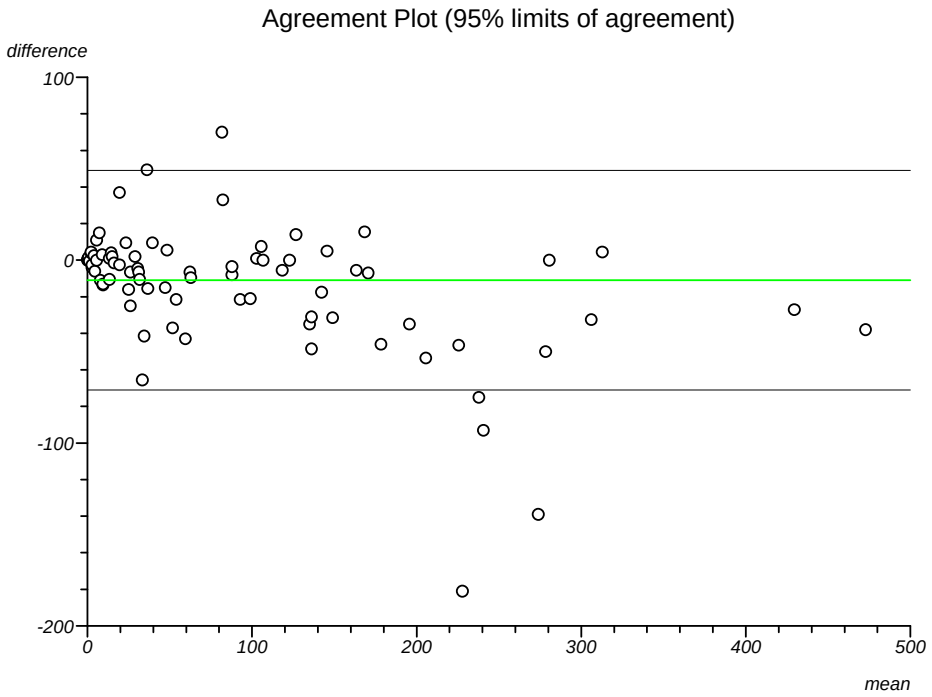

Figure 12 Agreement plot for inter-observer total 24-48h infarct volume in POSH cohort

For the presence of arterial occlusion in the POSH cohort the weighted Kappa for agreement was 0.782 ($p<0.001$, 95% CI 0.61 to 0.96). For recanalization the weighted Kappa was 0.777 ($p<0.001$, 95% CI 0.59 to 0.97).

2.11 Statistical analysis

Details of statistical analysis will be given in individual chapters. Clinical data were prospectively entered into a spreadsheet by one person (NM). Statistical analysis was mainly performed using PASW IBM SPSS statistics (SPSS Chicago, Illinois, USA, v 16-19). Where other packages have been used I have noted this in the text.

3 Animal models of post stroke hyperglycaemia

3.1 Introduction

3.1.1 The rationale for animal models of stroke

Animal models of human disease are often used to gain pathophysiological information that cannot be obtained from human experiments and to develop new therapeutic strategies.

In animal models we can control the severity, duration, location and cause of cerebral ischaemia. We can also control for variations in cerebral anatomy and co-morbid diseases in a way that is impossible in clinical research. It is also possible to manipulate physiological variables to ensure that experimental strokes can be reproduced if necessary.[433]

3.1.2 Problems with translational research in stroke

Preclinical evaluation of any new potential therapy is important. It is essential to know about any potential for toxicity before a new compound is used in humans. It is also important to have evidence that any new compound may have some therapeutic value.

A drug will usually be tested in animals before it is used in a clinical trial. While this has not always been the case historically new compounds that have never been used in humans are routinely tested in animals first. Animal studies may indicate potential human therapeutic efficacy. Unfortunately there have been many cases of a failure to translate the positive results of animal studies into successful clinical trials.[434]

The 1999 Stroke Therapy Academic Industry Roundtable (STAIR) guidelines were created to help with the translation of positive experimental neuroprotective trials into useful clinical treatments.[435] They have subsequently been updated after several failed major stroke trials.[436] These recommendations were as follows -

1. Define the dose-response curve

2. Define the time window in a well-characterized model

3. Blinded, physiologically controlled reproducible studies

4. Histological and functional outcomes assessed acutely and long-term[1]

5. Initial rodent studies, then consider gyrencephalic species

6. Permanent occlusion then transient in most cases

In the years since the initial STAIR guidelines were published no successful new treatments for acute ischaemic stroke have emerged and the guidelines are as yet unvalidated. The compound NXY-059 was thought to fulfil all the STAIR criteria but still failed to be clinically useful.[437]

Pre-clinical research that has failed to translate into workable clinical treatments has been re-examined. Systematic review and meta-analysis of basic research has suggested that publication bias exists and that pre-clinical research may have flawed methodology in some cases.[438, 439]

It is possible that experimental studies have not included enough animals to have statistically significant results. Historically sample size calculations have not always been included in basic scientific research papers and it has been suggested that many studies have just not been large enough to ensure that positive results have evolved from anything other than chance.[440]

More recently several journals have decided to only consider basic research papers for publication if there are clear descriptions of sample size calculations, inclusion and exclusion criteria, randomization, allocation concealment, reporting of animals excluded from analysis, blinded assessment of outcome and reporting of potential conflicts of interest and study funding.[441-443] While more basic studies containing observational, pilot or hypothesis generating data should still be published it should be made clear that these are only preliminary studies.

There may also be an issue with the choice of animals used for individual studies. Most experiments use young, male animals with no other co-morbidities. As the majority of

[1] For stroke recovery drugs an appropriate time window for histological studies may be several days or weeks after ischaemia to allow the drug to take effect. Behavioural studies should be carried out in the same time frame,

acute ischaemic strokes occur in older humans with various risk factors who are more likely to develop complications than young fit rats. An update of the STAIR recommendations suggests that studies should be repeated in older animals with co-morbid disease to better mimic the target human population. Efficacy studies should also be carried out in female animals.[436] Infarcts may be influenced by a multitude of factors including anaesthetic agents and all of these issues should be considered.[444]

3.1.3 Models of ischaemia

Stroke is a heterogeneous human disease so a variety of animal models are viable in basic research.

A variety of different types of models of cerebral ischemia exist. Ischaemia can be global (as in a cardiac arrest), hemispheric (as if a common carotid artery has been occluded) or focal (which may mimic a stroke in the vascular territory of the middle cerebral artery).[445] Focal ischaemic models are most appropriate for human ischaemic stroke. It is probably sensible to chose different models for lacunar stroke as opposed to cortical stroke.[446]

3.1.4 MCAO models

Several models of ischaemic middle cerebral artery (MCA) stroke exist. Some models have been in use since the 1930s[447]. The most widely used models involve unilateral middle cerebral artery occlusion (MCAO) in rats, mice or cats. These models can involve permanent occlusion of the MCA (pMCAO) or transient occlusion (tMCAO). Arterial occlusion can be induced by surgical ligation or cauterization of the MCA, intraluminal passage of a nylon suture or injection of an autologous thrombus into the common carotid artery.

The surgical method of MCA occlusion developed by Tamura *et al* in 1981 has also been widely used.[448] This technique involves a subtemporal approach to the MCA with diathermy to occlude the blood vessel. This method also allows access to more proximal regions of the MCA and creates infarctions that are similar to those produced by intraluminal suture. Infarctions are usually seen within 4 hours of vessel occlusion. The technique has been refined to identify anatomical sites in the MCA that will produce consistent infarcts when occluded.[449]

The intraluminal suture MCAO method is probably the most frequently used model of experimental ischaemic stoke[450, 451]. This technique is minimally invasive and can be used to induce both permanent and transient ischaemia in a controlled manner. The model involves inserting a monofilament into the internal carotid artery until blood flow is occluded in the MCA. Various technical modifications have been developed to improve the model.[452] Filament size is known to influence infarct size.[453] Problems with this technique include the risk of blood vessel rupture, hyperthermia and inadequate vascular occlusion.

In cats a transorbital approach can be used[454, 455]. This technique minimises manipulation and disturbance of the brain.

Experimental infarct volumes need to be consistent. The size of an infarct varies with the duration of ischaemia. For infarcts to be of a reproducible model at least 60 to 90 minutes of ischaemia are required.

Other models of MCA occlusion include photothrombosis models where a cortical infarct is induced by the systemic injection of a photoactive dye before irradiation with a light beam of a specific wavelength.[456] As the infarct is created quickly it is unlikely that any ischaemic penumbra exists in this model.[457] Endothelin-1 can be used to vasoconstrict the MCA causing cerebral infarction but this method allows less control than surgical techniques.[458]

There are several other surgical models which allow occlusion of the MCA. These models are often invasive and require craniotomy. They can be used in a variety of species.[459-464]

Table 3-1 Examples of benefits and drawbacks of animal models of MCA occlusion

Animal	Model	Benefits	Drawbacks[465]
Squirrel Monkey[459]	Transorbital approach to occlude MCA	Anatomy similar to human Reproducible infarct size Gyrencephalic brain	Ethical concerns Expensive Requires transorbital surgery Anaesthetics may have impact on infarct growth
Miniature Pig[460]	Focal cerebral ischemia	Reproducible Cheaper than primates Less ethical concerns	Larger and more expensive than rats
Cat[461]	Retro-orbital extradural	Easier physiological	Ethical concerns

	approach for occluding MCA	monitoring	Requires orbital surgery
Dog[463]	Occlusion of intracranial trunk arteries	Good control of infarct development is possible Consistent results	Extensive collateral cerebral circulation Ethical concerns Expensive Larger than rats Requires craniectomy

3.1.5 Embolic models

Embolic stroke models exist.[466] Human and homologous rat emboli have been used although infarct size may be variable.[467, 468] Human atherosclerotic plaques have been used to create emboli that are subsequently injected into rats.[469]A thoracotomy is often required to inject emboli.[470] Artificial embolic materials have been used although these may be less relevant to human stroke than clot based emboli.[471, 472] Multiple infarcts may be formed which makes precise reproducibility of lesions difficult.[473, 474] Not all animals will develop infarction even in more modern models.[470] When microspheres are used the evolution of infarcts appears to be prolonged.[475] These models are often in non-rodent species.[476-478]

3.1.6 Choice of species

The rat is often used as an animal model of stroke for several reasons. The cerebrovascular anatomy and physiology of the rat is similar to man.[433] The vascular anatomy of the gerbil, the cat and the dog are less similar to man. Table 3.1 summarises a few benefits and drawbacks of different models. Different strains of rat are fairly homogenous within a strain making reproducible studies possible. There is not as yet a standardised rat model. Intra-arterial suture occlusion models result in massive hemispheric infarction with short animal survival times.[435] In stroke recovery trials a more focal surgical technique such as the Tamura method may be better.[448] While the rat is a useful model species it is not perfect.

There are arguments that a distinct role exists for primate models of stroke recovery.[435] There are differences between the human brain and the rodent brain that may lead to a different response to a similar ischaemic insult. These differences are less apparent when the human brain is compared to the non-human primate brain. It would be easier to estimate human drug doses based on primate data than it is using rodent data. However

there are no standardised well recognised primate models of stroke recovery (as with rodents) and there are significant ethical concerns regarding the use of primates in medical research in western cultures.[479]

3.1.7 Models of hyperglycaemia - Streptozotocin

Streptozotocin is a broad spectrum antibiotic which was initially identified as an agent to create a model of type 1 diabetes mellitus in 1963.[480-482] Streptozotocin causes pancreatic islet cell destruction resulting in an insulin deficiency which mimics human type 1 DM. The severity of the diabetes is dose dependent.[483]

3.1.8 Models of hyperglycaemia - Dextrose infusions

Hyperglycaemia can also be induced by the infusion or injection of a dextrose solution. Animals can be infused with variable strengths of dextrose ranging from 5% to 50%. This will produce well controlled levels of hyperglycaemia although it may not precisely mimic a natural pathophysiological state.

Neither the streptozotocin model, nor the dextrose infusion model is likely to accurately reflect the pathophysiology of post-stroke hyperglycaemia.

3.1.9 Models of insulin resistance and Type II Diabetes Mellitus

It is possible that models of insulin resistance or Type II Diabetes may be more relevant in the experimental observation of post-stroke hyperglycaemia. The majority of human patients with PSH do not have type 1 diabetes mellitus and are not infused with highly concentrated dextrose solutions. Models have been developed for the 'metabolic syndrome' which may be more useful

The Zucker rat has been used as a model of metabolic syndrome. Normally rats do not develop atherosclerosis meaning that in the past they were considered to be poor models of cardiovascular disease. More recently genetic rat models of cardiovascular disease have been developed.

The fatty Zucker rat was first described in 1961[484]. The Zucker rat strain has a spontaneous mutant gene (*fa* or fatty) that affects the action of the adipocyte peptide hormone leptin. Leptin is a key element in the regulation of food intake through the inhibition of the release of hypothalamic neuropeptide Y. The Zucker also has a degree of

glucose intolerance, variable hyperglycaemia, hyperinsulinaemia, insulin resistance and a tendency to obesity.[485, 486] These features are only seen in the homozygous *fa/fa* animals and are not seen the heterozygous Fa/*fa* or the homozygous Fa/Fa.[487] The heterozygote and homozygous Fa/Fa Zucker rats are lean and metabolically normal.

The Zucker rat may be more representative of the average human stroke patient and may be a better model for post-stroke hyperglycaemia than animals treated with streptozotocin or dextrose infusions.

3.1.10 The need to re-examine the basic scientific evidence for post-stroke hyperglycemia

The rationale for the UK Glucose Insulin Stroke Trial (GIST-UK) was at least partially based on animal models[143, 313, 315, 488]. Since the publication of GIST-UK, a number of studies in other clinical groups have raised concerns about the safety of insulin treatment in acutely hyperglycaemic patients[242, 259, 283, 284, 298]. As GIST-UK was negative with a significantly worse outcome in patients whose blood glucose fell by more than 2mmol/l there is a need to reconsider some of the original basic experimental studies.[242]

We elected to explore the data on pathophysiology of hyperglycaemia in acute stroke, and undertook a systematic review of the literature and meta-analysis of studies in hyperglycaemia in animal models of focal ischaemia induced by middle cerebral occlusion (MCAO).

3.1.11 Assessment of quality of papers in basic scientific research

Several checklists have been proposed to help assess the quality of papers in basic scientific reseach.[440] Ideally a paper should make it clear exactly what a researcher has done to the extent that a reader could reproduce the original experiment.

3.2 Methods

3.2.1 Systematic review

Studies of hyperglycemia in animal models of MCAO were identified from OVID medline (1950- March 2009) and EMBASE (1980-March 2009). The search strategy is specified in appendix 1.

Titles were screened for relevance and abstracts for all potentially relevant papers were read by one investigator. We also performed hand searches of abstracts of scientific meetings including the 2008 World Stroke Conference, the 1981 - 2009 International Stroke Conferences, the 2005-2009 Brain meetings, the 1992 - 2009 European Stroke Conference and Marburg Conferences from 1994-98, and screened reference lists of identified publications.

3.2.2 Inclusion criteria

We included models of focal ischaemia induced by middle cerebral artery occlusion (MCAO) where data were presented on infarct volume, defined either histologically or on brain imaging. The subset of papers that included data on the use of insulin, while meeting other inclusion criteria, was identified for additional analysis. We excluded models of global or forebrain ischemia, and studies whose data did not include volumetric data.

3.2.3 Data extraction

We extracted data from the included papers on species, strain, gender and weight of animals; model and timing of ischemia; presence or absence of reperfusion; number of animals and experimental groups; experimental interventions; method of induction and timing of hyperglycemia; level of hyperglycemia; insulin use: timing of outcomes; method of measuring infarct and mean final infarct size and standard deviation.

Studies that clearly reported method of inducing hyperglycemia and volume of final infarction with standard deviation were included in further analyses.

If published data were incomplete, we contacted authors to obtain further information [489-495].

3.2.4 Meta-analysis

Data on infarct volume were recorded. To allow for different species in studies, the effect size was normalised to the mean of the control group (assumed 100%) before analysis in Review Manager 5.0.2 (Cochrane Collaboration) and StatsDirect, version 2.7.3 (StatsDirect Ltd, Cheshire, UK) by means of a DerSimonian-Laird random effects model that expresses the difference between groups as a weighted mean difference (WMD) for effect size, and 95% confidence interval. The significance of difference between groups

was assessed by partitioning heterogeneity and using the chi-squared distribution with n-1 degrees of freedom where n equals the number of groups.

Several stratified analyses were planned. We looked for a differential effect due to either streptozotocin or dextrose. The insulin studies were stratified by control group (normoglycaemic or hyperglycemic). We also compared permanent and transient MCAO models. In order to allow for multiple comparisons we adjusted the significance level using the Bonferroni method to $p<0.02$.

3.2.5 Study Quality

We assessed the quality of the individual papers analysed in this study using a modified version of the CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data in Experimental Stroke) score [438, 440], omitting a score for neuroprotective properties of anaesthetic agents used, and therefore giving a maximum score of 10 points.

3.2.6 Assessment of bias

Funnel plots comparing standard error of the mean treatment effect against effect size for each study were obtained and analysed by Egger's method to identify possible publication bias [496].

3.3 Results

3.3.1 Identification of papers

The initial search produced 1482 titles that were screened to identify 178 abstracts that were read in detail. From these abstracts, 57 papers were initially identified for data extraction. A further 15 papers were identified by hand searches.

Twenty-two papers reported data in a format suitable for meta-analysis [312, 313, 316, 319, 488, 497-512]. One further paper was included when an author kindly contacted us with additional data for analysis [491]. Six papers had insufficient data to include in the meta-analysis (see Table 3.1). The remaining papers did not report infarct size, but included measurement of cerebral blood flow [513-515], brain biochemistry analysis [516], blood brain barrier function [517], genetic analysis, evaluation of tissue energy states [314, 518, 519], behavioural tests [520] and immunohistochemical analysis.

From the 23 papers included, a total of 36 different comparisons of infarct size between hyperglycemia and normoglycemic controls after MCAO were described (see Table 3.2). These experiments used a total of 664 animals. In two cases 2 comparisons used the same control group. This is noted below table 2. Hyperglycemia was induced with dextrose infusion or injection in seven cat and one rabbit experiments. In rats, streptozotocin was used in 18 comparisons while dextrose infusion was used in 10. One study had useable data on infarct size but was excluded from analysis as it used a photosensitising model to induce local infarction instead of MCAO [313].

Streptozotocin was given 48 hours before the experiment in 8 papers. In other studies streptozotocin was administered at earlier times (72 hours earlier in 2 studies; 4 days earlier in 1 study; 7 days earlier in 1 study; 5-6 weeks earlier in 3 studies and i 4 months earlier in 1 study: note that these numbers do not add up to 18 as some studies included more than one comparison group).

Dextrose infusions were started between 15 and 120 minutes (median time 30 minutes) before MCA occlusion in the comparisons where hyperglycemia was induced before occlusion. Dextrose infusion concentration varied from 10% to 50%. In the comparisons where hyperglycemia was induced after MCAO this was done with an injection of 50% dextrose 5 to 20 minutes post ictus.

In 9 papers the frequency of monitoring of blood glucose was unclear. Glucose concentration was reported at the time of arterial occlusion and at an unstated time after occlusion in the remaining papers. Peak blood glucose values ranged from 18.4 to 31.2mmol/l in hyperglycaemic groups, and from 4.6 to 11.1mmol/l in control groups. In the hyperglycaemic groups the mean blood glucose was 23.9 mmol/l (95%CI 22.4-25.3). In the control groups the mean glucose was 8mmol/l (95%CI 7.3-8.7)

3.3.2 Study Quality

Study quality was generally low based on the modified CAMARADES score (median 3/10, range 1-6 points). All papers were published in peer reviewed journals. Blood pressure monitoring was documented in 20/24 papers while temperature monitoring was documented in 22/24. Random allocation of animals to experimental groups was documented in only 6/24 papers, while blinded assessment of outcome was only detailed in 7/24. No papers documented blinded induction of ischemia, sample size calculations or a clear conflict of interest statement. The complete quality score table is included as Appendix 2.

3.3.3 Assessment of Bias

Funnel plots (Figure 13, graphs A-C) suggested possible publication bias, with paucity of small, negative studies (particularly using the streptozotocin model), but formal statistical analysis was not significant (Egger's bias test 2.88 (95% CI = -1.53 to 7.29, p = 0.1925) [496].

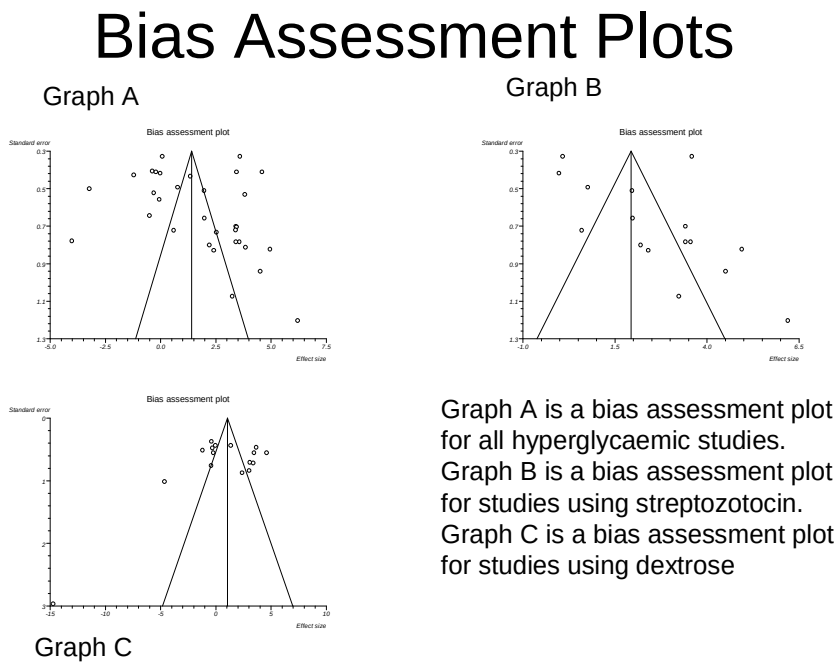

Figure 13 - Bias Assessment Plot for Effect of Hyperglycaemia on Infarct Size

3.3.4 Measurement of effect size

Infarct size was quantified by tissue staining (2,3,5-Triphenyltetrazolium Chloride [TTC] in 9 studies, cresyl violet in 4 studies, and hematoxylin and eosin in 11 studies) or magnetic resonance imaging (MRI). Two histology studies used both cresyl violet and hematoxylin and eosin staining. One study reported only MRI measurement, and three others undertook both MRI and histology. Where both techniques were used, we included MRI data as reported by the original authors. Infarct volume was measured at times ranging from 3 hours after arterial occlusion up to 2 weeks.

3.3.5 Streptozotocin Model and Dextrose Infusion Models

Hyperglycaemic animals had significantly larger infarcts (effect size 94, 95% confidence interval 69.1 - 118.9, $p<0.00001$), but streptozotocin was associated with greater exacerbation of infarct volume compared to dextrose alone (effect size 140.3, CI 104.8-175.9, $p<0.00001$ versus 48.3, CI 14.8-81.9, $p=0.005$). There was significant statistical heterogeneity between studies with chi-squared equal to 1208.7 with 32 degrees of

freedom (p<0.00001). There was also statistical difference between sub-groups (chi-squared =186.3, df =1, p<0.00001). See Figure 14 below.

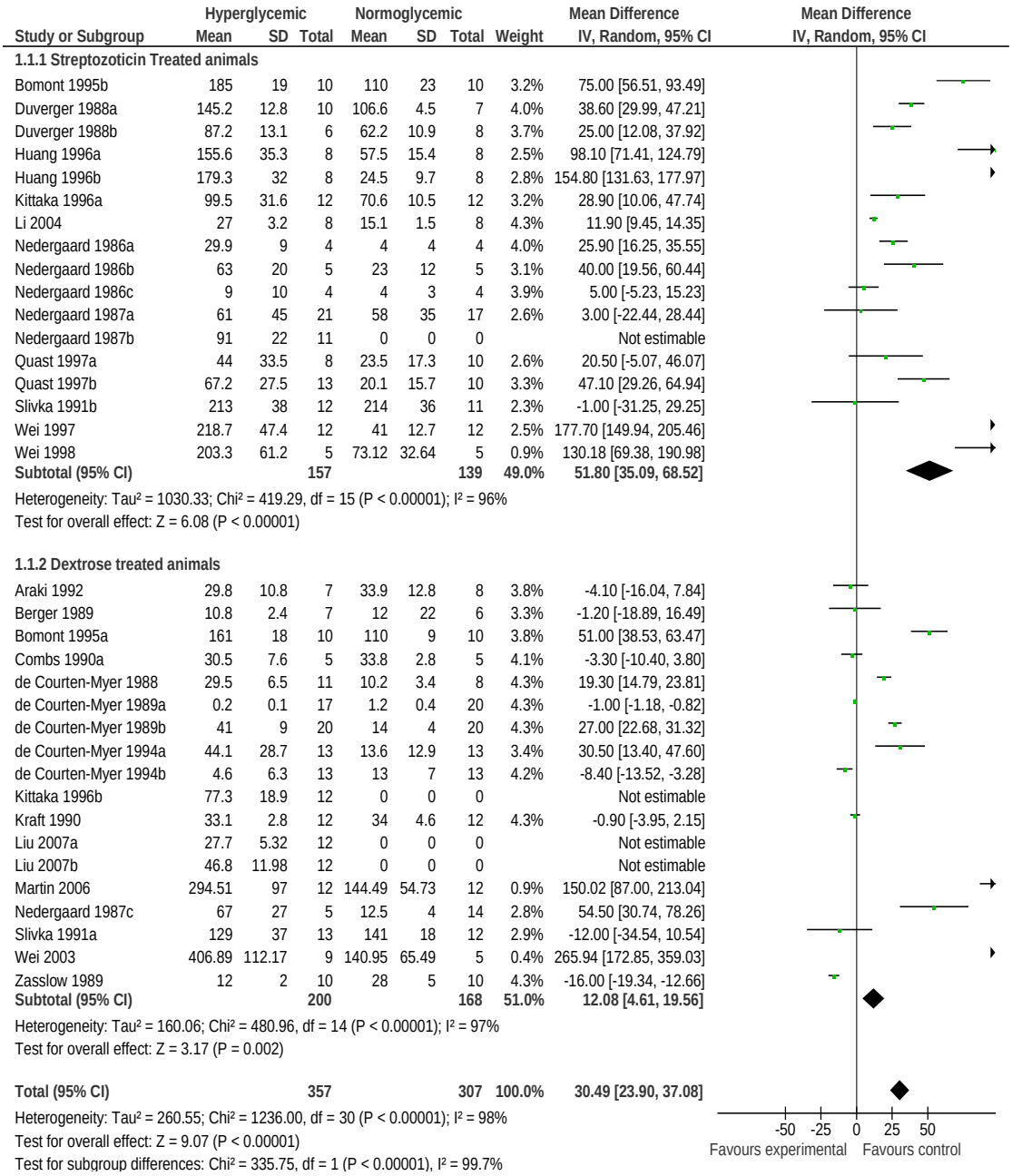

Figure 14 Meta-analysis of effect of hyperglycaemia on infarct size

3.3.6 Permanent versus Transient MCAO

The streptozotocin model was associated with larger infarcts in both transient and permanent MCAO. In 9 comparisons involving 190 streptozotocin-treated animals with permanent MCAO, the effect estimate for infarct size was 55.2 (95% CI 31.2 to 79.1, p<0.0001). There was statistically significant heterogeneity between studies (chi-squared =99.3, df=8, p<0.00001). In 8 comparisons involving 115 streptozotocin treated animals

with transient MCAO the effect estimate for infarct size was 319.1 (95% CI 191 to 447, p<0.000001). There was statistically significant heterogeneity between studies (chi-squared =116.6, df=7, p<0.00001)

In 10 comparisons involving 227 dextrose-treated animals with permanent MCAO the effect size was less than for STZ at 43.1 (95% CI -0.05 to 86.2, p=0.05) with statistically significant inter-study heterogeneity (chi-squared = 509, df=9, p<0.00001), and in 6 comparisons involving 140 animals with dextrose-induced hyperglycaemia and transient MCAO there was no significant effect on infarct size (effect size 19.2, 95% CI.-42.9 to 81.2, p = 0.54). There was statistically significant heterogeneity between studies (chi-squared = 149.8, df = 5, p<0.00001)

3.3.7 Effects of Insulin Treatment

In comparing insulin treatment with either hyperglycaemic or normoglycaemic control groups, infarct volume was smaller with insulin, but not significantly so.

Insulin did not significantly reduce infarct size (10 comparisons, n=194, effect size -13.4, 95% CI -41 to -5, p = 0.01) and results were heterogeneous in a statistically significant manner (chi-squared = 54.8, df=7, p<0.00001). (See Table 3.3 and figure 15)

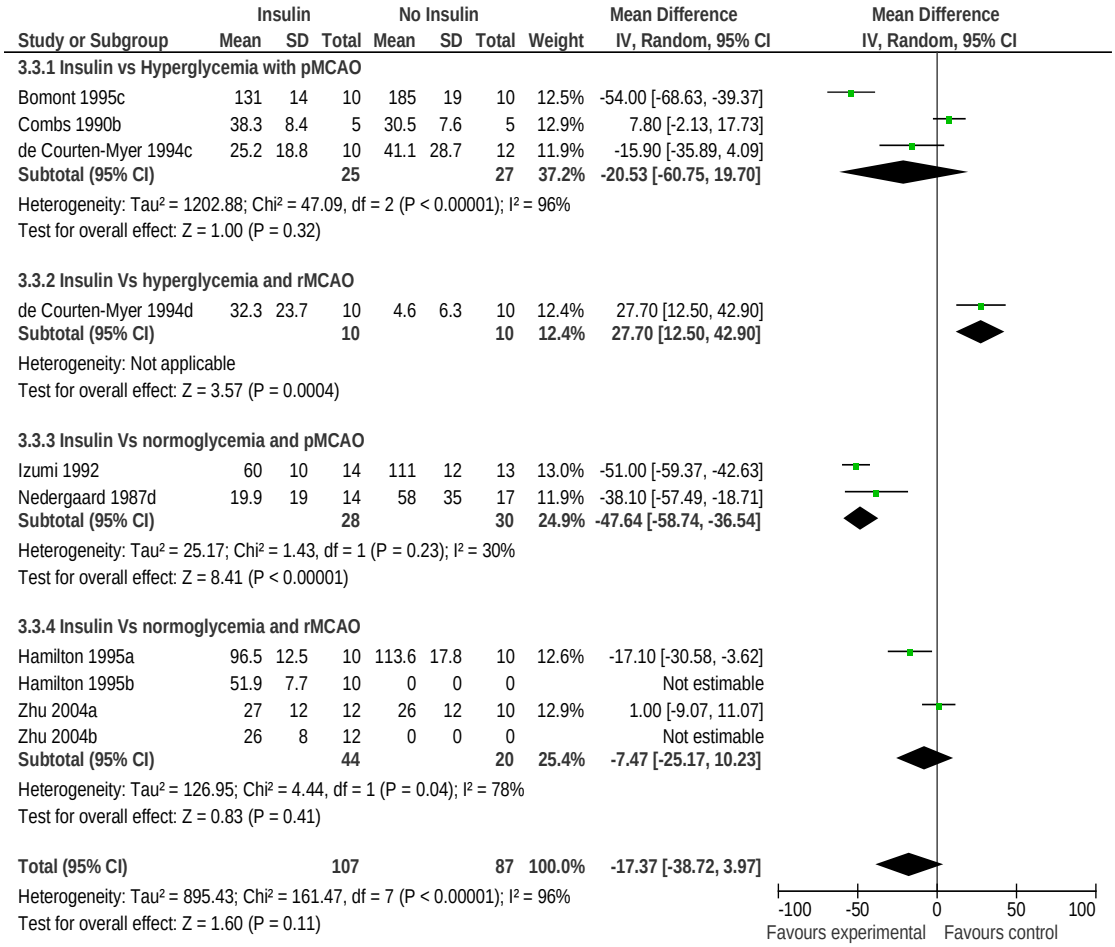

Figure 15 Meta-analysis of effect of insulin on infarct growth

3.4 Discussion

Whilst a large body of clinical observational evidence indicates that hyperglycaemia in the acute phase of stroke is associated with poorer outcomes [146], typically defined by death or dependence 90 days after the event, few clinical studies have addressed the mechanism by which this may occur [176, 336]. Many mechanistic proposals are based on inferences drawn from animal models of focal ischaemia. In particular, the rationale for acute intervention to lower blood glucose (usually by administration of insulin) is based on an assumption that the adverse effect of hyperglycaemia relates predominantly to exacerbation of acute infarct evolution. However, insulin treatment carries significant risks, particularly hypoglycaemia (Finfer and Heritier 2009; Griesdale *et al* 2009) and the clinical evidence supporting an acute effect of hyperglycaemia is mainly observational and open to alternative interpretations. Data relating hyperglycaemia to early infarct volume can alternatively be explained by "stress" hyperglycaemia resulting from more severe infarcts, for example. Recent observations that hyperglycaemia carries a higher risk of symptomatic intracerebral haemorrhage in patients treated with intravenous alteplase may be confounded by a similar effect [179, 182]. An association of glucose concentration with lactate concentration in the infarct core, and with infarct growth in a small observational study using MRI (Parsons et al 2002) is mechanistically plausible, but without an intervention arm, could not discount the possibility that the relationship with blood glucose is not causal. In addition, the assumption that lactate itself is toxic in the ischaemic brain is not necessarily correct, with other evidence suggesting that lactate is instead produced as an alternative metabolic substrate and is beneficial [176, 332]. Only one small clinical trial has attempted to address these mechanistic hypotheses using advanced imaging, and found that insulin treatment reduced blood glucose and lactate concentration in brain, but had no effect on infarct growth [336].

Our systematic review yields findings that suggest that existing animal model data have limited relevance to the clinical situation, and that further studies may therefore be required in order to inform clinical study design.

First, while hyperglycaemia at the time of focal ischaemia onset increases infarct size, this is predominantly due to its large effect in the streptozotocin rat model, which simulates Type 1 Diabetes Mellitus [480]. Hyperglycaemia induced by dextrose infusion has a much smaller effect on infarct size, although still significant. Significant heterogeneity of effect size was present among both STZ and dextrose models. While the animal data may be

informative with respect to a biological effect of hyperglycaemia on acute infarct volume, the clinical inferences that can be drawn are very limited. Dextrose infusion models typically administered hypertonic solution (20-50% dextrose) to give blood glucose exceeding 20mmol/l, a level considerably greater than is typically encountered in clinical practice (fewer than 2% of subjects in one study), and so the clinical relevance of findings in this model system is unclear. While only a minority of stroke patients have established diabetes mellitus (overwhelmingly type 2 diabetes), a high proportion of patients with acute hyperglycaemia after stroke are found to have unrecognised insulin resistance when followed (impaired glucose tolerance, metabolic syndrome, or undiagnosed type 2 diabetes) [231, 521]. No study of infarct volume used animals that model the insulin resistant phenotype typical of patient populations.

The underlying mechanism for the difference in final infarct size between dextrose models and STZ models is uncertain. In models of global ischemia the anatomical distribution and severity of brain damage in the STZ model is similar to that seen in animals acutely infused with dextrose [522]. It has been suggested that STZ may increase the rate of apoptosis in models of focal cerebral ischemia [523]. The diabetic state induced by STZ may damage the microvasculature of the rat brain or impair the compensatory mechanisms that would normally protect from ischemia. The difference in effect size raises a central question of whether the adverse effect arises from high glucose, or from lack of insulin.

The effect of insulin was non-significant, although the effect size estimate is consistent with reduction in infarct volume. However, as insulin may reduce infarct volume when compared with normoglycaemic control groups, it is possible that insulin does not act via reducing blood glucose in these model systems, and we cannot infer that reducing blood glucose (as opposed to administering insulin) represents an effective intervention. As the results of these studies are heterogeneous a degree of caution is necessary when interpreting them.

To allow for comparisons involving different species and models we used weighted mean differences in infarct volume normalised to the mean volume of the control group [524, 525]. Given the small group sizes, and the possible exclusion of animals that either died or exhibited no evidence of infarction from group mean infarct volume measurements - commented on in only a handful of studies – the magnitude of the effect size can be regarded only as an approximation.

Methodological quality of animal experiments is a significant concern as several reviews suggest that studies that do not report items such as blinding of outcomes and randomisation are more prone to bias than more rigorous studies [440]. Quality scores have been based on criteria developed for pre-clinical evaluation of therapeutic interventions (e.g. the STAIR criteria) which have gone through several iterations over time, rather than physiological studies such as those predominantly reported here. The median quality score using a slightly modified system used by the CAMARADES group [438] was only 3 (out of a possible 10), and only two papers scored 6, which likely reflects a predominance of older papers and different standards of documentation for physiological studies (e.g. items such as conflict of interest statements are not likely to be perceived as necessary, in contrast to drug treatment studies). The feasibility of blinding in some circumstances – such as streptozotocin pre-treated animals, and dextrose infusions in animals monitored by regular blood sampling – is also unclear.

We did not identify significant publication bias by a conventional analysis, but a recent publication highlights alternative methods such as 'trim-and-fill' analysis [526] to estimate the effect of publication bias on efficacy outcomes in systematic reviews and meta-analysis of animal models of stroke [527]. This method estimates the number of unpublished studies that may exist based upon the estimated proportion of unpublished data from the Egger Regression. While this approach suggested that up to one sixth of studies were unpublished (with effect sizes potentially altered by one third) the relevance of publication bias to physiological studies rather than therapeutic agents is not clearly established. Even with an effect of this magnitude on overall estimates, hyperglycaemia would have a highly significant adverse effect on infarct volume.

In summary, while animal focal ischaemia models indicate exacerbation of infarct volume by acute hyperglycaemia, this effect reflects a particularly detrimental effect in a model of type I (insulin deficient) diabetes, with both a smaller effect size and considerable heterogeneity in acute hyperglycaemia induced by dextrose infusion, which may represent a situation analogous to "stress hyperglycaemia". No study has reported the effects of hyperglycaemia in an insulin resistant model, which is potentially the most clinically relevant scenario. Few studies have investigated the effect of insulin on infarct volume, and since the concentrations of blood glucose induced in the model systems have generally greatly exceeded those relevant to clinical practice, we have no adequate data to support current clinical guidelines suggesting intervention at concentrations of >140mg/dl (7.7mmol/l) or greater.[294]

Whilst a large body of clinical observational evidence indicates that hyperglycaemia in the acute phase of stroke is associated with poorer outcomes, typically defined by death or dependence 90 days after the event, few studies have addressed the mechanism by which this may occur. Many mechanistic proposals are based on inferences drawn from animal models of focal ischaemia. In particular, the rationale for acute intervention to lower blood glucose (usually by administration of insulin) is based on an assumption that the adverse effect of hyperglycaemia relates predominantly to exacerbation of acute infarct evolution. However, insulin treatment carries significant risks, and the clinical evidence supporting an acute effect of hyperglycaemia is predominantly observational and open to alternative interpretations.

Data relating hyperglycaemia to early infarct volume can alternatively be explained by "stress" hyperglycaemia resulting from more severe infarcts, for example. Recent observations that hyperglycaemia carries a higher risk of symptomatic intracerebral haemorrhage in patients treated with intravenous alteplase may be confounded by a similar effect. An association of glucose concentration with lactate concentration in the infarct core, and with infarct growth in a small observational study using MRI is mechanistically plausible, but without an intervention arm, could not discount the possibility that the relationship with blood glucose is not causal. In addition, the assumption that lactate itself is toxic in the ischaemic brain is not necessarily correct, with other evidence suggesting that lactate is instead produced as an alternative metabolic substrate and is beneficial.[176, 332] Only one small clinical trial has attempted to address these mechanistic hypotheses using advanced imaging, and found that insulin treatment reduced blood glucose and lactate concentration in brain, but had no effect on infarct growth.[528]

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Table 3-2 Summary of excluded studies

Study	Hyperglycemic agent	No. Animals Normal Glucose	Size Infarct Normal Glucose	No. Animals Hyperglycemia	Size infarct Hyperglycemia	Reason for exclusion
Kamada 2007[489]	STZ	6	100 graph	6	300 graph	Incomplete data on infarct size
Rizk 2006[490]	STZ	6	3.02 +/- 2.4	6	Approx 10x greater	No report of infarct size
Gisselsson 1999[493]	DEX	Unclear	115 graph	Unclear	125 graph	Incomplete data on animal numbers and infarct size
Li 1998a[529]	DEX	None	None	?6	50	Incomplete data on animal numbers and infarct size
Quast 1995[492]	STZ	7	60 graph	7	400 graph	Incomplete data on infarct size
Zhang 2003[494]	STZ	6	100 graph	6	500 graph	Incomplete data on infarct size

Comparison	Hyperglycemic agent	Permanent or reversible MCAO	Species	Strain	**Time of induction of hyperglycemia with dextrose**	Experimental glucose level (mmol/l)	Control glucose level (mmol/l)
Araki 1992	Dextrose	Reversible	Cat		After	Above 27	Below 8.9
Berger 1989	Dextrose	Permanent	Rat		Before	19.2	8.2
Bomont 1995a	Dextrose	Permanent	Rat		Before	30.8	Below11.1
Bomont 1995b	Streptozotocin	Permanent	Rat			30.1	Below11.1
Combs 1990	Dextrose	Permanent	Cat		Before	25.5	12
De Courten-Myer 1989a	Dextrose	Reversible	Cat		Before	22	6
De Courten-Myer 1989b	Dextrose	Permanent	Cat		Before	22	6
De Courten-Myer 1994a	Dextrose	Permanent	Cat		Before	12.9 – 24.7	elow 9.2
De Courten-Myer 1988	Dextrose	Permanent	Cat		Before	20	6.5
De Courten-Myer 1994b	Dextrose	Reversible	Rat		Before	18.2-22.5	Below 9.4
Duverger 1988a	Streptozotocin	Permanent	Rat	Fischer		25.3	8.3
Duverger 1988b	Streptozotocin	Permanent	Rat	Wistar		30.6	8.8
Huang 1996a	Streptozotocin	Permanent	Rat			25	8.1
Huang 1996b	Streptozotocin	Reversible	Rat			25.8	8
Kraft 1990	Dextrose	Permanent	Rabbit		Before	Above 22.8	Below 9.2
Li 2004	Streptozotocin	Permanent	Rat			24.3	6.1
Liu 2007a	Dextrose	Permanent	Rat		Unclear	22.4	4.8
Liu 2007b	Dextrose	Reversible	Rat		Unclear	22.7	4.8
Nedergaard 1986a	Streptozotocin	Reversible (10min)	Rat			Above 20	Below 9.2
Nedergaard 1986b	Streptozotocin	Reversible (15min)	Rat			Above 20	Below 9.5
Nedergaard 1986c	Streptozotocin	Reversible (5min)	Rat			Above 20	Below 9.5
Nedergaard 1987a	Streptozotocin (2 days)	Permanent	Rat			25	7.3
Nedergaard 1987b	Streptozotocin (4 months)	Permanent	Rat			28	7.3
Nedergaard 1987c	Dextrose	Permanent	Rat		After	32	7.3
Quast 1997a	Streptozotocin	Permanent	Rat			26.5	9
Quast 1997b`	Streptozotocin	Reversible	Rat			26.5	9
Slivka 1991a	Dextrose	Permanent	Rat	SHR	Before	22.2	7
Slivka 1991b	Streptozotocin	Permanent	Rat	SHR		26.4	8.8
Wei 1997	Streptozotocin	Reversible	Rat			20.9	6.1
Wei 1998	Streptozotocin	Reversible	Rat			25.6	7.2
Wei 2003	Dextrose	Reversible	Rat		Before	19.6	4.5
Zasslow 1989	Dextrose	Permanent	Cat		Before	31.2	11.6
Martin 2006	Dextrose	Reversible	Rat		Before	18.4	8.6
Kittaka1996a	Streptozotocin	Reversible	Rat			15.5	4.3
Kittaka1996b	Dextrose	Reversible	Rat		Before	14.8	4.3^{2}

Table 3-3 Characteristics of studies included in the study

Study	*Target blood glucose with insulin treatment*	*Type of insulin*	*Timing of insulin*	*Baseline status*	*Species*	*Strain*	*Blood glucose levels (mmol)*	*Type of MCAO*	*Control*
Bernaudin and MacKenzie (1998), 3rd comparison c	Normal	Bovine insulin (Sigma)	Infusion started 2 days earlier	Diabetic streptozotocin	Rat	Fischer	9.8	Permanent	
Combs *et al* (1990), 2nd comparison b	Normal	Regular human insulin (Squibb Novo Inc., Princeton, NJ, USA) Somatostatin was also received	Before and after	Glucose infusion	Cat		24.9 initially, 11.4 at 4 hours	Permanent	
de Courten-Myers *et al* (1994), 3rd comparison c	Hyperglycaemic	Regular human insulin	Bolus before then infusion	Normoglycaemic	Cat		Between 2.9 and 7.6	Permanent	Dextrose infusion
de Courten-Myers *et al* (1994), 4th comparison d	Hyperglycaemic	Regular human insulin	Bolus before then infusion	Normoglycaemic	Cat		Between 3.3 and 3.7	Reversible	Dextrose infusion
Zhu (2004), 1st comparison a	Normal	Bovine crystalline zinc insulin (CZI) and longer-acting zinc insulin suspension	60 minutes before	Normoglycaemic	Rat	Wistar	5 before ischaemia, 2.5 at 3 hours after ischaemia	Reversible	Normoglycaemic
Zhu (2004), 2nd comparison b	Normal	Bovine CZI and longer-acting zinc insulin suspension	20 minutes after ischaemia	Normoglycaemic	Rat	Wistar	11.2 before ischaemia, 2.2 at 3 hours	Reversible	Normoglycaemic
Hamilton (1998), 1st comparison a	Normal	Porcine/bovine CZI and longer-acting zinc insulin suspension (Lente)	50 to 70 minutes before	Slightly hyperglycaemic with glucose	Rat	Sprague	Between 3.4 and 3.6	Reversible	Normoglycaemic
Hamilton (1998), 2nd comparison b	Hyperglycaemic	Porcine/bovine CZI and longer-acting zinc insulin suspension (Lente)	50 to 70 minutes before	Normoglycaemic	Rat	Sprague	Between 7 and 10.1	Reversible	Normoglycaemic
Inoué (1992)	Normal	Actrapid S (Novo, France) fast, short acting	Immediately after MCAO	Normoglycaemic	Rat	Fischer	4.7	Permanent	Normoglycaemic
Nedergaard and Diemer (1987), 4th comparison d	Hyperglycaemic	Crystalline porcine insulin (Leo)	2 hours before	Hyperglycaemic	Rat	Wistar	2	Permanent	Normoglycaemic

MCAO, middle cerebral artery occlusion.

4 A Systematic review of therapeutic interventions for Post-Stroke Hyperglycemia in Humans

4.1 Introduction

Although clear causality between post stroke hyperglycemia and poor clinical outcome has not been established there have been various attempts to control blood glucose levels with the intention of improving clinical outcome.

The majority of these attempts have relied upon an intravenous infusion of insulin running at a rate that varies in proportion to the level of blood glucose as measured using a capillary blood glucose monitoring machine. While this approach may appear to be mechanistically sensible in some ways there are several problems with this system, some that are universal to all patients and some that are specific to patients who have suffered from a stroke

These include

Dysphagia – many stroke patients are unable to swallow and cannot have a normal diet. This can result in malnutrition and an increased risk of hypoglycaemia when exposed to insulin. If feeding is instituted via a nasogastric tube the patient may become hyperglycaemic again.

Inability to speak – Stroke may cause aphasia or dysarthria making it difficult for patients to express themselves. A patient with communication problems may be unable to tell clinical staff of symptoms of hypoglycaemia that may require alteration of an insulin infusion rate.

Reduced level of consciousness – in a severe stroke a patient may have a reduced level of consciousness. In a drowsy patient the symptoms of hypoglycaemia may be masked by the effects of stroke. This situation may be analogous to the risk of nocturnal hypoglycaemia in insulin treated diabetes mellitus.

These stroke related problems may increase the risk of a patient becoming hypoglycaemic without any overt physical symptoms or signs.

4.1.1 Definition of hypoglycaemia

Hypoglycaemia is considered present when blood glucose level is below 2.8mmol/L (50 mg/dl). Although 3.3 or 3.9mmol/L (60 or 70 mg/dl) is commonly cited as the lower limit of normal glucose, different values (typically below 40, 50, 60, or 70 mg/dL) have been defined as low for different populations, clinical purposes, or circumstances. A normal healthy person can occasionally have a glucose level in the hypoglycemic range without symptoms or disease.

Most cases of hypoglycaemia are due to exogenous insulin administration although it can be seen in fasting, severe liver disease and insulinoma. Many prescription drugs can cause hypoglycaemia as a side effect.

4.1.2 Risk and consequences of hypoglycaemia

The use of intravenous insulin puts a patient at risk of hypoglycaemia with resultant neurological injury and other complications. Hypoglycaemia can quickly cause coma, cardiac dysrhthymia and death. Neurological symptoms can include altered mental function, seizures, visual disturbance, speech disturbance, ataxia and focal motor deficits. Indeed acute stroke patients should always be checked for hypoglycaemia before a diagnosis of stroke is made.

A small functional magnetic resonance imaging study involving 6 patients showed a generally decreased level of activity in motor areas when plasma blood glucose level was experimentally lowered to 2.5mmol/l for approximately 40 minutes.[530]

4.1.3 The use of intensive insulin regimes in other patient groups

The vogue for the use of intensive insulin regimes in critically ill patients was kick-started in 2001 with the publication of a study by Van den Berghe and colleagues in the *New England Journal of Medicine*.[303] This study involved mechanically ventilated patients admitted to a surgical intensive care unit (ICU) and was performed

on a prospective, randomised controlled basis. A total of 1548 patients were enrolled into this study before being randomly allocated to either intensive therapy (target blood glucose 80-110mg/dl) or conventional therapy (insulin infusion only starting if blood glucose exceeded 215mg/dl with a target of 180-200mg/dl).

Van den Berghe *et al* found that mortality dropped from 8% in the conventional care group to 4.6% in the intensive insulin group ($p<0.04$). The paper concluded that intensive insulin therapy improved outcomes in a surgical intensive care unit. This paper is highly cited (2553 times by 2/2/2010) and has had a visible impact on the medical literature and clinical practice as seen in Figure 16.

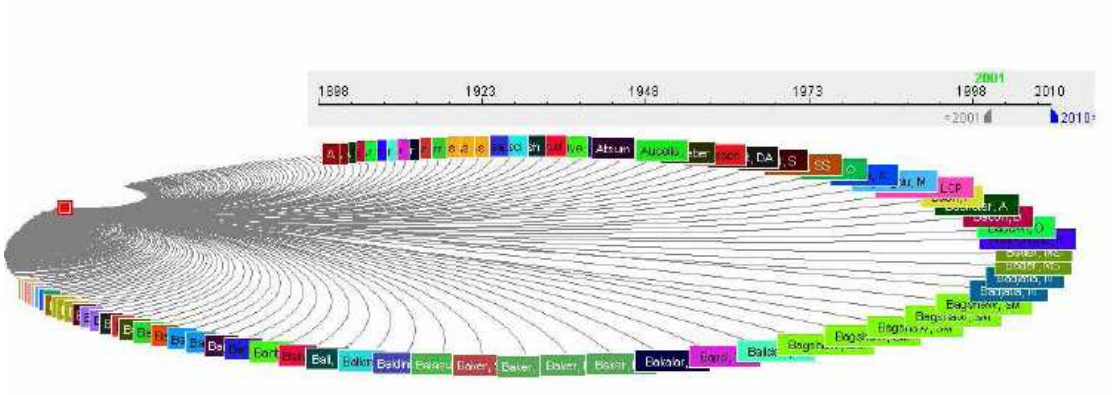

Figure 16 Graphical representation of citation map for Van Den Berghe et al 2001 from ISI Web of Science (accessed 2/2/2010)

More recently a meta-analysis performed by Griesdale and co-authors was published in 2009.[531] This meta-analysis included 26 trials involving a total of 13 567 patients. The pooled relative risk (RR) of death with intensive insulin therapy compared with conventional therapy was 0.93 (95% confidence interval [CI] 0.83–1.04). Only 14 trials reported hypoglycaemia and among these trials the pooled RR with intensive insulin therapy was 6.0 (95% CI 4.5–8.0). Different targets of intensive insulin therapy (glucose level $\leq$ 6.1 mmol/L v. $\leq$ 8.3 mmol/L) did not influence either mortality or risk of hypoglycaemia. The paper concludes that intensive insulin therapy significantly increases the risk of hypoglycaemia with no overall mortality benefit for critically ill patients.

Another recent meta-analysis looked at 29 randomised control trials including 3 of the trials discussed later in this chapter. A total of 8432 patients were included in these trials. There was no difference in hospital mortality between tight glucose control and usual care (21.6% vs. 23.3%; RR, 0.93; 95% confidence interval 0.85-1.03). Tight glucose control was associated with a significantly increased risk of hypoglycaemia (glucose < 40mg/dL; 13.7% vs. 2.5%; RR, 5.13; 95% CI 4.09-6.43).[306]

A post-hoc analysis of the DIGAMI data suggested that hypoglycaemia during initial hospitalisation after myocardial infarction was not a risk factor for mortality or morbidity in patients with type II Diabetes Mellitus although hypoglycaemic episodes were more prevalent in patients who were at risk for other reasons.[532]

In a retrospective cohort study of 2582 patients with Type II DM treated on general medical wards hypoglycaemia was observed in 7.7% of admissions.[533] Multivariate analysis of this cohort suggested that each additional day on which a hypoglycaemic event occurred increased the odds of inpatient death by 85.3% (p=0.009) and the risk of death within a year by 65.8% (p=0.0003). Inpatient death was more likely with lower levels of hypoglycaemia with risk of death increasing threefold for every 10mg/dl drop in lowest blood glucose level

In a series of papers published in Critical Care Medicine in 2006 Vriesendorp and colleagues looked at the consequences of and risk factors for hypoglycaemia in an intensive care unit.[534, 535] The hypoglycaemic population of 156 studied was too small to clearly identify a causal link between hypoglycaemia and poor outcome although there were 3 cases where a causal link was likely. Risk factors for hypoglycaemia were found to include continuous intravenous haemofiltration, decrease in nutrition without adjustment of insulin dose, diabetes mellitus, insulin use, sepsis and inotropic support were associated with hypoglycaemia.

Another study attempted to identify risk factors for hypoglycaemia in medical/surgical intensive care unit (ICU) patients and to assess the association between hypoglycaemia and mortality in this group[536]. In this paper hypoglycaemia was defined as a blood glucose level below 2.2mmol/l. The 523 patients in the study had been enrolled in a randomised control trial where patients with an admission

blood glucose of above 6.1mmol/L and assigned to either intensive or conventional insulin therapy.

Hypoglycaemia was observed in 16% of patients and was more common in the intensive insulin group (adjusted odds ratio, 50.65; 95% confidence interval, 17.36 – 147.78; $p < 0.0001$). After adjustment for potential confounding factors hypoglycaemia was not significantly associated with increased mortality (adjusted hazard ratio, 1.31; 95% CI, 0.70 –2.46; p=0.40) although patients with an admission glucose of below 10mmol/L had an increased mortality with hypoglycaemia (adjusted hazard ratio, 4.43; 95% CI, 1.36–14.44; p =0.01). There was also a non-significant trend towards increased mortality with blood glucose below 1.2mmol/L **(**adjusted hazard ratio, 2.56; 95% CI, .85–7.70; p= 0.10).

A 2006 combined analysis by Van den Berghe and colleagues used the pooled data set from both the original 2001 New England Journal of Medicine paper and from a later 2006 paper in the same journal.[303, 304, 537] This combined analysis supported their original findings. As these positive results are not supported by later meta-analysis and these randomised control trials have been staged in a single centre it is possible to hypothesise that the host intensive care unit may be of particularly high quality and may be undertaking additional procedures that are not carried out elsewhere. All patients were given 200-300g of intravenous glucose from admission to ICU and additional nutrition (enteral or parenteral) was started as soon as possible.

From the evidence that is currently available I do not believe that intensive insulin therapy should be used routinely in intensive care unit due to an uncertain clinical benefit and an increased risk of hypoglycaemia. The concerns that I have over the use of such treatment regimes in ICU are also applicable to stroke units.

In 2011 new guidelines were published by the American College of Physicians making recommendations for the use of intensive insulin therapy for the management of glycaemic control in hospitalized patients.[538] The first recommendation was that intensive insulin therapy should not be used in non-surgical ICU/medical ICU patients with or without diabetes mellitus. This was a strong recommendation based on moderate quality evidence. The second recommendation was that intensive insulin

therapy should not be used to normalise blood glucose levels in surgical or medical ICU patients with or without diabetes mellitus. The final recommendation was that if insulin therapy is used in these patients a target blood glucose level of 7.8 to 11.1mmol/L would be appropriate.

ACP Clinical Practice
American College of Physicians
GUIDELINES

Summary of the American College of Physicians Guideline on the Use of Intensive Insulin Therapy for the Management of Glycemic Control in Hospitalized Patients

Disease or condition	Inpatient hyperglycemia
Target audience	Internists, hospitalists, and other clinicians
Target patient population	Adults with inpatient hyperglycemia
Interventions	Intensive insulin therapy
Outcomes	Mortality
Recommendations	*Recommendation 1:* ACP recommends not using intensive insulin therapy to strictly control blood glucose in non-SICU/MICU patients with or without diabetes mellitus (Grade: strong recommendation, moderate-quality evidence). *Recommendation 2:* ACP recommends not using intensive insulin therapy to normalize blood glucose in SICU/MICU patients with or without diabetes mellitus (Grade: strong recommendation, high-quality evidence). *Recommendation 3:* ACP recommends a target blood glucose level of 7.8 to 11.1 mmol/L (140 to 200 mg/dL) if insulin therapy is used in SICU/MICU patients (Grade: weak recommendation, moderate-quality evidence).
Clinical considerations	• This guideline refers to hospitalized patients with hyperglycemia. • Critically ill medical and surgical patients who are hyperglycemic have a higher mortality rate. • Most clinicians agree that prevention of hyperglycemia is an important intervention. • The range of optimal glucose level is controversial. A few studies show that IIT improves mortality, whereas most have shown that patients who receive IIT have no reduction in mortality and have a significantly increased risk for severe hypoglycemia.

IIT = intensive insulin therapy; MICU = medical intensive care unit; SICU = surgical intensive care unit.

Figure 17 – The American College of Physicians guidelines on the use of intensive insulin therapy for glycaemic control in hospitalized patients

4.1.4 Insulin and the insulin receptor

Insulin is a hormone of metabolic homeostasis that is essential for human life. Insulin promotes the uptake of blood glucose into liver, muscle and fat cells. Glucose is stored as glycogen in the liver and the muscles. If insulin is not present the body uses fat for energy and glucose levels in the blood stream rise. In Type I diabetes mellitus there is a deficiency of endogenous insulin while in Type II diabetes mellitus insulin may be present at a normal or increased level but the target tissues are insensitive to insulin.

Insulin is a small protein containing 51 amino acids and has a molecular weight of 5808 Da.[539] Insulin is a polypeptide hormone that is synthesised in the B cells of the

islets of Langerhans (located in the pancreas). Initially proinsulin is produced before it is cleaved into mature insulin and C-peptide. Insulin secretion is principally regulated by glucose although insulin release can also be stimulated by some amino acids and inhibited by epinephrine.

Insulin binds to a receptor on the cell surface. The insulin receptor is a tyrosine kinase receptor which phosphorylates other proteins within the cell.[540] One such protein is the 'insulin receptor substrate 1' (IRS-1) protein which increases the number of high affinity glucose transporter (GLUT4) molecules on the outer membrane of insulin responsive tissues.[541] GLUT4 is transported from intracellular vesicles to the cell surface of muscle or adipose tissue cells where it potentiates the uptake of glucose from the blood into these cells.[542]

4.1.5 Vascular effects of insulin

Insulin is thought to have certain vascular effects on both the vascular endothelium and the vascular smooth muscle cells (VSMCs). Insulin has vasodilatory effects and may inhibit apoptosis of vascular endothelium. Excess insulin may have damaging effects on large arteries. Insulin may cause VSMCs to contract in the short term. Over longer periods of time hyperinsulinaemia may sensitise VSMCs promoting hypertension and atherogenesis.[543]

4.1.6 Central nervous system effects of insulin

Historically it was thought that insulin had no direct effect on the brain and only impacted on central nervous system (CNS) function by producing peripheral hypoglycaemia. This view was challenged in 1978 when Havrankova and colleagues identified insulin receptors in the brain.[544]

A positron emission tomography study used 18-flurodeoxyglucose to examine the effects of basal insulin levels on global and regional brain glucose uptake in 8 healthy men.[545] Endogenous insulin production was suppressed with somatostatin and subjects were given an insulin infusion followed by a saline infusion. Insulin appeared to regulate human brain glucose uptake, most prominently in the cortical areas.

It is now thought that insulin may have CNS effects which influence both glucose and energy homeostasis and it has been suggested that an abnormal CNS response to insulin may have a key role in the relationship between Type II Diabetes Mellitus and obesity.[546] Indeed, a link between the brain and diabetes was initially hypothesised in 1854 when the renowned French physiologist Claude Bernard observed that diabetes (glucosuria) could be induced in animals by puncturing the floor of the fourth ventricle.[227] Supporters of this idea argue that unless brain tissue is spared from the tissue insulin insensitivity features of Type II DM the action of insulin on the CNS must be abnormal.[547] Figures 17 and 18 illustrate the role that the brain is thought to play in the regulation of blood glucose.

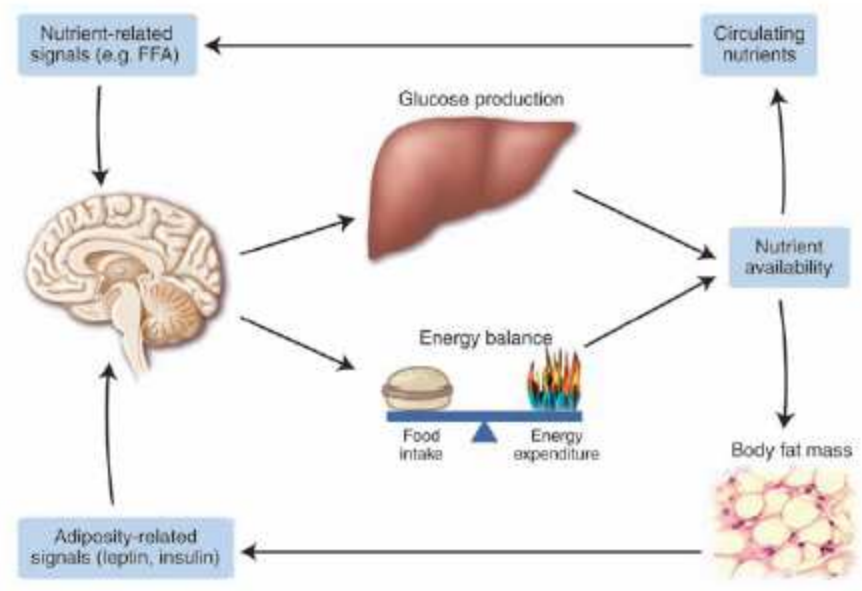

Figure 18 – The role of the brain in the regulation of glucose levels (Schwartz et al 2005)

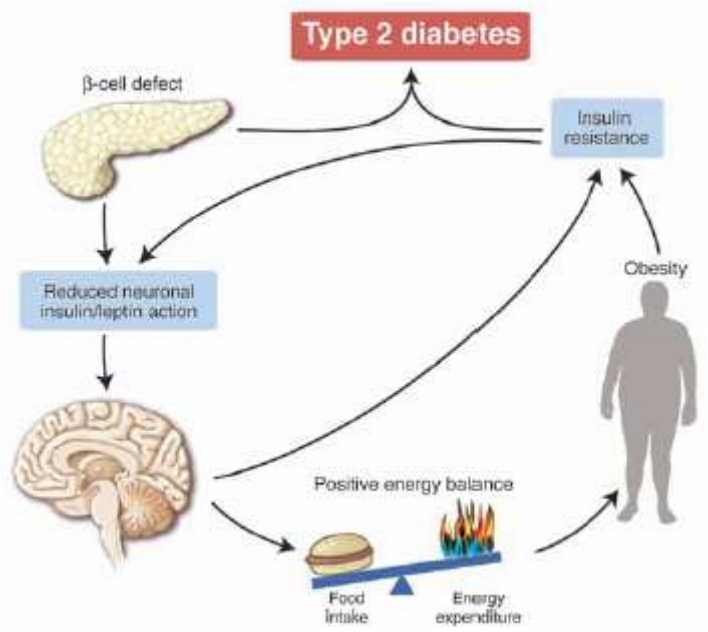

Figure 19 - Possible role of the brain in the relationship between glucose, insulin, Type II diabetes and obesity (Schwartz et al 2005) [548]

As well as having a prominent role in metabolic homeostasis insulin may also have a role in the regulation of neuronal survival, learning and memory.[549] Both Type 1 and Type 2 diabetes mellitus are associated with cognitive changes. Type 2 DM is clearly

associated with accelerated cognitive decline although the underlying mechanism is unclear. In the 1999 Rotterdam study Ott and colleagues established that patients with DM had almost double the risk of developing dementia (relative risk [RR] 1.9 [1.3 to 2.8]) and that patients treated with insulin were at the greatest risk (RR 4.3 [1.7 to 10.5]).[550] This increased risk with insulin treatment may reflect the severity of the underlying diabetes or it could be due to side effects of the insulin treatment. A later systematic review supports the findings of this study although the underlying pathological mechanism remains unclear.[551]

In a 2009 paper from the Journal of the American Medical Association, Whitmer and colleagues looked for an association between hypoglycaemia and increased risk of dementia in a population of patients with Type II Diabetes Mellitus.[552] This paper was a longitudinal cohort study involving 16667 patients with Type II DM between 1980 and 2007. One episode of hypoglycaemia was associated with an increased risk of developing dementia (HR, 1.42; 95% CI, 1.12-1.78) while two or more episodes were associated with an even greater risk (HR, 2.36; 95% CI, 1.57-3.55). Obviously we cannot say that hypoglycaemia causes dementia as an inability to recognise the warning signs of hypoglycaemia may just be an early feature of dementia.

4.1.7 Insulin therapy in stroke patients

Therapeutic interventions to lower blood glucose have relied upon insulin infusion, or subcutaneous injection with dose adjusted according to capillary blood glucose monitoring, but no single regime is generally agreed upon.[553] Both European and American guidelines recommend active intervention to lower blood glucose, but acknowledge inadequate evidence, and differ in their specific management advice.[293, 294] The largest single randomised, controlled trial (RCT) of glycaemic control after stroke to date was the Glucose-Insulin Stroke Trial (GIST-UK), which used combined glucose, potassium and insulin (GKI) infusion, and was neutral.[242] Other pilot studies using intensive insulin therapy for post-stroke hyperglycemia have been completed.[246, 259, 283, 554]

We undertook a systematic review of the published data on insulin treatment of post-stroke hyperglycemia. We examine the feasibility and safety of insulin treatment for

PSH. We have also combined data from the published literature to examine the effect of insulin on patient outcomes in PSH.

4.2 Methods

4.2.1 Identification of papers

We identified relevant papers with Ovid and Embase searches with a search strategy as detailed in appendix 1. Additional studies were identified from reference lists and conference abstracts for additional relevant studies. We checked the Stroke Trials Registry for relevant ongoing trials.[555] We used the ISI Web of Science citation index to identify papers citing studies that were already identified.

4.2.2 Data extraction

Data on study populations, care setting, insulin regimes, time-to-initiation of treatment, total insulin doses, change in blood glucose and hypoglycaemia incidence were extracted, together with data on mortality and functional outcome.

4.2.3 Meta-analysis

Meta-analysis was performed to look for the relationships between insulin exposure and mortality or clinical outcome.

Data from studies with clearly defined control groups that reported at least mortality rates were combined. The reporting of clinical outcomes was heterogeneous so we defined favourable clinical outcome as modified Rankin score (mRS) 0-2 or Extended Glasgow Outcome Score (eGOS) favourable (moderate disability and good outcome).to produce a dichotomised ‘good’ clinical outcome.

StatsDirect, version 2.7.3 (StatsDirect Ltd, Cheshire, UK) was used to generate a DerSimonian-Laird random effects model that expresses the difference between groups as an odds ratio, and 95% confidence interval.

4.3 Results

4.3.1 Papers identified

We identified 17 studies involving 2587 patients.[246, 266, 273, 283, 284, 298, 299, 554, 556-561] These included one randomised control trial (RCT) of 933 patients, 9 pilot studies containing between 10 and 116 patients, and 6 cohort studies, one of which included 851 patients. One RCT involved a mixed population of neuroscience intensive care unit patients with a subset of 15 stroke patients.[561] One paper used the control group from another paper by the same group.[264, 560] There were 1421 patients in RCTs and 1038 in cohort studies

Data from some recently completed or ongoing trials were not available for analysis at the time of this search. Information on these studies (GRACE, Insulinfarct[562])[3] can be obtained at http://www.strokecenter.org/trials/

4.3.2 Study populations

The study populations are described in Table 4.1. Thirteen studies included only patients with acute ischaemic stroke although GIST and one other study also included patients with intracerebral haemorrhage. One study included ischaemic stroke patients as a subgroup of neuro-intensive care patients. Eight studies included patients with type II diabetes mellitus whilst excluding diabetic patients who were dependent on insulin. The proportion of patients with DM varied from 0% to 91% whilst the lower limit of plasma blood glucose for inclusion varied between 5.6 mmol/l and 9.4mmol/l.

Table 4-1 Characteristics of included studies

Study (year)	Type of study	Setting	Number of Patients	Number with diabetes	Glucose level for inclusion	Target glucose range	Mean onset to treatment	Treatment duration (h)
GIST[242] (2007)	RCT	Ward	933	154 (16.5)	6	4 - 7	13.3	24
Walters[259] (2006)	RCT	Ward	25	13 (52)	8	5 - 8	9.1	48
THIS[563] (2008)	RCT	Ward	46	42 (91)	8.3	<7.2	<12	Up to 72
GRASP[246] (2009)	RCT	Ward	74	44 (59)	6.1	3.8-61 tight	12.3 tight	120
MISS[298] (2008)	RCT	Ward	40	13 (32.5)	Unclear	4.4 - 6.1	17.2 intensive 19.5 control	120
SELESTIAL[554] (2010)	RCT	Ward	40	12 (30)	7	4 - 7	20.8	24-72
Vriesendorp[564] (2009)	RCT	Ward	33	10 (30)	6.1	Not specified	13.2 basal	120
Scott[143] (1999)	RCT	Ward	53	10 (19.9)	7	4 - 7	<24	24
Staszewski[266] (2011)	RCT	ICU	50	0	7	4.5 - 7	<12	24
Bruno[284] (2004)	Cohort	Ward	24	21 (87.5)	9.4	3.9 - 7.2	Unclear	Up to 72
Kruyt[264] (2009)	Cohort	Ward	10	2 (20)	6.1	4.4 - 6.1	<24	120
Putaala[565] (2010)	Cohort	Ward	851	113 (13.3)	8	<8	'with tpa'	At least 48
Azevedo[558] (2009)	Cohort	ICU	34	Unclear	Unclear	<7.7	Unclear	Unclear
Kim[273] (2009)	Cohort	ICU	115	47 (41)	5.6	4.4 - 7.2	<48	24
Kanji[561] (2009)	Cohort	ICU	15/100	0	9.1	7 - 9	Unclear	At least 24
Fukuda[557] (2006)	Cohort	ICU	116	Unclear	8.3	<8.3	Unclear	Unclear

4.3.3 Level of care

In 5 studies patients were managed in ICU while in the remaining 11 studies patients were managed in stroke unit or on a ward.

4.3.4 Description of insulin regimes

Five studies used a Glucose-Potassium Insulin (GKI) regime while 10 used variable rate insulin IV infusions (Table 4.2). One cohort study used subcutaneous insulin injections for glucose levels above 8mmol/L switching to an IV infusion if glucose was above 16mmol/L.

All of the infusion protocols required frequent monitoring of capillary blood glucose. Initially CBG was checked every 1-2 hours with every insulin infusion. In all cases CBG was checked at least every 2 hours. Dose adjustments were required frequently for GKI regimes: in GIST, 41.2% of patients required at least one dose change; in SELESTIAL, a mean of 4.8 dose changes per patient was required. RCTs using insulin sliding scales have not reported how regularly doses are adjusted.

Control groups in RCTs were heterogeneous.[273, 284]

Table 4-2 Properties of insulin protocols and glycaemic outcomes

Study	Method of insulin delivery	Definition of hypoglycaemia (mmol/L)	Incidence of hypoglycaemia (%)	Glucose monitoring (hours)
GIST[242]	GKI	<4	16	2
Walters[556]	IVI	Not stated	8	2
THIS trial[283]	IVI	3.33 - 4.44 (moderate) <3.33 (severe)	35	1
Bruno[284]	IVI	<3	46	1-2
MISS trial[298]	IVI	<3.3	25	1
SELESTIAL[554]	GKI	<4	80	1-2
Vriesendorp[564]	IVI	Not stated	3.8(basal) 1.65 (meal)	1-2
Scott[143]	GKI	<4	17.9	1-2
GRASP[246]	GKI	<3	30 tight 4 loose	1-4
Staszewski[266]	IVI	<3.3	8	1-4
Kruyt[264]	IVI	<3.5	20	1-2
Azevedo[558]		Not stated	Group A -10 Group B – 46	Not stated
Kim[273]	GKI	<4.4	79.1	2
Fukuda[557]	IVI	Not stated	Not stated	Not stated
Kanji[561]	IVI	Mild <5	2 (severe)	1-2
Putaala[281]	SC and IVI	Not stated	Not stated	1

GKI – glucose, potassium and insulin infusion, IVI – intravenous insulin infusion SC – subcutaneous insulin

4.3.5 Glycaemic outcomes

Hypoglycaemia incidence was not reported for every study, and definitions varied. Three papers reported only symptomatic hyperglycaemia, while 11 studies reported incidence of biochemical hypoglycaemia using 7 different definitions, ranging from <2.2mmol/l to <5mmol/l. (Table 4.2) Symptomatic hypoglycaemia rates varied between 2% and 46%. In an ITU setting symptomatic hypoglycaemia was present in between 2% and 9.6% of cases. In a ward setting the rate varied from 4% to 46%. Biochemical hypoglycaemia was seen in between 4% and 79.1% of patients treated with insulin. In GIST the hypoglycaemia rate was 16%. .

Different studies report variable effects of insulin on glucose level. These differences could be due to many factors such as monitoring variations or protocol violations. Where stated the mean difference in blood glucose between insulin and control arms varied from 0.57mmol/L in GIST to 3.7mmol/L in THIS. Mean daily insulin dose varied from 13.3 units over 24 hours to 81.6 units over 24 hours.

4.3.6 Meta-analysis of mortality outcomes

Only RCTs were included in the meta-analysis. Mortality data were reported in 8 studies, and functional outcomes in eight studies, 7 of which used the modified Rankin Scale and one of which used the extended Glasgow Outcome Scale.

Insulin had a neutral effect on mortality, odds ratio 1.16 (95% CI 0.89 to 1.51, p= 0.286, figure 19).

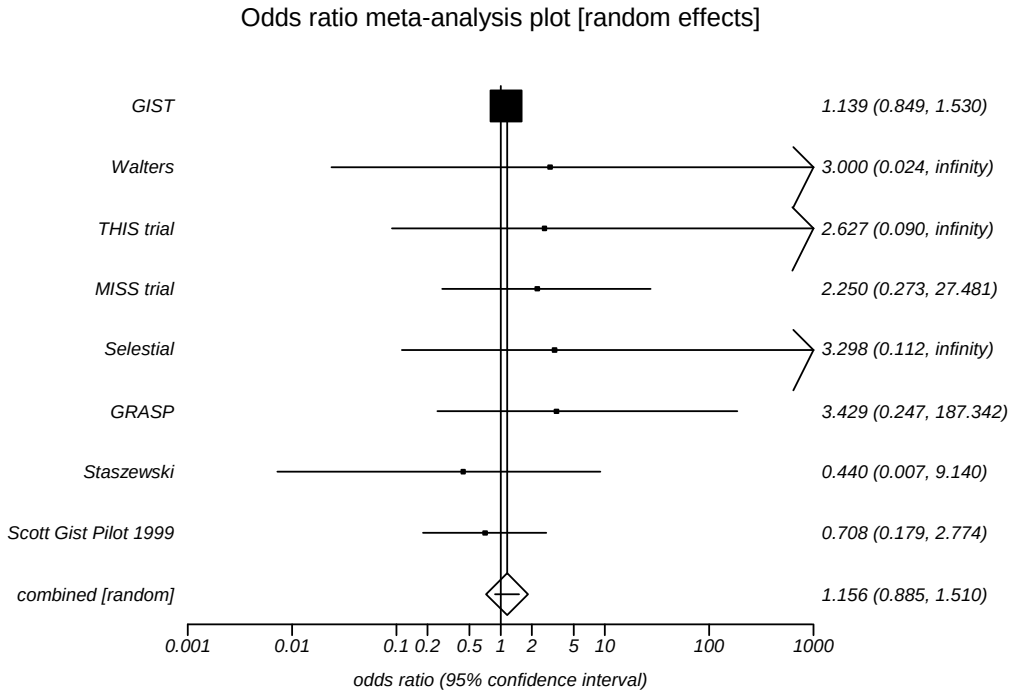

Figure 20 - Meta-analysis of effect of insulin treatment on mortality

Insulin also had a neutral effect on favourable functional outcome (odds ratio for favourable clinical outcome 1.03 (95% CI 0.69 to 1.54, p=0.8845, figure 20).

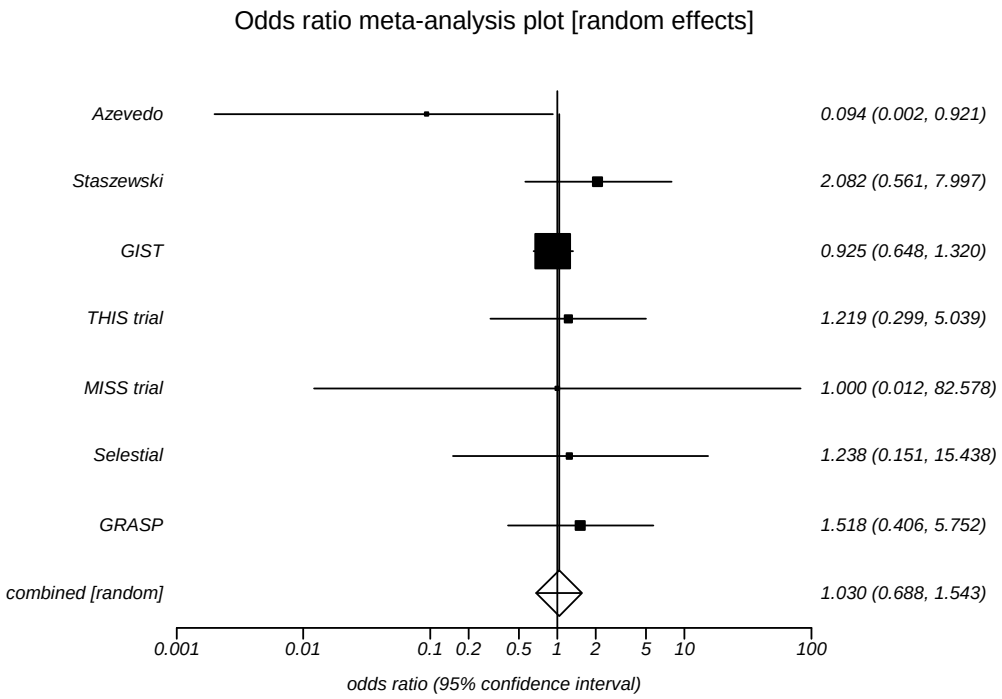

Figure 21 - Meta-analysis of effect of insulin treatment on clinical outcome

4.4 Discussion

Hyperglycaemia is consistently associated with poor outcomes after stroke, and active intervention to lower blood glucose is recommended by all major international guidelines on stroke care, although with poor grades of evidence supporting these recommendations.[146] The small number of patients included in RCTs may reflect in part the inclusion of protocols for glucose monitoring and intervention in routine stroke unit care, but also may relate to the considerable workload involved in delivering intensive insulin protocols, which is itself a consequence of the potential risks of hypoglycaemia.[566] The only RCT designed to have sufficient size to reliably detect even a large effect of intervention on clinical outcomes, the UK Glucose-Insulin Stroke Trial (GIST), recruited only around one third of the planned sample size, in addition to achieving only very minor reductions in glucose concentration in the active treatment arm compared to the control arm.[242] All other RCTs were small, predominantly single centre studies, and were intended as pilot trials only. There was marked heterogeneity of target populations, treatment regimes (insulin delivery method, target glucose concentration, duration, and monitoring frequency) and outcome reporting. Systematic review of the available data should therefore be interpreted with caution, but highlights a number of issues that will require to be addressed by planned trials.

4.4.1 Safety and feasibility

Effects of insulin on both mortality and functional recovery were neutral, but with confidence intervals that include the possibilities both of significant benefit and significant harm. This is consistent with the absence of any clear effect on outcome in a non-randomised cohort of acute thrombolysis patients in whom the majority of hyperglycaemic patients were treated with insulin.[281]

However, the wider context of trials investigating intensive insulin regimes for glycaemic control is important to consider. Meta-analysis of intensive insulin therapy in critically ill patients suggests that there is no overall mortality benefit and an increased risk of hypoglycaemia.[531] All insulin regimes are labour intensive and require close monitoring of patients. Even within intensive care or stroke unit environments, and as part of an RCT, the incidence of biochemical hypoglycaemia in

stroke patients was reported to be between 4% and 79.1%. In the large meta-analysis published by Weiner and colleagues examining the risks of tight glucose control in critically ill adults there was a significantly increased risk of hypoglycaemia (glucose <2.2mmol/L, 13.7% vs. 2.5%, RR 5.13, 95% CI 4.09 to 6.43).[306] Since hypoglycaemia may itself exacerbate brain injury, this is a potentially serious side effect. The potential harm from biochemical (as opposed to symptomatic) hypoglycaemia needs also to be considered.[173] In the GIST-UK trial, patients who had a decrease in plasma glucose of 2mmol/L or more in the first 24 hours were more likely to die than those who had a decrease of less than 2mmol/L (34% vs. 22%; OR 1.15 (0.86-1.51); p=0.009). Other insulin regimes have achieved greater reductions in glucose, for example by a mean of 3.7mmol/L in the THIS trial, but at the penalty of an incidence of biochemical hypoglycaemia of 35%. This was not prevented by hourly monitoring and symptomatic hypoglycaemia was only reported in 13% of cases.

It may be possible in future to improve safety by the use of real-time continuous glucose monitoring, but this is expensive and not widely available.[214] Computerised protocols have also been developed and may be used to improve the safety of insulin infusions in the future.[567]

4.4.2 Efficacy of regimes

Stroke trials to date have arguably largely failed to achieve their main biochemical goals, and therefore may differ from trials in other therapeutic areas, which have either targeted patients with higher blood glucose concentrations, or have more aggressively maintained blood glucose within a narrow range, or both.[300, 301, 303] The populations involved in stroke trials have generally had only mild hyperglycaemia at the time of trial entry, and in addition have documented significant decrease in blood glucose over time in control groups, probably as a consequence of lack of oral intake and avoidance of intravenous fluids containing sugars. These factors have ensured that in most trials there was only a small difference in glycaemic control between intervention and control arms. In GIST-UK the mean glucose concentration at entry was 8.43mmol/L, and the mean difference in glucose concentration between GKI-treated patients and controls after 24 hours of treatment was only 0.57mmol/L.[242]

The timing of initiation of insulin may be important, but is difficult to define from existing literature. Arguments that favour very early intervention are supported by observational data from thrombolysis trials and registries, that indicate a poorer outcome when hyperglycaemia precedes IV thrombolysis,[178, 568] possibly due to accelerated ischaemic damage by hyperglycaemia if present before blood vessel recanalization post-thrombolysis.[197, 198] Exacerbation of infarct growth by hyperglycaemia also supports the relevance of an early influence on the evolution of ischaemic damage.[569] In addition, any neuroprotective effect of insulin would be expected to be of greatest benefit if commenced early. Arguably, therefore, a mean time to start insulin of at least 9.1 hours after stroke onset in the reviewed trials may have missed the potential therapeutic window. Since the majority of the trials included here were intended only as pilot studies with biomarkers such as glycaemic control as the primary end-point, conclusions regarding effects on clinical end-points must be regarded with caution. On the other hand, the detrimental effect of hyperglycaemia appears to include patients in whom glucose is increased at any time within a much longer period, up to 48h after onset of ischaemia with sustained, rather than isolated hyperglycaemia, and later time points, being of much greater prognostic relevance than “admission” hyperglycaemia.[160, 161, 267, 281] If later intervention is undertaken, as may be justified in light of these data, then an additional factor to consider is the very high proportion of patients in whom isolated (>80%) or sustained (>50%) hyperglycaemia is evident.

The mechanism by which hyperglycemia exacerbates ischaemic brain damage remains unclear. A correlation of ischaemic lesion growth and infarct core lactate concentration on MRI supports the hypothesis that exacerbation of lactate accumulation due to anaerobic glycolysis in the face of hyperglycaemia is a specific mechanism for the harmful effects.[176] However, reduced lactate accumulation with GKI infusion was not associated with attenuation of infarct growth in the SELESTIAL trial,[554] and there is doubt regarding the role of lactic acidosis, since lactate may have neuroprotectant properties[332] and under certain circumstances may be the preferred energy substrate for the brain.[323] Acidosis in the presence of hyperglycemia and not lactate *per se* may be responsible for the aggravated cerebral ischaemia seen with hyperglycemia.[331]

4.4.3 Time of starting insulin

The timing of starting the insulin infusion may be important. If insulin does have a neuroprotective role in acute ischaemic stroke it would be logical to predict that the greatest benefit would be gained if insulin was administered in the hyperacute phase at the same time as tPA. While penumbra may persist up to 12 hours after onset of ischaemia the size of penumbra will diminish with time reducing any potential neuroprotective benefit from insulin.

The mean time to start insulin was at best 9.1 hours after stroke onset. There is a need for further trials looking at the administration of insulin in hyperglycaemic patients who are due to receive thrombolytic therapy. Hyperglycemia during ischaemia rapidly accelerates brain damage in stroke patients treated with tPA.[197] In the hyperacute setting a small bolus of insulin before tPA administration may have a protective benefit and further clinical trials involving insulin boluses instead of infusions may be useful.

4.4.4 Alternative approaches to the treatment of post stroke hyperglycemia

These data raise further questions about potential treatments for post-stroke hyperglycaemia.

4.4.5 Should we be using insulin for post stroke hyperglycemia?

As I have already discussed in my chapter on animal models of post stroke hyperglycemia I do not believe that there is good animal evidence on the relationship between hyperglycemia and infarct growth. There is certainly not satisfactory animal evidence on the use of insulin after stroke. I believe that some further basic scientific work is really required before we expose more patients to the potential risks of hypoglycaemia from insulin therapy. The current evidence from clinical trials in stroke suggests that there is a clear danger of hypoglycaemia in these patients without any evidence of benefit. Indeed in GIST there appeared to be more benefit from a saline infusion than from an insulin infusion. The evidence from clinical trials of

intensive insulin therapy in other clinical areas certainly does not support routine use of such protocols.

4.4.6 Glucagon-like peptide analogues

The future for the management of post-stroke hyperglycaemia may lie in glucagons-like peptide analogues and agonists such as liraglutide. GLP agonists work by binding to a membrane GLP receptor increasing insulin release from pancreatic beta cells.

GLP-1 analogues have been given in an infusion to fasted, healthy subjects for 48 hours with no apparent increased risk of hypoglycaemic episodes.[570] This industry funded study involved 8 healthy subjects and was a randomised, double-blind placebo-controlled crossover study. Two hypoglycaemic episodes were seen during GLP-1 infusion and 1 hypoglycaemic episode was seen during placebo infusion. Hypoglycaemia was defined as plasma glucose less than or equal to 2.8nM with neuroglycopaenic symptoms. After the infusion of the study drug a 3 hour oral glucose tolerance test was carried out to identify possible reactive hypoglycaemia on re-feeding.

In another pilot study with a prospective open randomised crossover design 8 clinically stable subjects with type II DM were given insulin infusions and GLP-1 regimes to normalize blood glucose after breakfast.[571] GLP-1 was found to achieve normoglycaemia more rapidly than insulin. GLP-1 also produces lower maximum glucose levels and lower glucose levels at 2 hours and 4 hours. One symptomatic episode of hypoglycaemia occurred in the insulin group and no symptomatic hypoglycaemia was observed in the GLP-1 group.

4.4.7 Insulin or GLP-1 analogue bolus before tPA

Another therapeutic option may be to give a small bolus dose of insulin before initiation of tPA therapy in patients who are clearly hyperglycaemia (i.e. blood glucose >10mmol/l). In such cases only a very small dose of insulin may be necessary and hopefully the risk of hypoglycaemia associated with insulin infusion

would be reduced. It may even be more sensible to try a bolus of a GLP-1 analogue in the acute situation to further reduce the potential risk of hypoglycaemia.

4.5 Conclusions

Despite the consistent association of hyperglycaemia in the 48h after stroke with higher risk of death or dependence, there is no evidence that insulin treatment is associated with reduced mortality or favourable functional outcome in randomised controlled trials to date, although the majority of RCTs have not been designed as efficacy trials, recruited only small numbers of subjects, and the confidence intervals for clinical endpoints include the possibility of both significant benefit and significant harm. All insulin regimes tested to date in stroke have been labour-intensive, requiring frequent monitoring and dose adjustment. Fluid volumes infused are large when insulin and dextrose are used in combination regimes such as glucose-potassium-insulin infusion. Differences in blood glucose have been small in most trials and the risk of biochemical hypoglycaemia moderately high. There is wide variation in treatment threshold, target, and duration, and in reporting standards for hypoglycaemia. Large differences in blood glucose have been achieved only in very small numbers of patient

5 The prognostic outcome of stroke based on blood glucose measurement

5.1 Introduction

While it is known that post stroke hyperglycemia is common and linked with poor clinical outcomes there are still many uncertainties.[146, 147] It is not known if there is a clear cut-off level at which blood glucose becomes pathogenic and we do not know the best way to measure blood glucose level to accurately predict prognosis.

Depending on definition, between 50 and 80% of patients may be classified as hyperglycaemic.[146] Different methods of measuring glucose have been used although it is unclear which method is most practical and prognostically accurate.[214]

Historically interest has focused on the admission blood glucose level although blood glucose is a constantly changing physiological parameter and can change markedly in a short period of time.[160] The definition of 'admission' is vague and can represent an uncertain period of time. Admission can mean the time at which a patient arrives in the hospital emergency department or it can mean the time at which a patient arrives in an Acute Stroke Unit. Indeed, sustained hyperglycemia predicts infarct expansion and hyperglycemia within 48h is more prognostically important than single measures

Post-stroke hyperglycemia (PSH) predicts poor outcome from stroke, but there is no agreed definition of PSH. Hyperglycemia is an independent predictor of lesion growth and poor functional outcome, although intervention to reduce glucose levels does not appear to affect lesion growth.[176, 336]

The GLIAS study suggested that a blood glucose of >155 mg/dL (>8.6mmol/L) at any time within the first 48 hours from stroke onset, and not the isolated value of admission glycaemia is associated with poor outcome independently of stroke severity, infarct volume, diabetes, or age.[161]

5.1.1 The definition of post-stroke hyperglycemia

There is no clear, formally agreed definition of post-stroke hyperglycemia. In acute stroke research there is some variation in definition of hyperglycemia. Blood glucose values ranging from 6mmol/L to 11mmol/L have been used to define hyperglycemia. .[242, 243, 246, 285] I have discussed this in my introductory chapter and I have tabulated 99 studies that demonstrate the range of glucose values that have been used to define PSH (see Table 1.1 in Chapter 1).

The American Diabetes Association defines diabetes by a fasting plasma glucose level of greater than 7mmol/L. The guidelines also suggest that this value should be used in epidemiological studies.[572]

Epidemiological studies looking at the prevalence of admission hyperglycemia in acute stroke patients cannot guarantee that all patients are fasting and many may present immediately after eating, increasing blood glucose to postprandial levels. One may conclude that admission blood glucose above 7mmol/L is not automatically diagnostic of a dysglycaemic state. Pragmatically a cut off value of 7mM based on the ADA guidelines may be sensible to use in studies while acknowledging that various uncontrollable factors will affect admission glucose level.

5.1.2 Continuous blood glucose measurement

One observational study from Australia looked at the influence of hyperglycemia on infarct growth.[160] In this study 25 patients underwent MRI scanning within 24 hours of stroke onset (median imaging time – 15 hours). Follow-up MRI examinations were carried out at approximately 5 and 85 days. Using a continuous glucose monitoring system (CGMS) plasma glucose was monitored every 5 minutes for 72 hours. Additional capillary blood glucose was monitored every 4 hours.

Baird and colleagues were able to ascertain that the mean glucose produced by the CGMS and the mean capillary blood glucose correlated with infarct volume change on acute and sub-acute diffusion weighted MRI. Multiple regression analysis was performed for change in infarct volume as a continuous variable. Mean capillary glucose and mean CGMS levels above 7mmol/L were found to be independent variables that appear to influence infarct growth. Other variables included in the model were NIHSS score dichotomised at 13, prior glycaemic control represented by dichotomised HbA1C, treatment with rt-PA and

time to initial imaging. The relationship between mean glucose level and infarct growth appeared to be independent of these other variables.[160]

While this study is small it does provide some possible pathophysiological evidence to support the hypothesis that persistent hyperglycemia is associated with greater infarct growth.

A later paper by the same group attempted to more clearly define the temporal profile of hyperglycemia after stroke.[214] A total of 59 patients were monitored with the continuous glucose monitoring system for 72 hours. Monitoring began between 5 and 44 hours after stroke onset and continued for a median of 69 hours.

This paper found that at 8 hours after stroke onset 100% of 21 patients with known diabetes and 50% of 38 patients without known diabetes had blood glucose levels above 7mmol/l. Presence of diabetes was defined by a clear history or use of anti-diabetic medication. After an early phase of hyperglycemia glucose levels dropped in most patients by 14-16 hours by which point only 11% of non-diabetic and 27% of diabetic patients were hyperglycemic. There was a further late phase of hyperglycemia at between 48 and 88 hours after stroke onset that was observed in 27% of non-diabetic and 78% of diabetic patients. I have reproduced a figure from this paper below (Figure 21). It was also noted that 34% of the non-diabetic patients and 86% of the diabetic patients were hyperglycaemic for at least a quarter of the monitoring period.

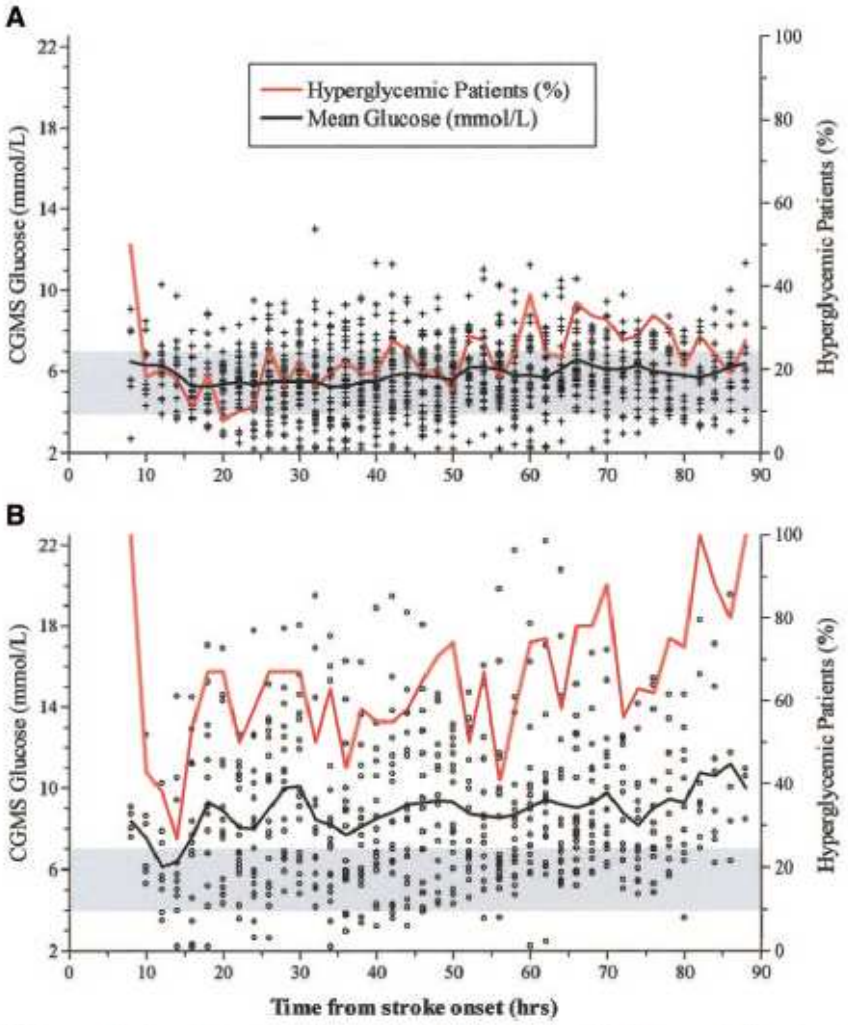

Figure 1—*CGMS glucose values for patients without (A) and with (B) diabetes in sequential 2-h time epochs illustrating an early and late hyperglycemic phase in both groups. The gray box represents the euglycemic range (4–7 mmol/l), the black line is the mean glucose value (left axis), and the red line is the proportion of patients with hyperglycemia (right axis) across sequential time points from stroke onset.*

Figure 22 - Temporal profile of glucose levels after stroke (from Allport et al)

5.1.3 The GLIAS approach

The Glycemia in Acute Stroke study (GLIAS) organised by the Spanish Society of Neurology investigated the prognostic value of capillary blood glucose levels in acute stroke patients.[161] A total of 476 patients were recruited into the study within 24 hours of stroke onset. The median time between stroke onset and emergency department arrival was 5 hours. Capillary blood glucose was checked on admission and 3 times per day over the initial 48 hours.

The GLIAS study aimed to find the glucose threshold value that had the highest predictive power for a poor outcome. Receiver operating characteristic curves were determined to show the predictive value of maximum capillary glucose values during the first 48 hours. The area under the curve was 0.656 (95% confidence interval 0.592 to 0.720, $p<0.01$) and

a glucose value of 155 mg/dl (8.6mmol/l) was identified as the optimum cut-off level for poor outcome at 3 months. This level was associated with a hazard ratio of 2.7 (95% CI 1.42-5.24) for poor outcome after adjustment for factors such as age, diabetes, admission blood glucose level, infarct volume and baseline stroke severity. There was also a hazard ratio of 3.8 (95% CI 1.79-8.1) for death at 3 months.

5.1.4 Which blood glucose level best predicts outcome in stroke thrombolysis patients?

The Australian studies make mechanistic sense. It may be more plausible that a sustained high average blood glucose level would affect patient outcomes more profoundly than a single high reading. While the GLIAS study does report that patients with diabetes have a higher mean glucose level over 48 hours and while it is mentioned that diabetes does not correlate with poor outcome there is no explicit analysis of the relationship between mean glucose level and outcome.

Neither of these studies focused on patients undergoing IV thrombolysis. The proportion of patients treated with thrombolysis was not stated in the GLIAS study while in the 2003 paper by Baird and colleagues only 16% received IV rt-PA.[160, 161] Elevated blood glucose pre-thrombolysis has been identified as a predictor of poor outcomes including outcome at 24 hours,[181] mortality,[183] functional outcomes[179] and symptomatic intracranial haemorrhage.[178, 182]

We therefore undertook an analysis of the relationship between glucose profiles and clinical outcome in a population receiving IV thrombolysis for acute ischaemic stroke, specifically exploring alternative indices of glycaemic state to compare the optimal predictive index for functional outcome as measured by the modified Rankin scale.

5.2 Methods

Patients treated with r-tPA, between May 2003 and November 2008, were identified from our local Safe Implementation of Thrombolysis in Stroke (SITS) database.[14-16] Capillary blood glucose is checked as part of routine clinical care every 4 hours in our unit. Case notes were obtained where possible and glucose profile data were extracted. Additional glycaemic indices (diabetic state, insulin treatment, HbA1C level were noted where available.

Capillary blood glucose (CBG) was measured 4 hourly for 48h in acute patients. We reviewed all patients with complete 48h CBG records who received IV rt-PA and compared the relative risk (RR) of unfavourable 3 month modified Rankin score (mRS)>1 using admission blood glucose (ABG), weighted mean 48h CBG (MCBG), and hyperglycemia defined as two or more elevated CBG (THBG) readings. Hyperglycemia was defined as glucose >126mg/dl (7mmol/L). Additionally patients with a single blood glucose level of above 8.6mmol/L were identified.

Patient outcome was recorded with a 3 month modified Rankin score. Data on patient age, pre-treatment National Institutes of Health Stroke Scale (NIHSS) score and onset to treatment time were also extracted from the database.

Rankin scores were dichotomised to show favourable outcome (mRS<2) and poor outcome (mR≥2).

5.2.1 Statistical analysis

Statistical analysis was performed using SPSS 15 (SPSS Inc, Chicago, USA) and STATSDIRECT (StatsDirect Ltd, Cheshire, UK) software. Fisher's exact test was used to compare proportions in 2 by 2 tabulated data. Binary logistic regression analysis was carried out using SPSS.

5.3 Results

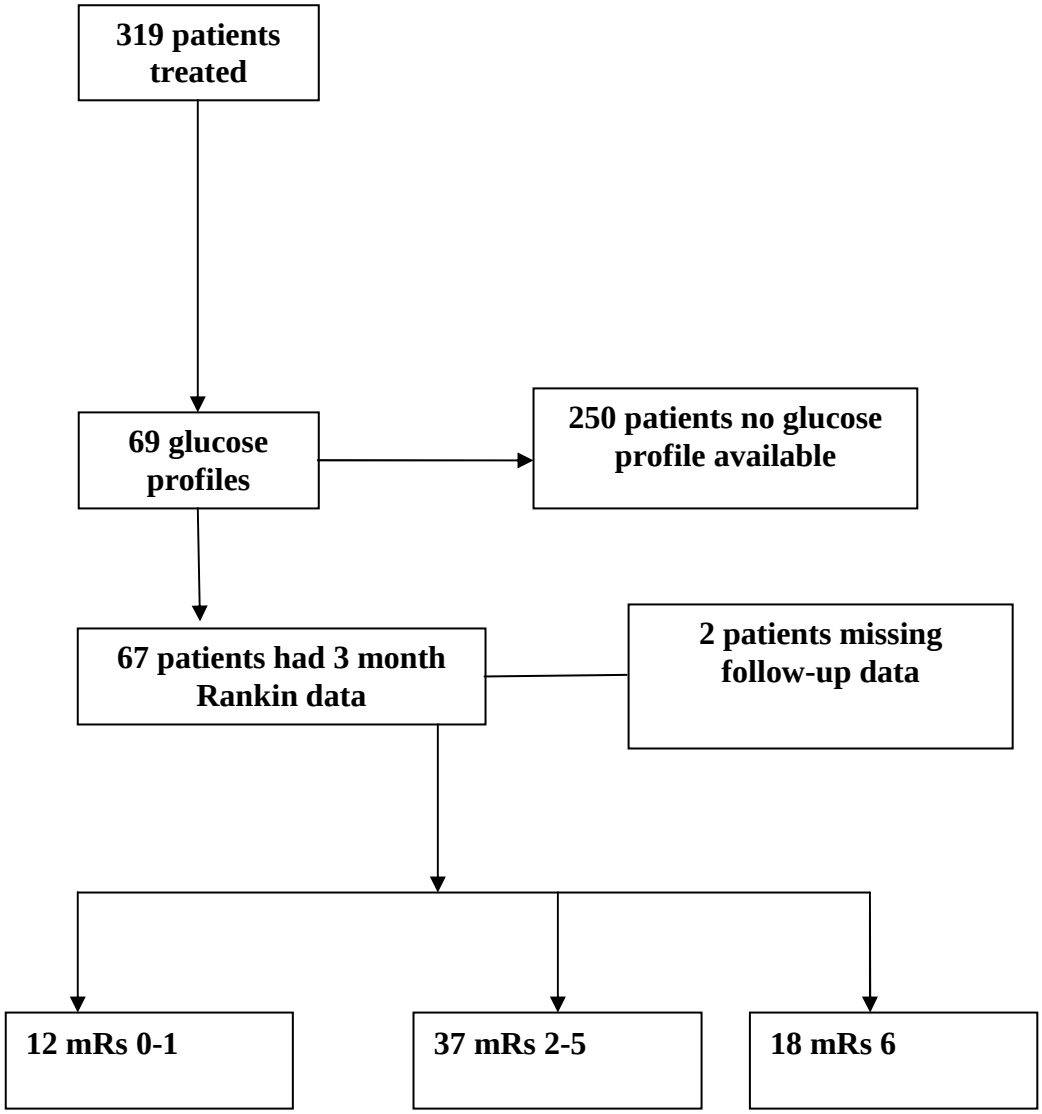

Figure 23 - Population profile for SITS database glucose profile study

Data from 69 patients were analysed. In this group 9 patients (13%) had a diagnosis of diabetes while 12 patients (17%) were treated with some form of insulin infusion. Glycosylated haemoglobin (HbA1c) was checked in 11 patients. In these 11 patients the mean HbA1c was 7.3 (range 5.9-12.3). Outcome data in the form of a 3 month modified Rankin score (mRS) were available for 67 patients.

Table 5-1 Population characteristics for patients included in study

Male	35 (50.7%)
Mean Age	68 (66-70)
Known DM	9 (13%)
Median NIHSS (IQR)	14 (7-19)
tPA treatment <3h	50 (72.5%)
Median time to t-PA treatment (minutes)	170 (165-175)
Smoking History	18 (26.1%)
Known AF	20 (29%)
Hypertension	41 (59.4%)
Known cardiac failure	2 (2.9%)
Previous stroke	12 (17.4%)

The relative risk of mRS>1 was greatest with MCBG (RR 4.04, 95% CI 1.46-14.46, p = 0.0013) compared to ABG (RR 3.49, 1.25-12.57, p = 0.0092) or THBG (RR 0.93, CI 0.64-1.63, p=0.753). Using the GLIAS value of 8.6mmol/L the RR was 0.97, CI 0.77-1.26, p=0.811.

Relative risk of death was also greatest with MCBG (RR 2.72, 1.35-5.35, p=0.0053) compared to either ABG (RR 1.68, 0.77-3.59, p=0.19) or THBG (RR 1.54, 0.67-3.85, p=0.33). Using the GLIAS value of 8.6mmol/L the RR was 1.27, CI 0.58-2.88, p=0.557).

Outcome data for each of the glucose measurement groups is graphically represented in Figures 5.2-5.4.

5.4 Conclusion

In a stroke thrombolysis population, relative risk of both unfavourable functional outcome and death was greatest for mean capillary blood glucose over 48h compared to either admission blood glucose or two elevated blood glucose readings.

If outcome from stroke is dependent on amount of glucose exposure it seems logical that a mean glucose value should have more prognostic significance than an arbitrary isolated glucose value. Certainly the mean capillary glucose value appears to have a greater positive predictive value than an isolated admission glucose, two glucose values above 7 or the presence of one glucose reading of greater than of equal to 8.6mmol/L, the cut-off point identified by the GLIAS study.[161] The results presented here are not definitive. This is a small study population so it is difficult to adjust for other known prognostic factors that could confound the outcomes.

We still do not know if a causal relationship exists between PSH and outcome for stroke. PSH is certainly associated with a poor outcome but there is no reliable evidence that any clinical intervention to alter glycaemic state improves outcome.[6, 10, 242, 283, 573]

Ours study suggests that reliance on single admission blood glucose readings to risk stratify patients in randomised controls trials of insulin may be ill advised. Our small study suggested that a mean 48 hour blood glucose value may help risk stratify although we are left with a therapeutic black hole. The relationship between mean capillary blood glucose, admission blood glucose and outcomes is further explored in Chapters 6 to 9.

6 Demographics and clinical outcomes for stroke patients recruited to the post-stroke hyperglycaemia and brain arterial patency study (POSH study)

6.1 Introduction

Post-stroke hyperglycaemia (PSH) is consistently associated with poorer outcome in terms of survival, disability and markers of brain injury such as infarct expansion.

Even modest post-stroke hyperglycaemia (PSH) is associated with a three-fold increase in odds of death at 30 days in non-diabetic patients.[146] However, in most previous studies, blood glucose (often single measurements) has been evaluated more than 12 hours after stroke onset, and sometimes as late as 72h after onset. Several studies have suggested that the adverse prognostic impact of PSH may simply reflect an association with stroke severity.[218, 219]

Continuous monitoring of tissue glucose concentration using a subcutaneous probe has shown that the profile of glucose over the first 48h after stroke is a better predictor of infarct expansion than point measurements.[160]

In analysis of routine observational data from a more intensive glucose monitoring regime commenced predominantly <6h after stroke onset 78% of patients develop PSH within 48h of stroke. For those with complete data commencing within 6h of onset, 100% of diabetics and 75% of non-diabetics developed hyperglycaemia within the first 48h after stroke.[521]

Further, pre-stroke glycaemic status (defined by glycosylated haemoglobin concentration [HbA_1c]), and not stroke severity, predicted PSH within the first 6 hours, whereas PSH 6-48h after onset showed trends towards an association with stroke severity. At later time-points, complex interactions between stroke severity, blood glucose and feeding emerged.[521]

The detrimental effects of PSH may be restricted to specific groups of patients, defined by pathophysiological processes, and that intervention to lower glucose may therefore be unnecessary (and might indeed be harmful) in patients who do not share these features.[528]

Firstly, only patients with metabolically compromised brain tissue (an ischaemic penumbra) on MRI were susceptible to infarct expansion with PSH.[160, 176] The likelihood of a penumbra being present declines over the first few hours after stroke onset, and clinical outcome correlates strongly with the volume of penumbral tissue that is salvaged through spontaneous or therapeutic reperfusion.

Secondly, PSH was associated with poorer outcome after IV alteplase only in patients with early arterial recanalization (<3h after treatment)[271] this might explain the association of PSH with poorer outcome after IV thrombolysis overall.[179] Both these sets of data support a more specific mechanism by which PSH may adversely influence the probability of survival of the ischaemic penumbra in patients within the first few hours after stroke.

Animal model evidence of the adverse impact of hyperglycaemia on infarct volume evolution may have limited relevance to the clinical presentation due to very high glucose concentrations and models representing type I diabetes predominantly (Chapter 3). In addition, evidence for the efficacy of insulin in preventing this adverse influence is inconclusive.[206] A recent clinical trial of insulin treatment for PSH reported divergent effects of treatment dependent on arterial patency, being associated with greater infarct expansion in patients with persistent occlusion, but less expansion than placebo in those with recanalization.[554] It appears therefore that early and late PSH may differ in their causes and pathophysiological significance, and that individual brain tissue vulnerability and vessel status further influence the effects of PSH

In the post stroke hyperglycaemia and brain arterial patency study (POSH) we aimed to investigate the relationship between post-stroke hyperglycaemia, infarct growth and brain arterial patency. The main results of this study are presented and discussed over the next few chapters. In this chapter I have presented the baseline demographics and clinical outcomes for the entire population of stroke patients recruited into the study. Later chapters concentrate on smaller subgroups of patients based on the availability and quality of the study imaging or on imaging findings.

6.2 Methods

The general methods for the POSH study are detailed in chapter 2. Patients were recruited prospectively as they were admitted to the Acute Stroke Unit in the Institute of Neurological Sciences at the Southern General Hospital in Glasgow.

6.2.1 Image analysis

The imaging for each patient was reviewed by two researchers (NM and XH). Clinically relevant data were additionally reported by experienced neuroradiologists. Discrepancies in imaging interpretation were adjudicated by an experienced stroke neurologist (KM).

6.2.2 Statistical analysis

Differences between groups were analysed using ANOVA for scale variables and Pearson's chi squared for categorical variables. If a significant difference between groups was discovered using ANOVA it was further analysed using the least significant difference method.

Binary logistic regression analysis was also carried out using SPSS to seek predictors of mortality. Univariate analysis included age, admission blood glucose, mean capillary blood glucose, atrial fibrillation, HbA1c, NIHSS score, systolic and diastolic blood pressure and thrombolytic treatment as a binary variable. All variables with $p<0.1$ in univariate analysis were entered into a forward stepwise conditional model. Findings were confirmed in a backwards stepping model beginning with all potentially predictive variables

Additionally we used SAS version 9.3 to perform a Rankin shift analysis using Cochran-Mantel-Haenzel statistics to look for a relationship between glycaemic status and outcome while correcting for admission variables (NIHSS, age).[574]

6.3 Results

Between January 1st 2009 and December 31st 2011 the acute stroke unit admitted 2128 patients.

The study recruited 113 patients between the 1st of January 2009 and the 31st of December 2011. From this group 108 had a clinical diagnosis of ischaemic stroke. The baseline demographics for this group are included in Table 6.1 below.

Serial capillary blood glucose concentrations were used to define 3 subgroups: admission hyperglycaemia (blood glucose >7mmol/l within 6h of stroke onset), delayed hyperglycaemia (blood glucose >7mmol/l 6-48h after stroke onset), and normoglycaemia. A recruitment flowchart in Figure 24 illustrates the different groups of patients.

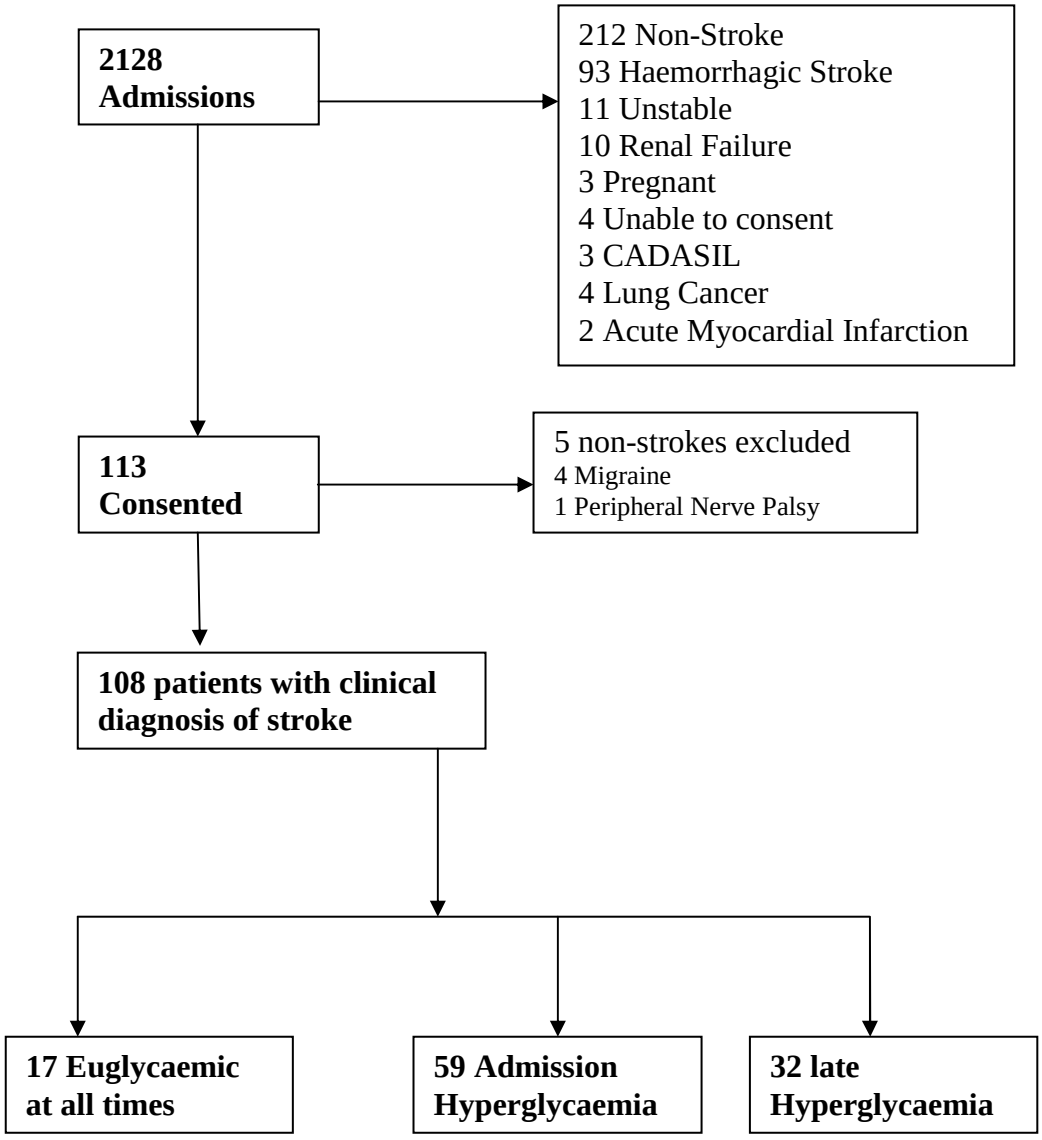

Figure 24 - Recruitment flowchart for patients in the POSH study

One subgroup contained 17 patients who were normoglycaemic during their admission, with blood glucose levels consistently below 7mmol/L. The 59 patients in the second subgroup were hyperglycaemic on admission with a blood glucose level of 7mmol/L or greater within 6 hours of stroke onset. The final subgroup of 32 patients became hyperglycaemic later during admission with a blood glucose level of 7mmol/L or greater 6 or more hours after stroke onset.

Table 6-1 Baseline demographics and clinical outcomes of stroke patients recruited into the POSH study

Group (n)	All patients (108)	Hyperglycaemia None (17)	Hyperglycaemia Admission (59)	Hyperglycaemia Late (32)	
Male Gender (n/%)	66 (61.1)	10 (59)	34 (57)	22 (69)	
Age ± SD (mean/years)	69.7 ± 11.5	62 ± 14.3 * ***	70.5 ± 10.5 *	72 ± 10.4 ***	p=0.009
Admission blood glucose ± SD (Mean mmol/L)	7.42 ± 3.28	5.82 ± 0.6 *	8.66 ± 3.92 * **	6.03 ± 1.48 **	p<0.001
Mean capillary blood glucose ± SD (Mean, mmol/L)	6.73 ± 1.6	5.4 ± 0.59*	7.41 ± 1.64* **	6.17 ± 1.27 **	p<0.001
HbA1c ± SD (Mean)	5.9 ± 1.1	5.4 ± 0.5 *	6.19 ± 1.3 *	5.72 ± 0.7	p=0.016
Pre-morbid Rankin (Median/IQR)	0 (0-0)	0 (0-0)	0 (0-1)	0 (0-0)	
Admission NIHSS (Median/IQR)	9 (5-18)	11 (4-17)	10 (6-19)	8 (5-16)	p=0.701
Admission Blood pressure ± SD (mmHg)	150/78 ± 23/15	152/78 ± 21/12	147/77 ± 25/18	154/79 ± 21/10	p=0.442
Smoking history (n/%)	50 (55.6)	10 (58.8)	32 (54.2)	18 (56.3)	p=0.941
Previous stroke (n/%)	27 (25)	3 (17.6)	14 (23.7)	10 (31.3)	p=0.547
Previous TIA (n/%)	24 (22.2)	2 (11.8)	12(20.3)	10 (31.3)	p=0.258
Atrial Fibrillation (n/%)	28 (25.8)	4 (23.5)	16 (27.1)	8 (25)	p=0.947
Hyperlipidaemia (n/%)	53 (49.1)	7 (41.2)	32 (54.2)	14 (43.8)	p=0.492
Diabetes (n/%)	20 (18.5)	0	18 (30.5)	2 (6.3)	p=0.002
Impaired glucose tolerance (n/%)	20 (18.5)	0	17 (28.8)	3 (9.4)	p=0.007
Peripheral vascular disease (n/%)	11 (10.2)	1 (5.9)	7(11.9)	3 (9.4)	p=0.76
Hypertension (n/%)	53 (49.1)	4 (23.5)	34 (57.6)	15 (46.9)	p=0.044
Stroke on imaging (n/%)	93 (86.1)	13 (76.4)	54 (91.5)	26 (81.3)	p=0.182
Occlusion (n/%)	71 (65.7)	10 (58.8)	41 (69.5)	20 (62.5)	p=0.644
Recanalization (n/%)	39/67	4/10(40)	24/41 (58.5)	11/20 (55)	p=0.4
Thrombolytic treatment (n/%)	76 (70)	10 (58.8)	44 (74.6)	22 (69)	p=0.44
Rankin 0-1 Day 30 (n/%)	17(15.7)	5 (29.4)	8 (13.6)	4(12.5)	p=0.48
Rankin 2-5 Day 30 (n/%)	79 (73.1)	11 (64.7)	43 (72.9)	25 (78.1)	
Rankin 6 Day 30 (n/%)	12 (11)	1(6)	8(14)	3(9)	
Supra-aortic large artery atherosclerosis (n/%)	31(28.7)	4 (23.5)	18 (30.5)	9 (28.1)	
Cardio-aortic embolism (n/%)	27 (25)	4 (23.5)	16 (27.1)	7 (21.8)	
Small artery occlusion (n/%)	9 (8.3)	2 (11.8)	4 (6.8)	3 (9.4)	
Other causes (n/%)	5 (4.6)	2 (11.8)	3 (5.1)	0	
Undetermined causes (n/%)	36 (33.3)	5 (29.4)	18 (30.5)	13 (40.6)	p=0.754

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG.

6.3.1 Differences in baseline characteristics

There were significant differences in age between the 3 groups. Euglycaemic patients were younger than those with admission hyperglycemia or later development of hyperglycemia (p=0.009). There was also an expected significant difference in admission blood glucose level (p<0.001) and mean capillary blood glucose (p<0.001). Baseline HbA1c level was also significantly different between the 3 groups (p=0.016). On post hoc analysis a significant difference in HbA1c was seen between the euglycaemic and admission hyperglycaemia groups (p=0.009).

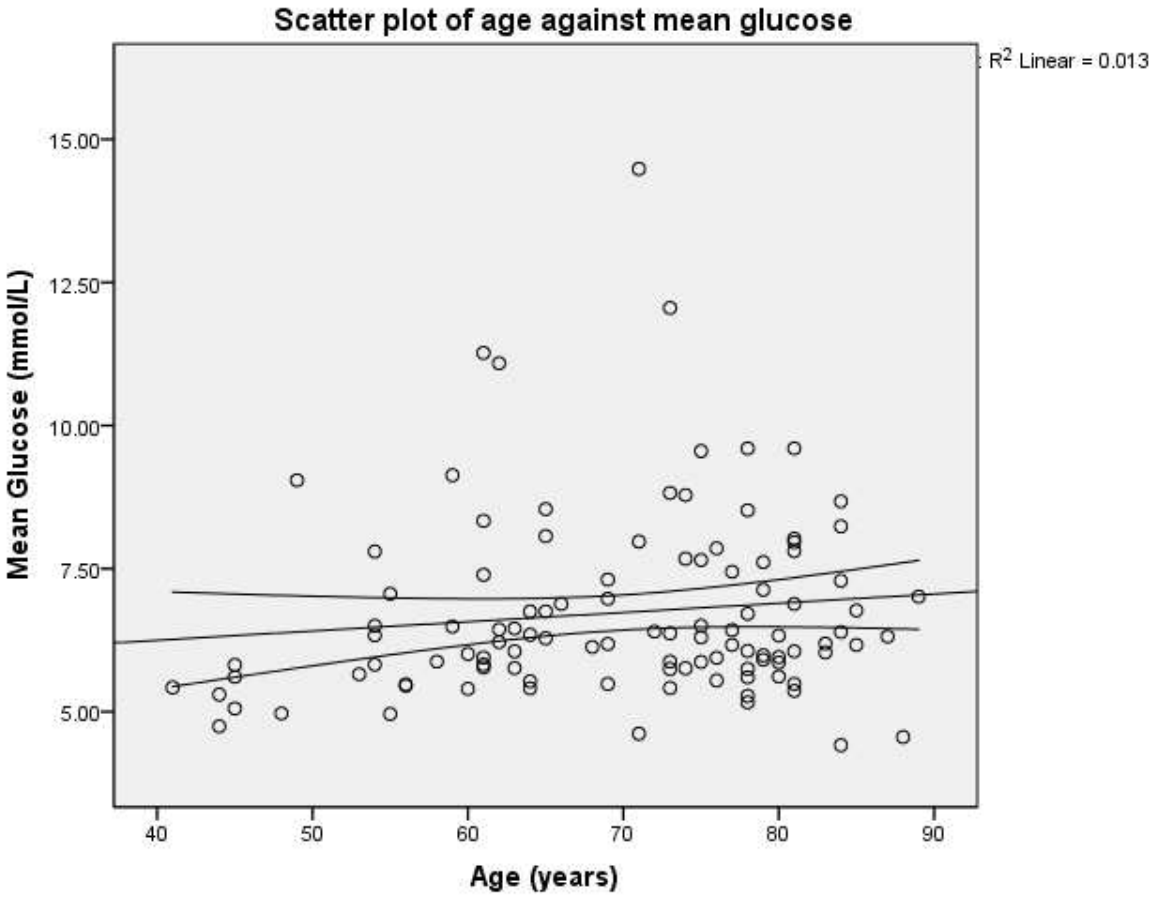

Figure 25 Scatter plot of mean glucose level against age

While there was a significant difference between age and mean glucose across glycaemic groups there was no statistical evidence of a correlation between these two variables using Pearson's correlation coefficient (p=0.233). Similarly age did not correlate with HbA1c (p=0.848) or admission blood glucose (p=0.835) although there was a correlation between age and systolic blood pressure (p=0.041).

Diabetes strongly predicted admission hyperglycaemia in binary logistic regression (p=0.003, OR 10.3, 95% CI 2.26 to 47.2) as did HbA1c (p=0.013, OR 2.1, 95% CI 1.16 to 3.64). Admission hyperglycaemia was not predicted by admission blood pressure although binary logistic regression for the absence of a history of hypertension gave an odds ratio of 0.47 with p=0.053 (95% CI 0.22 to 1.01).

Significant differences were also seen between glycaemic groups for past history of diabetes mellitus (p=0.002) and impaired glucose tolerance (p=0.007).

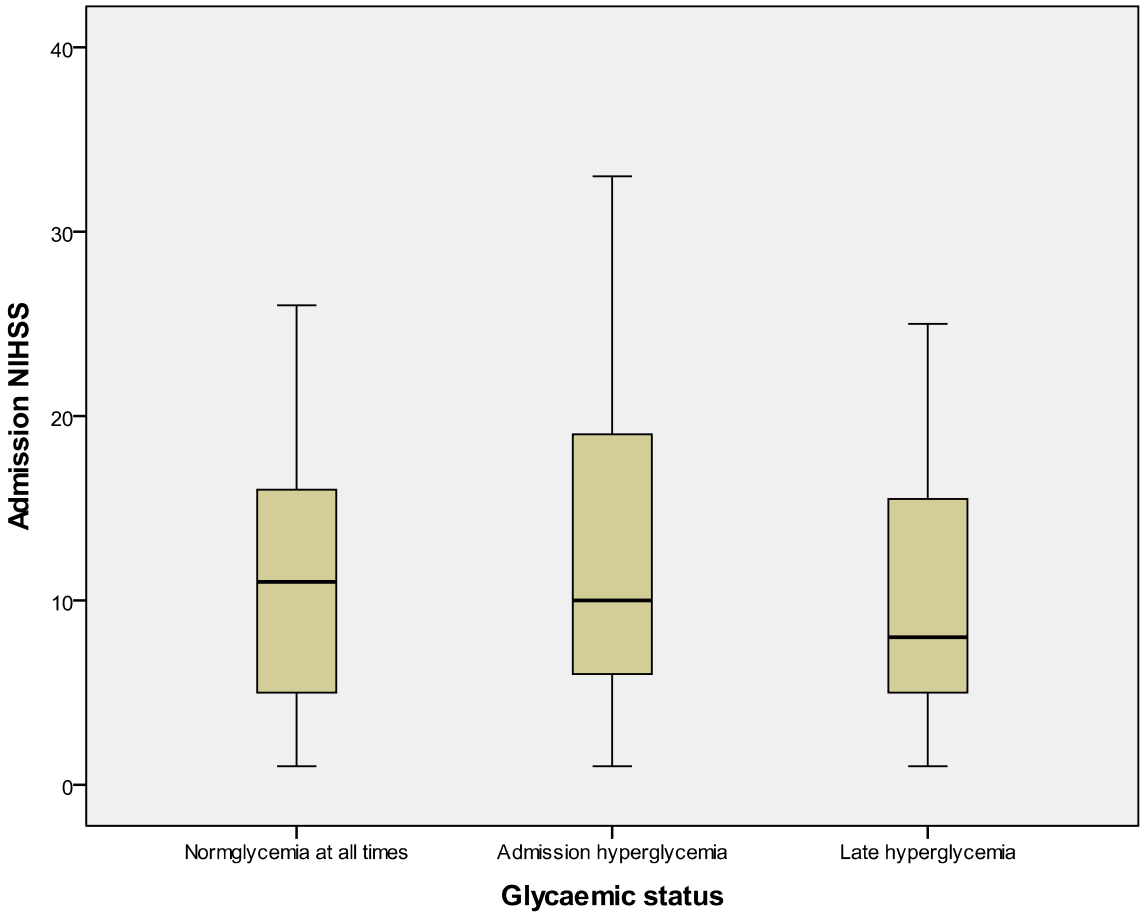

Figure 26 - Admission NIHSS in different glycaemic groups

There was no significant difference in median admission NIHSS between glycaemic groups (p=0.701) although there was a trend to lower NIHSS scores in the late hyperglycaemia group. This is illustrated in Figure 26.

6.3.2 Glucose Profiles

Detailed glucose profiles were collected for all the patients included in the study. The median number of glucose readings per patient was 12. The mean time to initial blood

glucose measurement was 1 hour and 25 minutes from stroke onset (standard deviation 1:02) and the maximum time to initial measurement was 4 hours and 45 minutes.

The glucose kinetics of the three glycaemic groups are illustrated in Figure 27. The mean glucose level of the euglycaemic group is consistently less than 6mmol/L. The admission hyperglycaemia group have an initial mean glucose level of 8.96mmol/L falling to a nadir of 6.49mmol/L at around 15 hours after stroke onset before rising again. The late hyperglycaemia group had a mean initial glucose level of 5.81mmol/L with levels staying below 6mmol/L until 16 hours after stroke onset when levels began to rise.

A high proportion of patients became hyperglycaemic during this study. The proportion of hyperglycaemic patients increased with time and increased as the threshold for hyperglycaemia was reduced (see Figure 28). With a threshold of 7mmol/L 83% of patients became hyperglycaemic within 48 hours while a threshold of 8mmol/L led to 63% of patients being classified as hyperglycaemic during that period.

Repeated measures ANOVA revealed a significant difference between groups based on glycaemic status ($p=0.001$). There was a significant difference on repeated measures between the euglycaemic group and the admission hyperglycaemia group (mean difference 2.32, 95% CI 1.16 to 3.47, $p<0.001$) although the difference between the euglycaemic group and the late hyperglycaemia group was not significant (mean difference 1.149, 95% CI -0.04 to 2.34, $p=0.059$).

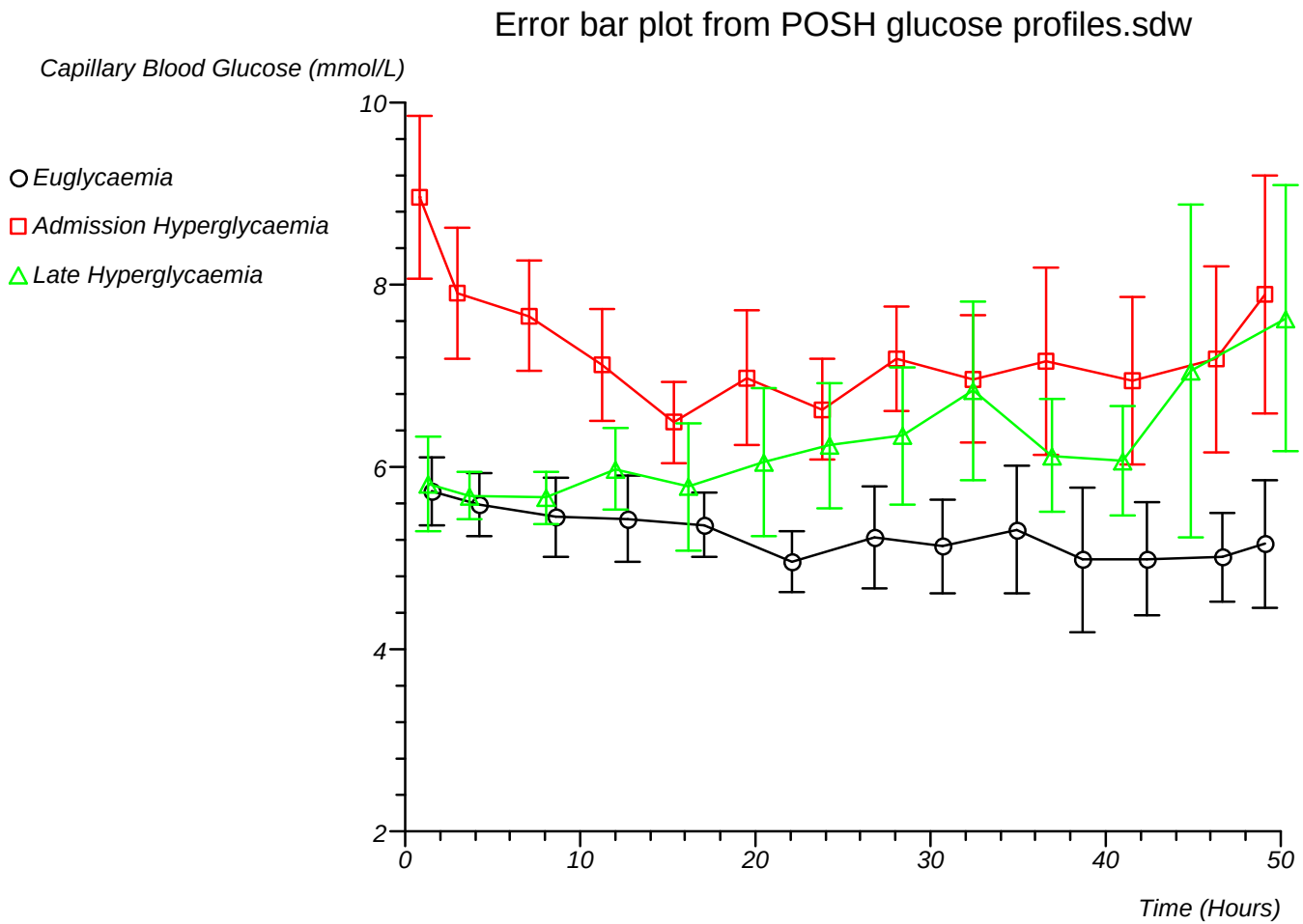

Figure 27 - Mean capillary blood glucose against time with error bars showing 95% CI

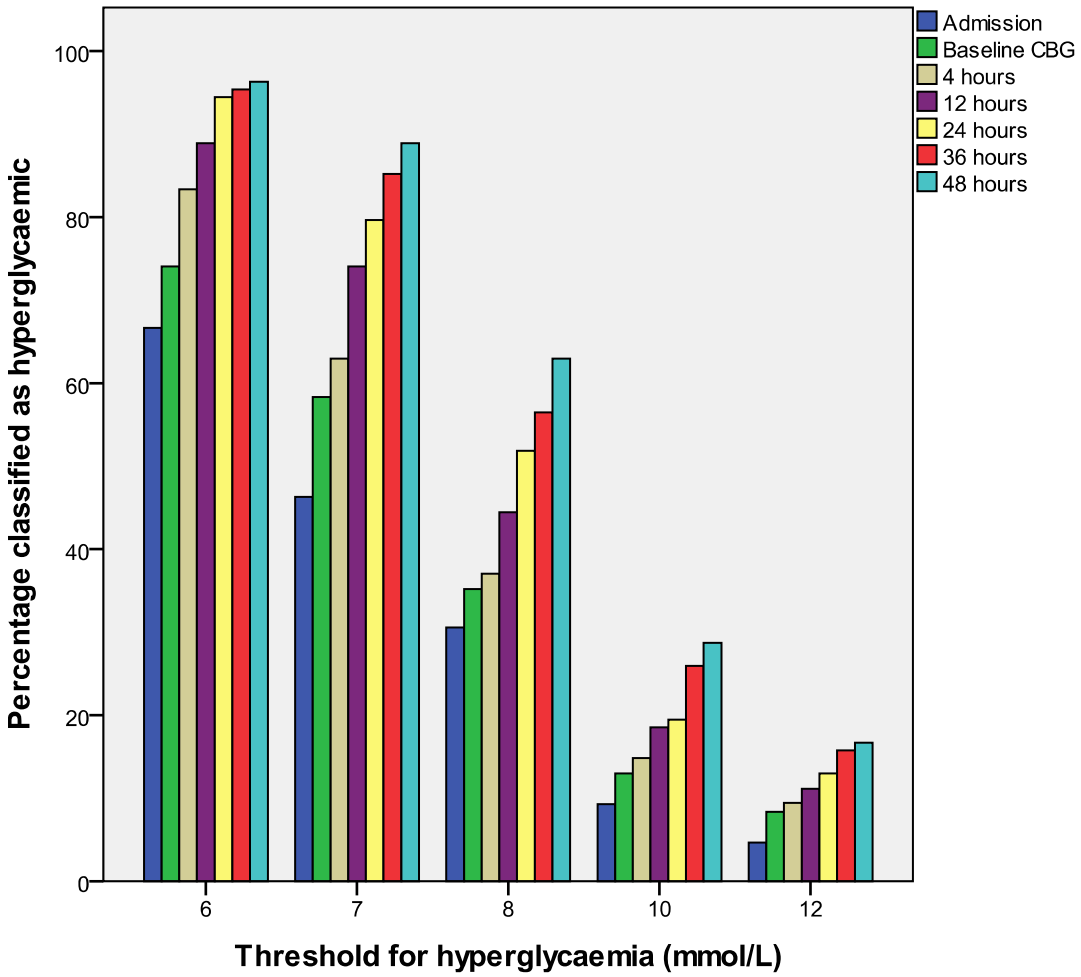

Figure 28 - Proportion of patients who have been hyperglycaemic increases with time and varies with glucose threshold

6.3.3 Treatment for Diabetes Mellitus

Of the 20 patients with diabetes mellitus 12 were treated with metformin. Five of these patients were treated with metformin and gliclazide. Three patients were treated with gliclazide alone. Four patients were treated with insulin. Two of these patients were treated with insulin and metformin. Three patients were on no treatment for DM.

No other patients in the cohort were treated for hyperglycaemia. Where possible, patients continued with their normal medications. One patient was given Lucozade for hypoglycaemia (CBG 2.2mmol/L).

6.3.4 Swallow and fluid management

Initial screening for problems with swallowing was carried out in the Acute Stroke Unit on the day of admission. Seven patients (40%) in the euglycaemic group had an initial swallowing problem, compared to 14 patients (44%) in the late hyperglycaemia group and 19 patients (32%) of the admission hyperglycaemia group. Information was missing in 24% of the normoglycaemia group, 27% of the admission hyperglycaemia group and 9% of the late hyperglycaemia group.

Four normoglycaemic patients, fifteen admission hyperglycaemia patients and two late hyperglycaemia patients were declared ‘nil by mouth’.

Intravenous fluids were prescribed to 12 normoglycaemic patients (70%), 43 admission hyperglycaemia patients (73%) and 23 late hyperglycaemia patients (73%). Two patients in the admission hyperglycaemia group and one patient in the late hyperglycaemia group were given 5% dextrose solution. All other patients were given saline solution. The mean volume of fluid given in 24 hours was 1853ml with no significant difference in volumes between groups ($p=0.647$) or infusion rate ($p=0.647$).

In chi-squared testing there was no significant difference in scores for point 10 on the NIHSS score (for dysarthria) between groups ($p=0.85$).

6.3.5 Cause of stroke

There were no significant differences in the cause of stroke between groups. In the cohort as a whole 29% of strokes were due to supra-aortic large artery atherosclerosis while 25% were due to cardio-aortic embolism. One third of strokes were of undetermined aetiology while smaller proportions were due to small artery occlusion (8.3%) or other causes (4.6%).

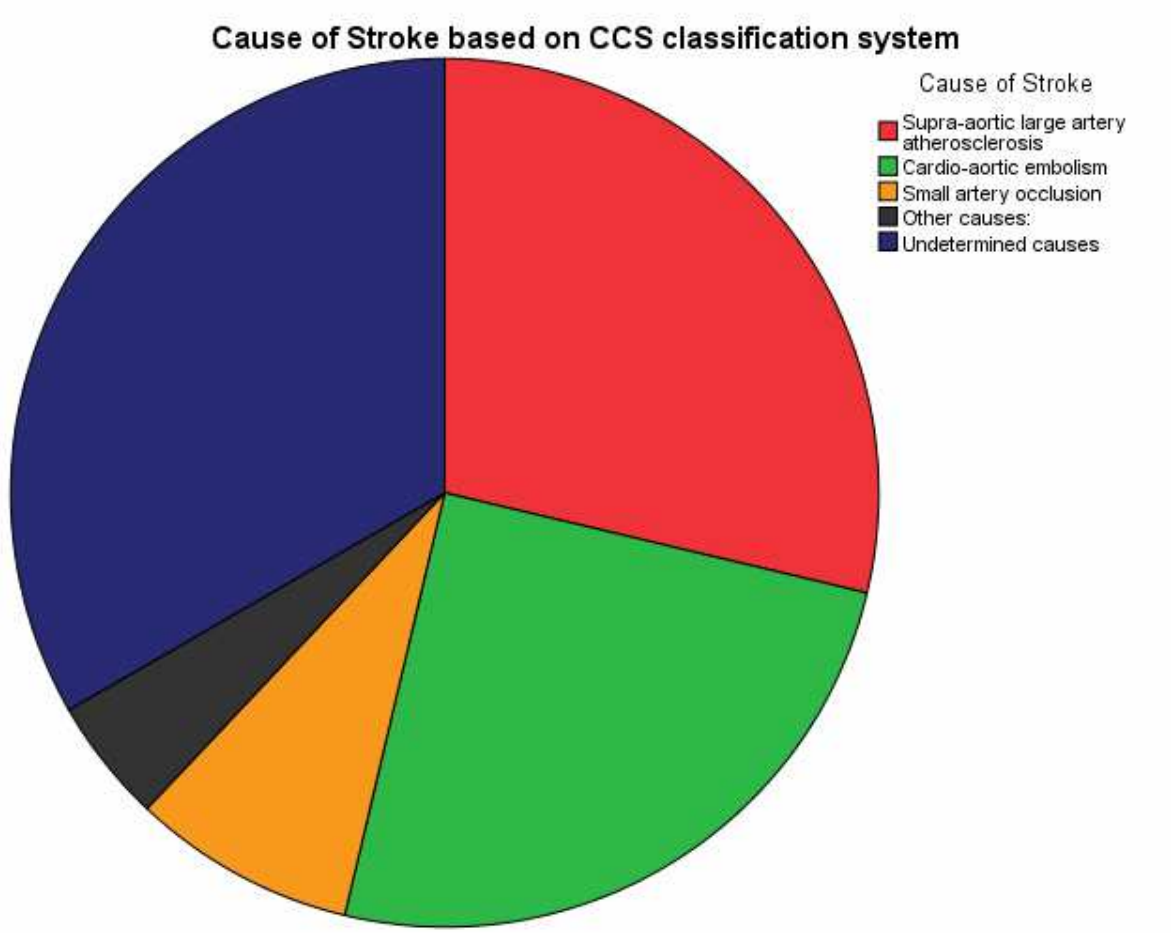

Figure 29 - Cause of stroke in general population using CCS classification

6.3.6 Clinical outcomes

There were no significant differences in clinical outcomes between groups. Overall 11% of patients died by day 30, 16% of patients had a 'good' outcome with modified Rankin scores of between 0 and 1 while 73% of patients had a 'poor' outcome with Rankin scores of 2 to 5.

We subsequently performed a shift analysis using Cochran-Mantel-Haenszel statistics to see if glycaemic status predicted outcome as graded by day 30 Rankin score. We corrected the analysis for age and admission NIHSS but we found no significant relationship between PSH and outcome Rankin score (p=0.308).

We also repeated the Cochran-Mantel-Haenszel shift analysis to see if admission NIHSS score was predicted by glucose group when corrected for age. Again there was no significant relationship (p=0.457).

We also used the Cochran-Mantel-Haenszel shift analysis to see if glycaemic category was predicted by admission NIHSS but again there was no significant relationship (p=0.549).

Binary regression analysis suggested that NIHSS score was a strong predictor of mortality (P=0.001, OR 1.2, 95%CI 1.078-1.359) in combination with mean capillary blood glucose (P=0.028, OR 2.36, 95%CI 1.1- 5.08) and HbA1c (P=0.023, OR 0.19, 95% CI 0.046-0.792). Other factors included in the regression did not have a significant effect.

6.4 Discussion

In this prospectively recruited series of stroke patients we found that a large proportion (54.6%) of patients became hyperglycaemic within 6 hours of stroke onset. Another 29.6% became hyperglycaemic over the subsequent 48 hours and only 15.7% of patients were observed to have glucose levels below 7mmol/L at all times.

These three groups appear to be very distinct. There are significant differences in admission glucose, mean capillary blood glucose and HbA1c between groups. The glucose kinetics of the 3 groups look different as graphically represented in Figure 6.3. In this group post stroke hyperglycaemia does not appear to be a regression to the mean as suggested by Wong and colleagues.[190] In their study they excluded all patients with diabetes which does not really reflect the reality of the acute stroke patient. They also did not check HbA1c leaving prior glycaemic status beyond a reported history of diabetes unexplored.

Repeated measures ANOVA confirmed that the euglycaemia group and the late hyperglycaemia group were significantly different. The difference between the euglycaemic group and the late hyperglycaemic group was not significant (p=0.059) although patients who were improving in some cases would have left hospital or

transferred to another resulting in missing capillary blood glucose values towards the end of the first 48 hours and exclusion from the model.

Despite this high incidence of hyperglycaemia after stroke only 18.5% of this cohort had an existing diagnosis of diabetes mellitus and the mean glycosylated haemoglobin level for the group was only 5.9%. There was a significant difference in mean glycosylated haemoglobin levels between group with the largest difference existing between the euglycaemic group and the admission hyperglycaemia group. This would suggest that the admission hyperglycaemia group had higher blood glucose levels over the weeks before their strokes. It is possible that the patients in the admission hyperglycaemia group had undiagnosed insulin resistance but we did not test fasting insulin levels on admission so we cannot perform a homeostasis model assessment to assess insulin sensitivity.[50]

The incidence of hyperglycaemia alters depending on the threshold chosen for hyperglycaemia. In this population 96% of patients were hyperglycaemic within 48 hours if a threshold of 6mmol/L was chosen, 83% with a threshold of 7mmol/L, 63% with a threshold of 8mmol/L and 29% with a threshold of 10mmol/L. If this was an acute study 31% of patients could have been recruited at 4 hours if 8mmol/L was the chosen threshold and 45% of patients with a threshold of 7mmol/L. This is in keeping with observations from the VISTA database.[267]

Some other observational studies have been unclear in the reporting of patients treated with insulin or oral hypoglycaemic agents during the study period.[197] In this population only 4 patients with known DM were treated with insulin during the admission. These patients were in the admission hyperglycaemia group. All patients who were using oral hypoglycaemic agents were in the admission hyperglycaemia group.

A very small proportion of the patients in this study had strokes that were felt to be due to small artery occlusion or other causes. This may be a factor of the nature of the inclusion criteria. All patients had to present to hospital within 6 hours of stroke onset and a patient with a small vessel or lacunar stroke may have less of a deficit leading to a delayed presentation. It is also possible that patients with small vessel occlusion were missed by the screening researchers who did not think that the presenting symptoms and signs represented an acute stroke. Almost all patients admitted to the Acute Stroke Unit at the Southern General come in an emergency ambulance and it is possible that doctors outside

the unit would not feel that a patient with a minor stroke related to small vessel disease needed such urgent attention.

Some differences were seen between different glycaemic groups. The euglycaemic patients were significantly younger than hyperglycaemic patients (p=0.009). The oldest patients were those who developed hyperglycaemia at a later time point in the admission. The admission hyperglycaemia patients were more likely to have a past history of diabetes mellitus or impaired glucose tolerance. They also had significantly higher glycosylated haemoglobin levels. The age related differences seen in these data may be explained by the increasing prevalence of type 2 diabetes mellitus with age.[575] Undiagnosed diabetes is often offered as an explanation for post-stroke hyperglycaemia.[230-233]

Known risk factors for stroke such as atrial fibrillation, hyperlipidaemia and hypertension were not significantly different between groups. Despite this the absence of a history of hypertension trended towards reducing the risk of admission hyperglycaemia (p=0.053, OR 0.47, 95% CI 0.22 to 1.01). In a meta-analysis of a large volume of clinical trial data from the VISTA archive a history of hypertension was seen to be predictive of post stroke hyperglycaemia within 48 hours of stroke.[267]

A non-significantly smaller proportion of patients had definite stroke on imaging, proven occlusions and thrombolytic treatment in the normoglycaemia group compared to the other groups. Baseline clinical stroke severity as assessed by NIHSS scale was similar with no significant statistical differences seen. There was nothing to suggest that stroke severity correlated with hyperglycaemia. This is in keeping with previous observations.[221, 222, 576]

In the regression analysis we see NIHSS as a strong predictor of 30 day mortality. This fits with the role of the NIHSS as an indicator of baseline stroke severity. Mean capillary blood glucose also appears to predict 30 day mortality as did HbA1c. This is in keeping with previous studies.[160, 176] Rankin shift analysis using Cochran-Mantel-Haenszel statistics to correct for age and baseline NIHSS did not show any relationship between glycaemic groups and 30 day outcome.

7 Assessment of the relationship between infarct growth and post stroke hyperglycaemia

7.1 Introduction

In this chapter I have tried to identify differences in the growth of irreversibly damaged tissue (infarct) after a stroke between patients in whom blood glucose remains consistently normal after a stroke, patients in whom blood glucose is increased early (within 6 hours of onset), and patients who develop hyperglycemia >6 hours after stroke onset.

7.2 Methods

7.2.1 Baseline demographics

The baseline demographics have been represented and reanalysed due to the change in population size between this chapter and chapter 6. Analysis of these demographics was performed in the same way as before.

7.2.2 Image analysis

Scans from all patients were analysed independently by two observers (NM and XH), who measured core and penumbra volume on CTP 24-28h infarct volume on the co-registered slices of the follow-up non-contrast CT (i.e. limited to the coverage of the CTP) and total brain 24-48h infarct volume using all slices. The mean of the volumes measured by the two observers was used in statistical analysis.

Infarct growth was calculated as 24-28h infarct volume – core volume and expressed both as absolute growth (ml) and as a percentage of CTP core volume.

Inter-observer agreement had already been established as detailed in Chapter 2.

7.2.3 Statistical analysis

Differences between groups were analysed using one way ANOVA for scale variables and chi squared for categorical variables. If a significant group effect was found by ANOVA, post-hoc between-groups comparisons were undertaken using the least significant difference (LSD) method

Linear regression analysis was carried out using SPSS to seek predictors of infarct growth, penumbral salvage and final infarct volumes. Univariate analysis included age, hypertension, Hba1c, atrial fibrillation, admission blood glucose, NIHSS score, systolic and diastolic blood pressure, thrombolytic treatment, occlusion status and evidence of recanalization. All variables with $p<0.1$ in univariate analysis were entered into a forward stepwise conditional model. Findings were confirmed in a backwards stepping model beginning with all potentially predictive variables.

Binary logistic regression analysis was also carried out using SPSS to seek predictors of recanalization and 30 day mortality. Univariate analysis included age, admission blood glucose, mean capillary blood glucose, atrial fibrillation, HbA1c, NIHSS score, systolic and diastolic blood pressure and thrombolytic treatment as a binary variable. All variables with $p<0.1$ in univariate analysis were entered into a forward stepwise conditional model. Findings were confirmed in a backwards stepping model beginning with all potentially predictive variables

7.3 Results

7.3.1 Population in subgroup analysis

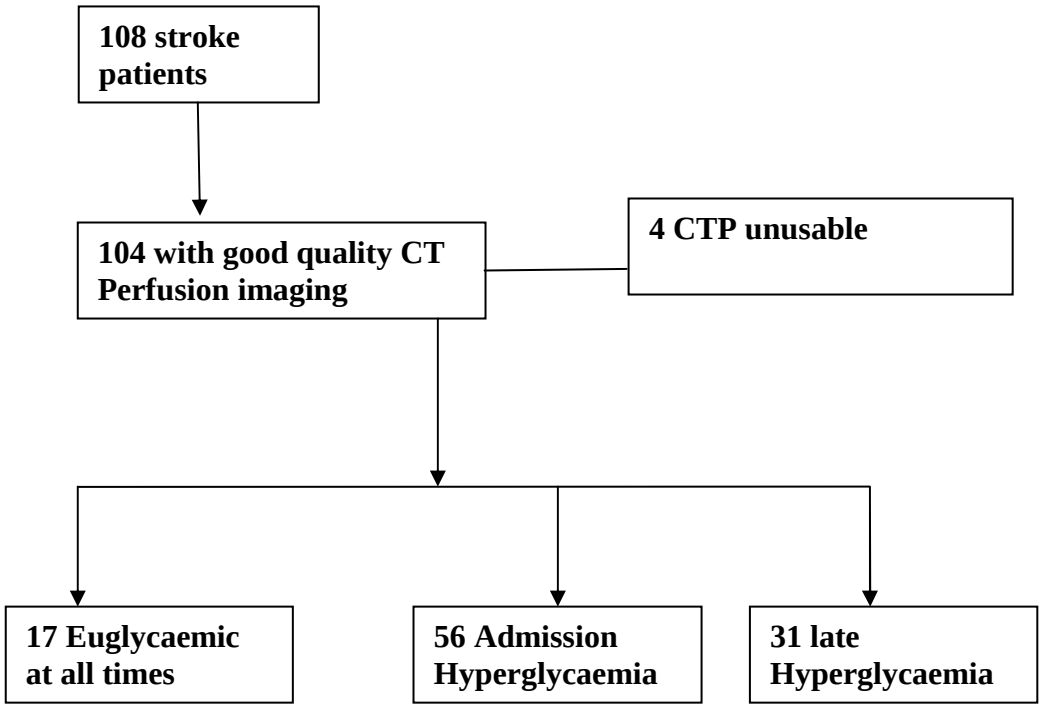

Figure 30 – Recruitment flowchart for patients with good quality CTP imaging

In 4 patients, CT perfusion imaging could not be processed due to movement artefact or other technical problems, leaving 104 patients with ischaemic stroke and analysable CT perfusion imaging. These patients were divided into 3 subgroups based on glycaemic status. Fifty six patients had admission hyperglycemia, 31 subsequently developed hyperglycemia and 17 were normoglycemic at all times.

Table 7-1 Demographics, clinical outcomes and imaging data for patients with CTP imaging

Group *(n)*	All patients (104)	Hyperglycaemia None(17)	Hyperglycaemia Admission (56)	Hyperglycaemia Late (31)	
Male Gender (n/%)	63 (60.6)	10 (59)	32 (57.2)	21(68)	
Age ± SD (mean/years)	69.53	62 ±1 4 *	70.5 ± 10.5 *	71.8 ± 10.5	p=0.01
Admission blood glucose ± SD (Mean mmol/L)	7.47 ± 3.3	5.82 ±0.6 *	8.79 ±3 .98 * **	6.05 ± 0.67 **	p<0.001
Mean capillary glucose ± SD (Mean, mmol/L)	6.75 ± 1.6	5.4 ± 0.59 *	7.47 ± 1.66 * **	6.17 ±1.29 **	p<0.001
HbA1c ± SD (Mean)	5.9 ± 1.1	5.4 ±0.5 *	6.2 ± 1.4 * **	5.7 ± 0.7 **	p=0.016
Admission NIHSS (Median/IQR)	9 (5-18)	11 (4-17)	9.5 (6-19)	8 (5-15)	p=0.659
Admission Blood pressure ± SD (mmHg)	151/78	152/78 ±21/12	148/77 ± 26/18	155/80 ± 21/10	p=0.801
Diabetes (n/%)	19 (18.3)	0	18 (32.1)	1 (3.2)	p<0.001
Impaired glucose tolerance (n/%)	19 (18.3)	0	17 (30.4)	2 (6.5)	
Thrombolytic treatment (n/%)	73 (70.2)	10 (59)	42 (75)	21 (67.7)	p=0.415
Occlusion on imaging (n/%)	67 (64.4)	10 (58.8)	38 (67.9)	19 (61.3)	p=0.644
Recanalization on imaging (n/%)	37/67 (55)	4/10(40)	23/38 (60.5)	10/19 (53)	p=0.4
Supra-aortic large artery atherosclerosis (n/%)	28 (27)	4 (23.5)	16 (29)	8 (26)	p=0.797
Cardio-aortic embolism (n/%)	26 (25)	4 (23.5)	15 (26.8)	7 (22.6)	
Small artery occlusion (n/%)	9 (8.7)	2 (11.8)	4 (7.1)	3 (9.7)	
Other causes (n/%)	5 (4.8)	2 (11.8)	3 (5.4)	0	
Undetermined causes (n/%)	36 (34.6)	5 (29.4)	18 (32.1)	13 (42)	
CT Perfusion Core volume (Mean± SD, ml)	18.9 ± 25.6	20.2 ± 19.5	21.3 ± 29.3	13.8 ± 20.7	p=0.409
CT Perfusion Penumbra volume (Mean± SD, ml)	23 ± 24.4	25.9 ± 26.5	22.2 ± 23.4	22.9 ± 25.5	p=0.868
Total Perfusion Lesion Volume ± SD (Mean, ml)	41.9 ± 45.3	46.1 ± 40	43.6 ± 48.4	36.6 ± 43.1	p=0.730
Co-registered 24-48h infarct volume ± SD (Mean, ml)	41.9 ± 55.7	33.5 ± 39.8	51.9 ± 63.2	28.5 ± 45.6	p=0.135
24-48h Infarct Volume ± SD (Mean, ml)	78.2 ± 97.1	56 ± 66.5	85.3 ± 11.7	44.3 ± 76.9	p=0.144
Infarct Growth ± SD (Mean, ml)	23.6 ±	14.6 ± 21.7	31.1 ± 46.1	14.7 ± 28.5	p=0.1
Penumbral salvage ± SD (Mean, ml)	0 ± 41.8	12.6 ± 32	-8.4 ± 48	8.2 ± 29.9	p=0.083
Rankin 0-1 Day 30 (n/%)	17 (16.3)	5 (29.4)	8 (14.3)	4 (12.9)	p=0.504
Rankin 2-5 Day 30 (n/%)	75 (72.1)	11 (64.7)	40 (71.4)	24 (77.4)	
Rankin 6 Day 30 (n/%)	12 (11.5)	1(6)	8 (14.3)	3 (9.7)	

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG

7.3.2 Baseline demographics

As seen in the entire study population, euglycaemic patients were significantly younger than hyperglycaemic patients (p=0.01) in the CTP sub-group.

Unsurprisingly the admission blood glucose level was significantly different between groups (p<0.001). Prior glycaemic status as defined by glycosylated haemoglobin was also significantly different between groups (p=0.016). There were also significantly more patients in the admission hyperglycaemia group who had a pre-existing diagnosis of diabetes mellitus or impaired glucose tolerance before admission.

There were no significant differences in cause of stroke between groups. (p=0.797)

There was no difference in baseline stroke severity as assessed by NIHSS score (p=0.659). Similar proportions of patients had angiographic evidence of occlusion between the groups (P=0.644) and similar proportions received thrombolytic treatment (p=0.415)

7.3.3 Occlusion counts

Overall 64% of patients had an occlusion on CTA. Thirty-five patients had M1 occlusions while 18 had M2 occlusion. Fifteen had an intracranial ICA occlusion while 13 had extracranial ICA occlusions. Twenty patients (19%) had tandem occlusions (2 or more sites of occlusion). There were no significant differences in occlusion counts between glycaemic groups More details are available in Table 7.2.

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Table 7-2 Site of occlusion

Occlusion	Overall (n/%)	Hyperglycaemia			
		None (n/%)	Admission (n/%)	Late (n/%)	
M1	35 (32)	6 (35)	18 (31)	11 (34)	**p=0.897**
M2	18 (17)	2 (12)	12 (20)	4 (12.5)	**p=0.531**
M3	7 (6.5)	1 (6)	3 (5)	3 (9)	**p=0.725**
Extracranial ICA	13 (12)	3 (18)	6 (10)	4 (12.5)	**p=0.703**
Intracranial ICA	15 (14)	3 (18)	7 (12)	5 (16)	**p=0.785**
Vertebral	1 (0.9)	1 (6)	0	0	**p=0.067**
PCA	7 (6)	0	4 (7)	2 (6)	**p=0.549**
Tandem occlusion	20 (19)	3 (18)	9 (15)	8 (25)	**p=0.518**

7.3.4 Infarct growth

There were no significant differences in baseline CTP core or penumbra volumes. There were no significant differences in follow-up infarct volumes between groups. However, despite the lack of statistical significance, mean co-registered follow-up infarct volumes and mean total follow-up infarct volumes were larger in the group with admission hyperglycaemia. The admission hyperglycaemia group also appeared to have greater infarct progression than the other groups. I have illustrated the distribution of infarct growth and 24-48 hour infarct volumes as box plots (Figures 7.3 and 7.4).

The difference between the mean infarct growth in the admission hyperglycaemia group and the late hyperglycaemia group was just non-significant, with p=0.058 in post-hoc analysis.

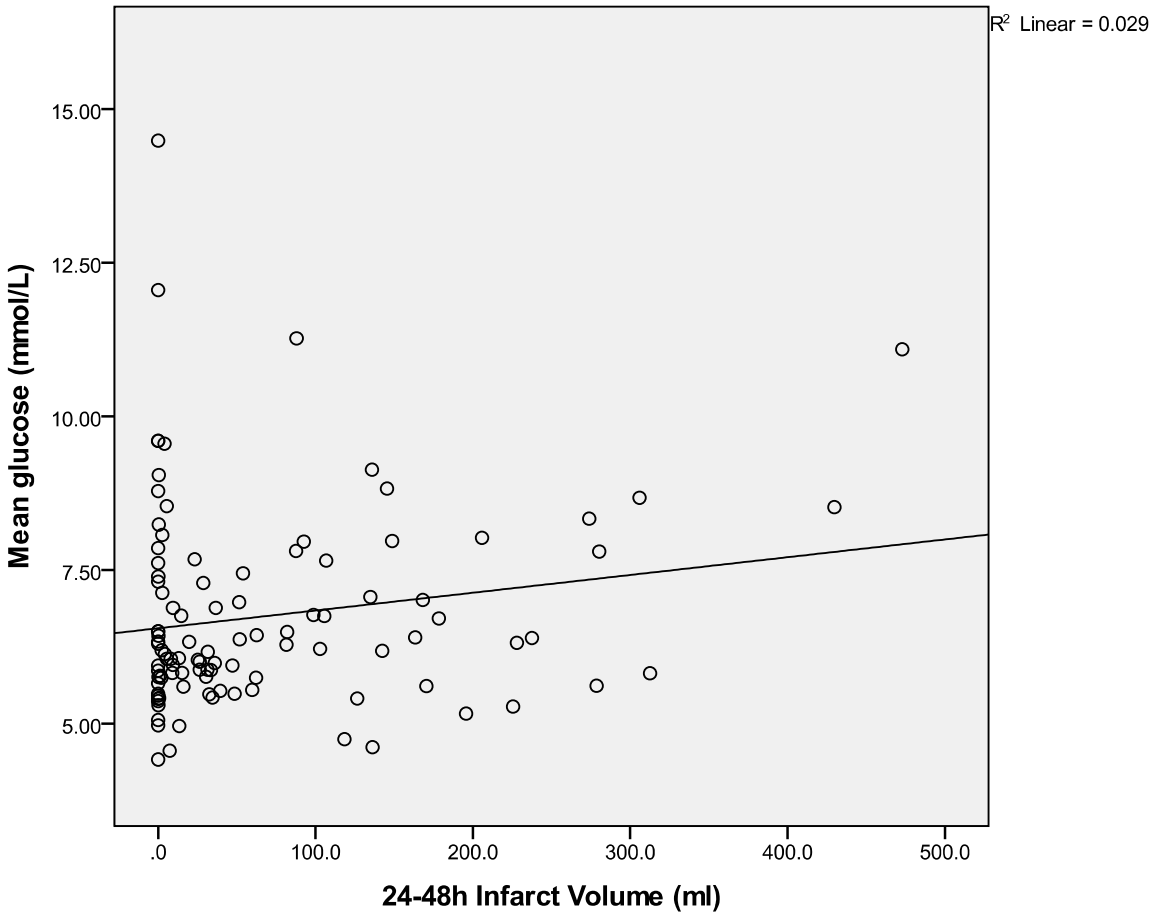

Figure 31 – Scatter plot of outcome infarct volume against mean glucose level

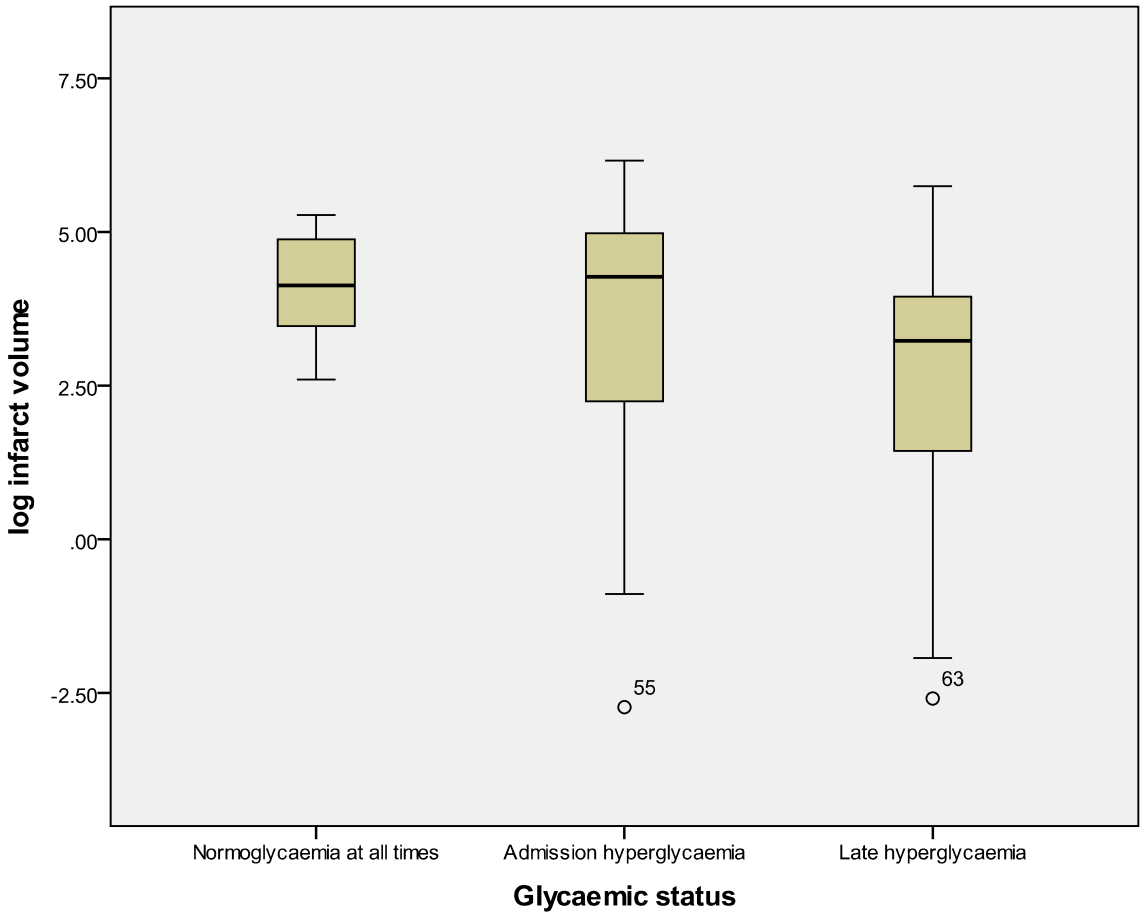

Figure 32 - Natural log of 24-48 hour infarct volume in glycaemic groups

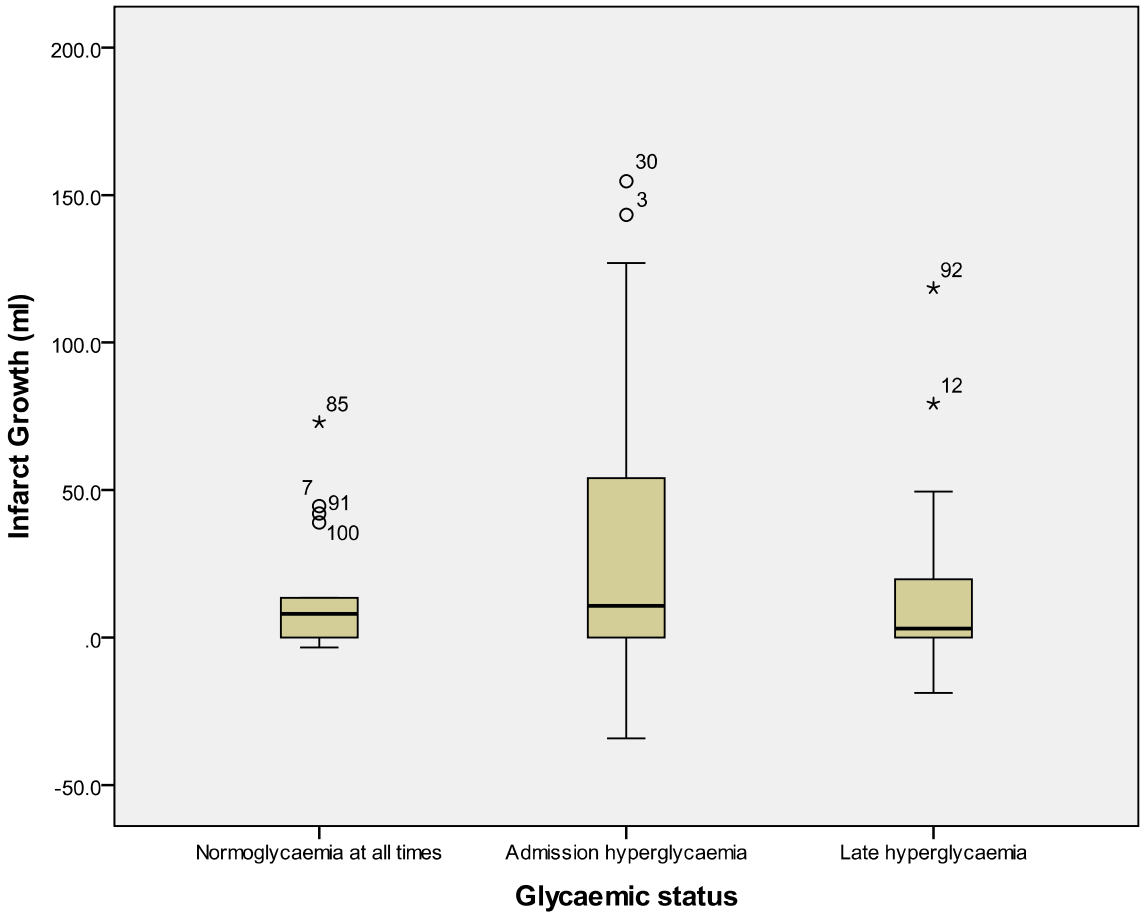

Figure 33 - Infarct growth in glycaemic groups

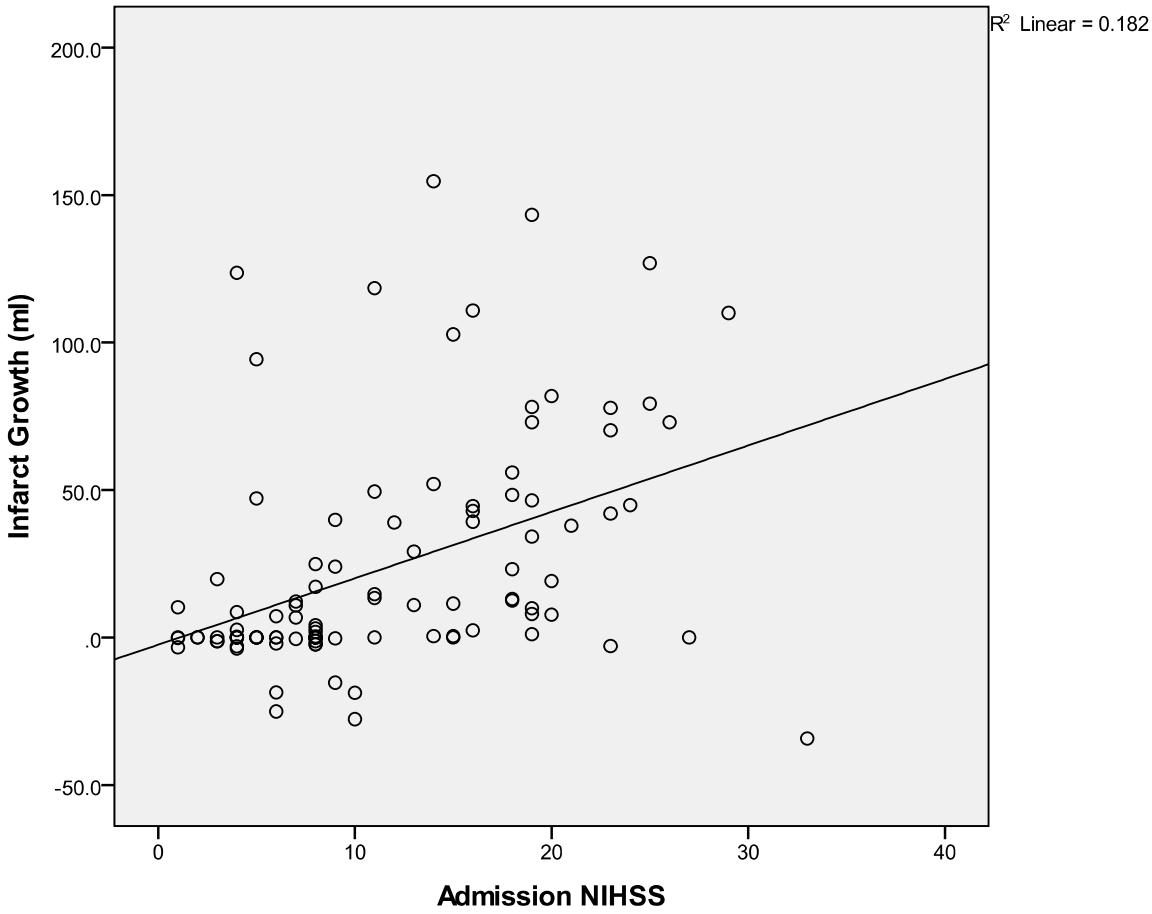

Figure 34 - Infarct growth in relation to admission NIHSS

In linear regression, admission NIHSS (p<0.001, OR 1.81, 95% CI 0.86 to 2.76), admission blood glucose (p=0.030, OR 2.493, 95% CI 0.25 to 4.74) and systolic blood pressure (p=0.066, OR 0.268, 95% CI -0.18 to 0.55) were predictive of infarct growth. R squared was 0.234. Variables excluded in the forward and backward regressions included thrombolysis, Hba1c, diastolic blood pressure, presence or absence of occlusion. When the model was repeated, substituting mean capillary blood glucose (MCBG) for admission blood glucose (ABG) R squared was 0.378. This model included admission NIHSS (p<0.001, OR 2.08, 95% CI 1.34 to 2.81), mean blood glucose (p=0.007, OR 5.17, 95% CI 1.43 to 8.9) and admission systolic blood pressure (p=0.004, OR 0.31, 95% CI 0.11 to 0.57). Age was forced into this model to correct for baseline imbalances.

Table 7-3 Coefficients in backwards regression for Infarct growth

Variable	P value	Odds ratio	95% Confidence interval
Admission NIHSS	<0.001	2.02	1.29 – 2.75
Admission Systolic blood pressure	0.008	0.3	0.08 – 0.53
Mean capillary blood glucose	0.009	5.06	1.3 – 8.81

7.3.5 Infarct growth in patients with initial core volumes of more than 10 ml

To look at infarct growth as a percentage we restricted the analysis to patients with a baseline core volume of 10ml or greater to reduce the influence of measurement error. When intra-observer reliability is assessed repeated measurements are often less consistent on smaller volumes. The baseline demographics and outcomes for this subset are shown in table 7.4.

In this selected subpopulation of patients with core lesions of 10ml or larger on perfusion imaging there were significant differences in age and glucose levels (admission and mean) as seen in the cohort as a whole. A high proportion of these patients had an occlusion on imaging (94%) with 100% of the admission hyperglycaemia patients having an occlusion compared to 91% of the late hyperglycaemia patients and 83% of the normoglycaemia patients (p=0.09). Core lesions were smaller in the normoglycaemia group but not significantly so.

Table 7-4 - Baseline demographics, imaging outcomes and clinical outcomes for patients with initial core volumes of more than 10ml

Group (n)	All patients (52)	Hyperglycaemia None (12)	Hyperglycaemia Admission (29)	Hyperglycaemia Late (11)	
Male Gender (n/%)	28 (53.8)	10 (59)	32 (57.2)	21(68)	
Age ± SD (mean/years)	70 ± 12.3	62.6 ± 14.9 * ***	73 ± 10.6 *	70.1 ± 11 ***	p=0.044
Admission blood glucose ± SD (Mean mmol/L)	7.54 ± 3.68	6.02 ± 0.5 *	8.81 ± 4.55* **	5.94 ± 0.88 **	p=0 .02
Mean capillary glucose ± SD (Mean, mmol/L)	6.59 ± 1.4	5.3 ±0.53 *	7.27 ± 1.47 * **	6.2 ± 0.66 **	p<0.001
HbA1c ± SD (Mean)	5.9 ±1.2	5.5 ± 0.5 *	6.3 ± 1.6 * **	5.6 ± 0.4 **	p=0
Admission NIHSS (Median/IQR)	16 (10-19)	13.5 (11-18.75)	18 (11.5-19.5)	11 (9-20)	p=0.271
Admission Blood pressure ± SD (mmHg)	149/24 ± 3/2	157/83±21/10	147/75 ± 29/19	144/79 ±22/10	p=0.408
Thrombolytic treatment (n/%)	45 (86.5)	9 (75)	27 (93.1)	9 (81.8)	p=0.265
Occlusion on imaging (n/%)	49 (94.2)	10 (83)	29 (100)	10 (91)	p=0.099
Recanalization on imaging (n/%)	26/49 (53.1)	4/10 (40)	18/29 (62.1)	4/10 (40)	p=0.147
CT Perfusion Core volume (Mean± SD, ml)	36.2±26.6	28.6 ± 17	39.8 ± 30.9	34.9 ±22.5	p=0.477
CT Perfusion Penumbra volume (Mean± SD, ml)	40.3 ± 22.3	36.6 ± 24.3	39.2 ± 20.6	47.3 ± 25.1	p=0.484
Total Perfusion Lesion Volume ± SD (Mean, ml)	76.5 ± 39.9	65.3 ± 31	78.9 ±43.1	82.2 ± 40.4	p=0.533
Co-registered 24-48h infarct volume ± SD (Mean, ml)	71.9 ± 61.5	47.5 ± 39.8	83.4 ± 68.5	68.3 ± 57.4	p=0.233
24-48h Infarct Volume ± SD (Mean, ml)	113.5 ± 112.1	79.4 ± 66.4	135.7 ± 129.4	92.4 ± 95.3	p=0.273
Infarct Growth ± SD (Mean, ml)	36.8 ± 45.7	21 ± 23.3	44.6 ± 53.2	33.3 ± 40.5	p=0.314
Infarct growth as percentage of core (%)	--10.5 ± 214	32.2 ± 27	-0.3 ± 134	-83.9 ± 418	p=0.408
Penumbral salvage ± SD (Mean, ml)	2 ± 51	17.8 ± 37	-4.4 ± 60	14 ± 46	p=0.386
Rankin 0-1 Day 30 (n/%)	6 (11.5)	2 (16.7)	3 (10.3)	1 (9.1)	
Rankin 2-5 Day 30 (n/%)	35 (67.3)	9 (75)	18 (62.1)	8 (72.3)	
Rankin 6 Day 30 (n/%)	11 (21.2)	1(8.3)	8 (27.6)	2 (18.3)	p=0.70

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG.

Outcome infarct volumes were on average smaller in the normoglycaemia group than the other groups (79.4ml vs. 135.7ml and 92.4ml, p=0.273). There was a non-significant trend for patients in the normoglycaemia group to have a higher percentage of infarct growth. Logarithmic transformation did not make this relationship significant.

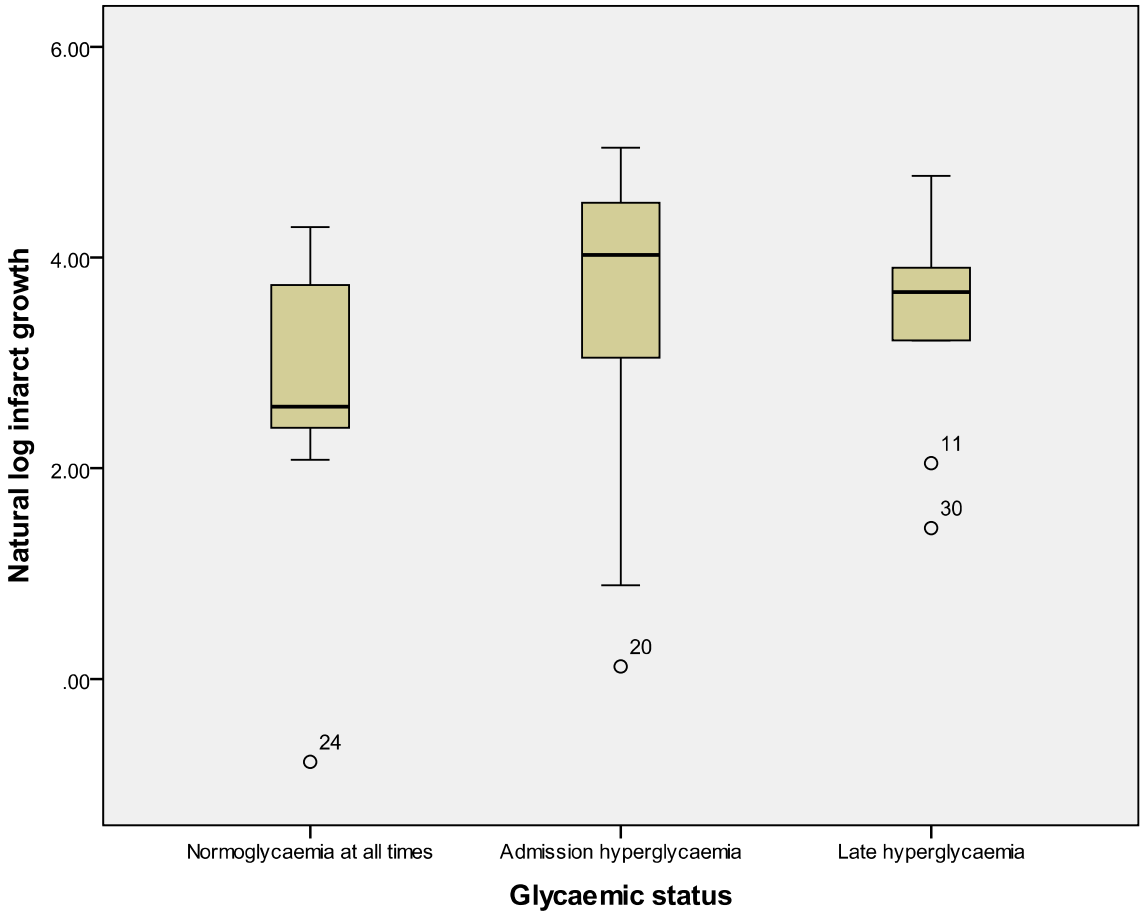

Figure 35 - Infarct growth in patients with an initial perfusion core volume of >10ml

I also looked for a relationship between mean capillary blood glucose and transformed infarct growth as illustrated below (Figure 36).

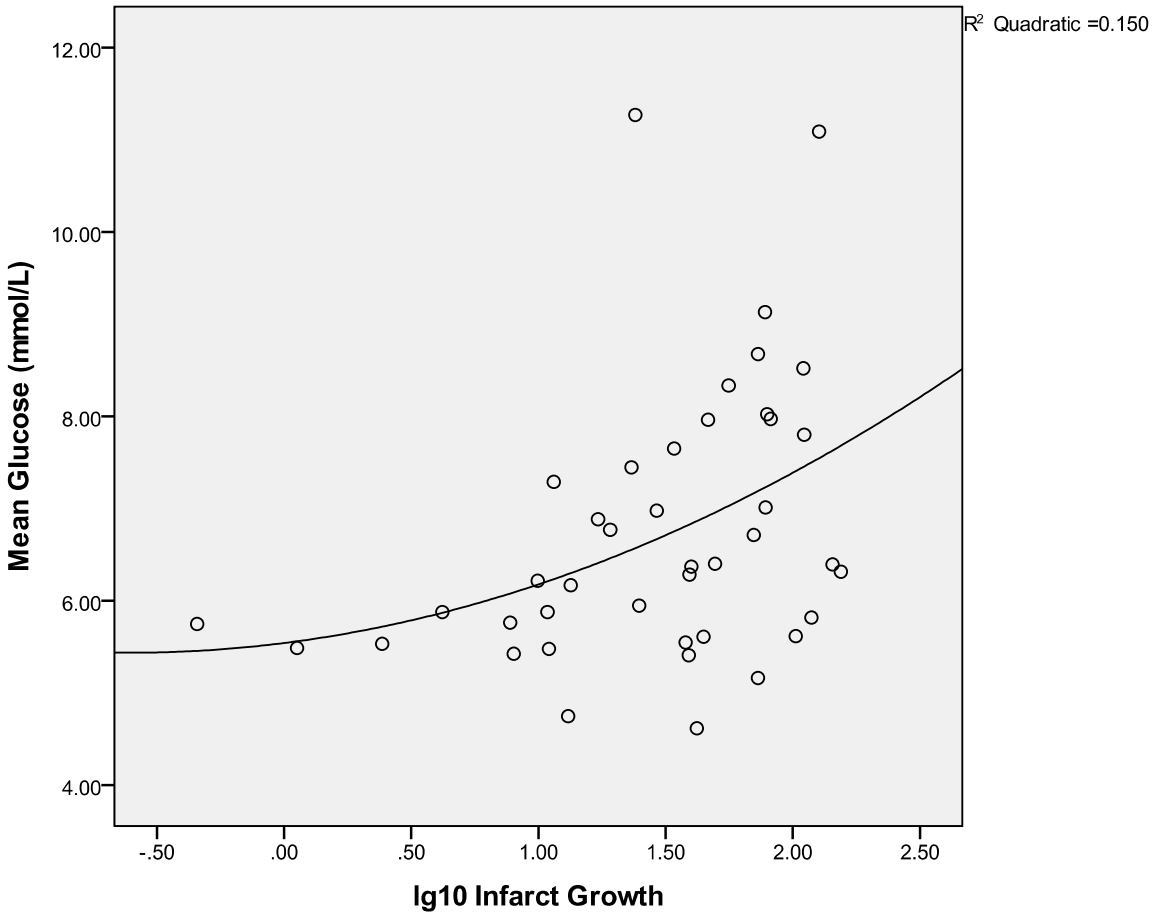

Figure 36 - Relationship between mean capillary blood glucose and transformed infarct growth in patients with baseline core perfusion lesion >10ml

There was a trend towards a difference in mean transformed infarct growth although this was not significant (p=0.16).

7.3.6 Penumbral salvage

As illustrated in the bar chart in Figure 37 there was a tendency for less penumbra to be salvaged in the admission hyperglycaemia group. This trend is non-significant.

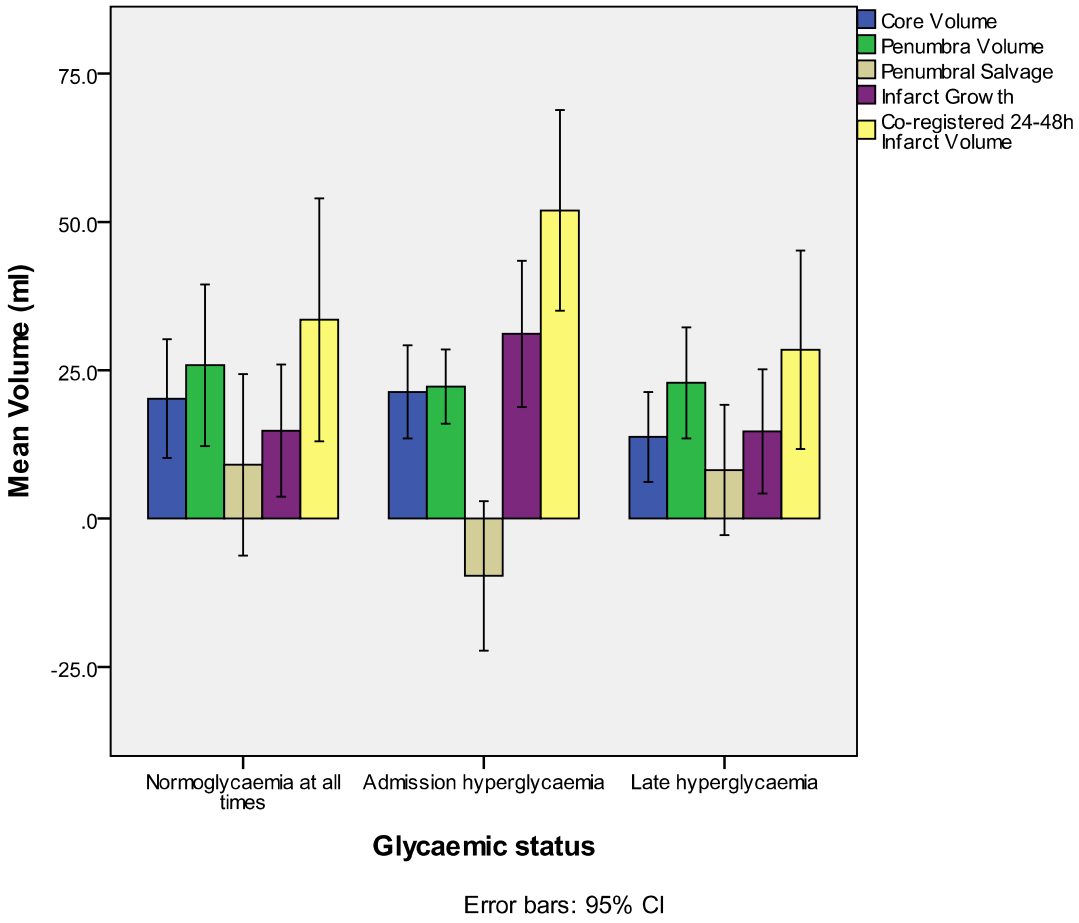

Figure 37 - Glycaemic status and imaging findings

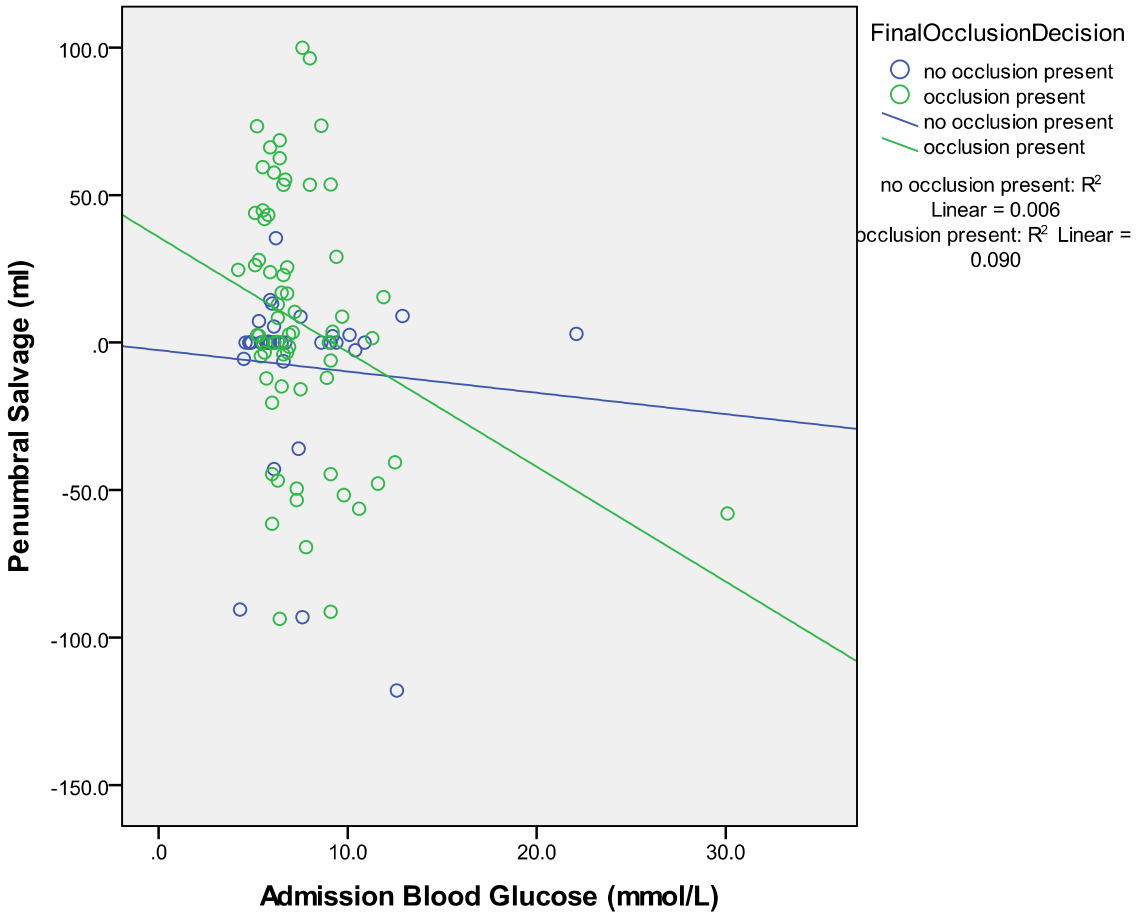

Figure 38 - Scatter plot of penumbral salvage and admission blood glucose

7.3.7 Recanalization

Using binary logistic regression a model containing admission blood glucose (p=0.005, OR 1.4, 95% CI 1.1 to 1.78), thrombolytic treatment (p=0.005, OR 5.29, 95% CI 1.66 to 16.8) and HbA1c (p=0.017, OR 0.41, 95%CI 0.19 to 0.85) was predictive of recanalization in 70.1% of cases.

Table 7-5 Variables in backward regression for prediction of recanalization

Variable	p value	Odds ratio	95% Confidence interval
HbA1c	0.017	0.41	0.19 – 0.85
Thrombolysis	0.005	5.29	1.66 – 16.8
Admission blood glucose	0.005	1.4	1.1 – 1.78

7.3.8 Clinical outcomes

There were no significant differences in clinical outcomes between groups based on day 30 modified Rankin scores when analysed with a 3x3 chi squared test when mRs was split into three groups. Modified Rankin score was divided into 0-1, 2-5 and 6 as well as 0-2, 3-5 and 6 but was still non-significant. The distribution of Rankin scores between glycaemic groups is illustrated in Figure 39. Rankin shift analysis using Cochran-Mantel-Haenszel statistics found no significant relationship between glycaemic status and outcome when corrected for age and admission NIHSS (p=0.329).

Binary logistic regression showed that mean core volume was a predictor for mortality at 30 days with an odds ratio of 2.27 (95% CI 1.41 to 3.67, p<0.001,) per 10 ml in a model that also included systolic blood pressure (OR 0.95, p=0.047, 95% CI 0.9 to 0.99). This model had a p value of <0.001.

Table 7-6 Variables in binary logistic regression for 30 day mortality

Variable	P value	Odds ratio	95% Confidence interval
Admission systolic blood pressure	0.047	0.95	0.9 - 0.99
Core perfusion lesion volume (per 10 ml increment)	<0.001	2.27	1.41 - 3.67

I repeated this regression in the subset of patients with a baseline core lesion of more than 10ml. Again a 10ml incremental increase in core volume was associated with increased risk of day 30 mortality (p=0.007, OR 1.53, 95% CI 1.12 to 2.09).

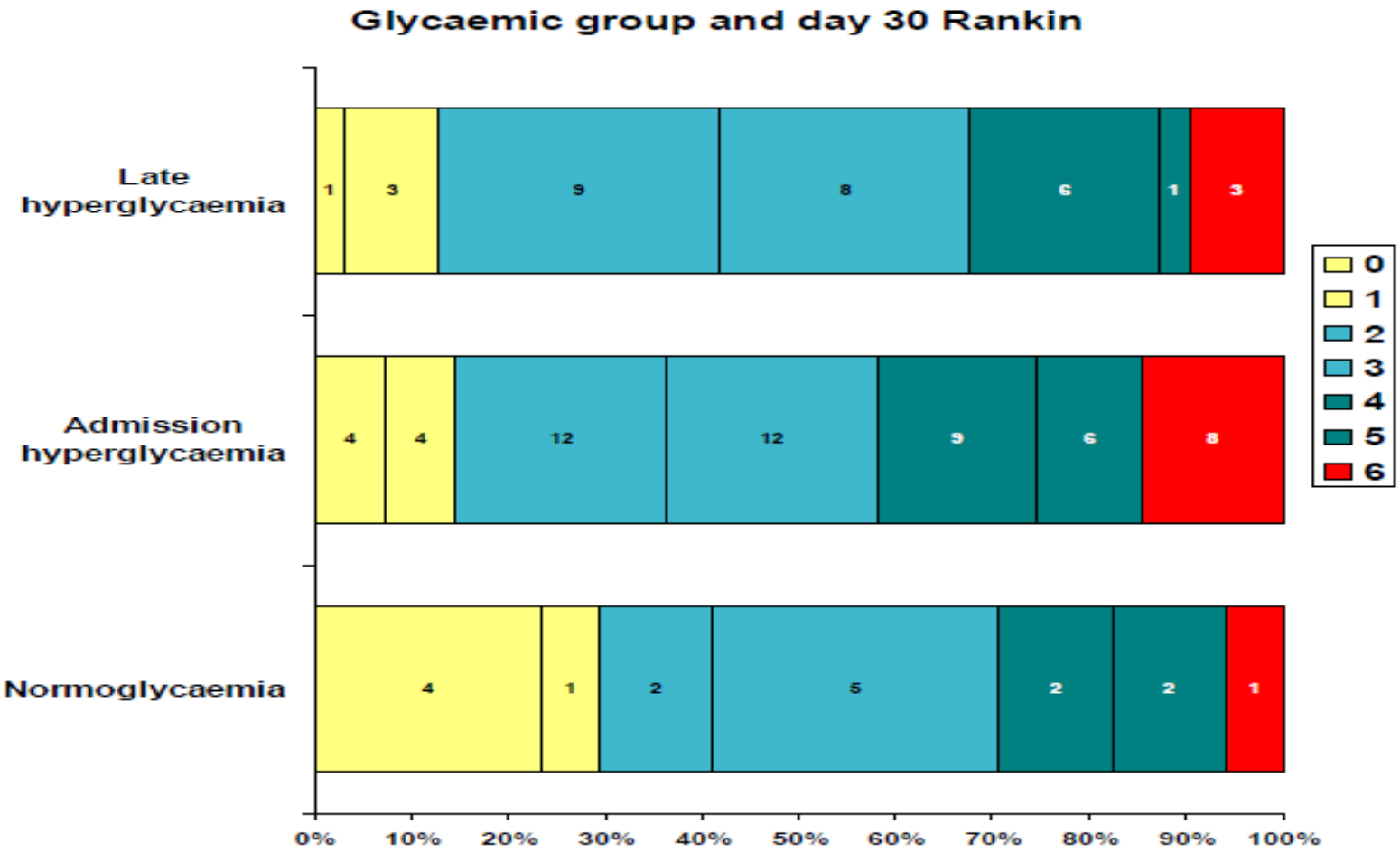

Figure 39 - Day 30 Rankin scores by glycaemic groups

7.4 Discussion

This chapter explores infarct growth in relation to blood glucose. The core lesion defined on CT perfusion imaging was compared with 24-48h infarct volume on co-registered non-contrast CT slices that covered the same area of brain as the CT perfusion scan.

Several interesting correlations were observed. Higher admission glucose and higher mean glucose were correlated with larger follow-up infarct volumes. Higher NIHSS scores correlated with greater infarct growth. It was also interesting to note that increasing core volume correlated with mortality while increasing systolic blood pressure was associated with reduced mortality.

Baseline demographics in the subgroup analysed in this population were similar to those described in Chapter 6 despite the loss of 4 patients due to inadequate CTP imaging. As before, significant differences were seen between glycaemic groups for age, admission blood glucose level and prior glycaemic status. Mean capillary blood glucose was significantly different between admission hyperglycaemia group and the other groups but not between the euglycaemic group and the later hyperglycaemic group. Other baseline clinical variables did not differ significantly across groups.

No significant difference in infarct growth between groups was seen although there was certainly a trend towards increased infarct growth in patients with admission hyperglycaemia when compared to the other groups. Follow up infarct volumes also trend towards being larger in patients with higher mean glucose levels. This trend was consistent when analysis was restricted to patients with a baseline core lesion volume of more than 10ml.

Overall penumbral salvage was not significantly different between groups ($p=0.075$). However, there was a trend towards less penumbral salvage in the admission hyperglycaemia group (AHG) compared to the late hyperglycaemia group and the normoglycaemia group (mean volume salvaged -8.4ml compared to 8.2ml and 12.6ml, post hoc p-values 0.076 and 0.07). Expressed as absolute difference in volume between baseline and 24-48h scans, infarct growth was twice as great in the AHG group compared to the LHG group, (mean AHG growth 31.1ml vs. mean LHG growth 14.7ml) but was just non-significant ($p=0.058$).

Interestingly in the group of patients with a baseline core perfusion lesion of more than 10ml the highest percentage of infarct growth was seen in the normoglycaemia group (32.2%) whilst the lowest percentage was seen in the late hyperglycaemia group (-89.3%).

There is a definite trend towards patients with admission hyperglycaemia having larger final infarcts with greater infarct growth despite similar baseline core volumes despite the lack of clearly significant results. Future studies should reproduce this result in a larger population to confirm a difference between these groups.

Both admission blood glucose and mean capillary blood glucose were correlated with 24-48h infarct volume. A higher admission NIHSS was associated with greater infarct growth while increased systolic blood pressure was associated with reduced infarct growth. Admission blood glucose, HbA1c and thrombolytic therapy were predictors of recanalization.

Admission hyperglycaemia was associated with reduced penumbral salvage although this finding should be confirmed in other studies. Late hyperglycaemia does not appear to be as damaging. The patients with late hyperglycaemia who had baseline core volumes of more than 10ml had the lowest percentage of infarct growth. The late rise in glucose levels could be an artefact of a patient recovering from a stroke and returning to a normal diet. Certainly hyperglycaemia developing only after 6 hours does not appear to have adversely affected the evolution of brain ischaemia in these patients.

Despite looking at outcome Rankin scores with different iterations and methods no significant correlation between glycaemic group and mRs was seen. The highest proportion of patients died in the admission hyperglycaemia group while the smallest proportion died in the normoglycaemia group.

Mortality was predicted by increasing core perfusion volume. The predictive value of perfusion core lesions has been described before.[577, 578] It is interesting to note that increasing admission systolic blood pressure was associated with reduced mortality. It is unclear why this may be although one could hypothesize that this may be a healthy physiological response where the brain is trying to increase cerebral blood flow to salvage penumbral tissue. It is also possible that patients with higher blood pressure will distribute rt-PA to a clot more efficiently.

Core volumes were not significantly different between glycaemic groups although when the analysis was restricted to baseline core volumes of 10ml or greater a trend emerged for the patients with admission hyperglycaemia to have larger core lesions. Repeated binary regression analysis in this population demonstrated the relationship between increasing core volume and risk of death.

Parsons and colleagues[176] performed acute MRI in 40 patients at a median of 4.5 hours after stroke onset, a similar time point to the patients included in this study,[176] and also reported a correlation between acute (admission) hyperglycaemia (glucose measured within 1h of MRI) and reduced penumbral salvage, consistent with our observations using different imaging methodology. While we also found greater final infarct size among those with admission hyperglycaemia, this was not statistically significant, and may reflect a more heterogeneous population. Our larger population sample had a lower median NIHSS with a wider intra-quartile range. The mean age of our population was lower at 69 (vs. 73) and we had a larger intra-quartile age range (61-78 vs. 64-78). Many factors may make our population different from the population in the Australian paper.

.The observation that hyperglycaemia is associated with increased transformation of penumbra into core has been made several times.[160, 202, 203] Certainly the EPITHET investigators felt that the beneficial effects of rt-PA were attenuated in diabetes.[203] The results in this chapter do support the theory that hyperglycaemia may increase infarct growth in many patients. They additionally suggest that not all hyperglycaemia is bad and that some patterns of hyperglycaemia may be better than others.

In the population studied in this chapter recanalization was predicted by a model including admission blood glucose, thrombolytic treatment and HbA1c. Recanalization will be looked at in more detail in Chapter 8 and Chapter 9.

8 Infarct growth in the context of arterial patency and glycaemic status

8.1 Introduction

The harmful effects of post stroke hyperglycemia may be restricted to specific groups of patients, defined by pathophysiological processes. Treatment to lower glucose may be unnecessary or even harmful in patients who do not share these features.

One important pathophysiological variable is the presence or absence of arterial occlusion. Patients with an arterial occlusion have worse outcomes than those who do not. A persistent arterial occlusion is associated with poorer outcomes while recanalization generally confers a better prognosis.

A recent clinical trial of insulin treatment for PSH reported divergent effects of treatment dependent on arterial patency, being associated with greater infarct expansion in patients with persistent occlusion, but less expansion than placebo in those with recanalization.[554] The InsulInfarct study with compared a subcutaneous insulin treatment regime with an intensive insulin infusion regime found that the more intensive insulin regime was associated with greater infarct growth in patients with arterial occlusions.[562] It appears therefore that early and late PSH may differ in their causes and pathophysiological significance, and that individual brain tissue vulnerability and vessel status further influence the effects of PSH.

In this section of the study we aimed to define the interaction of early and delayed hyperglycaemia with arterial patency and brain perfusion in acute stroke patients.

8.2 Methods

As detailed previously all patients underwent CTP and CTA on admission, in addition to routine CT. Plain CT and CTA (limited to Intracranial vessels) were repeated at 24-48h to establish final infarct volume and arterial patency (persistent occlusion or recanalization). Angiographic imaging was only repeated if there was evidence of an occlusion on the initial scan.

8.2.1 Image analysis

The analysis of the CTP scans and the plain CT scans was performed as detailed in the previous chapter. The admission and follow-up scans were again read by two observers (NM and XH) who looked for presence or absence of an arterial occlusion on the admission scan and for evidence of recanalization in the follow-up imaging. If there was any disparity between observers a final decision was made by an experienced stroke neurologist who could also make reference to neuroradiology reports (KM).

8.2.2 Analysis of infarct growth

Infarct growth was defined as the difference between core perfusion volume lesion and final co-registered infarct volume. Patients were divided into groups based on glycaemic status as described in the previous chapters. These groups were further subdivided by presence or absence of arterial occlusion and then by recanalization status.

8.2.3 Statistical analysis

Differences between groups were analysed using ANOVA for scale variables and chi squared for categorical variables. If a significant difference between groups was discovered using ANOVA it was further analysed using the least significant difference method.

Linear regression analysis was carried out using SPSS to seek predictors of infarct growth, penumbral salvage and final infarct volumes. Univariate analysis included age, hypertension, Hba1c, atrial fibrillation, admission blood glucose, mean capillary blood glucose, NIHSS score, systolic and diastolic blood pressure, thrombolytic treatment, occlusion status and evidence of recanalization. All variables with $p<0.1$ in univariate analysis were entered into a forward stepwise conditional model. Findings were confirmed in a backwards stepping model beginning with all potentially predictive variables.

8.3 Results

8.3.1 Patients

104 patients had ischaemic strokes with analysable CT perfusion imaging. These patients were divided into 3 subgroups based on glycaemic status.

8.3.2 Patients with occlusions

Sixty seven patients had angiographic evidence of arterial occlusion while 37 patients had no evidence of occlusion. Of the patients with occlusions 38 (56.7%) were hyperglycaemic on admission, 19 (28.4%) subsequently became hyperglycaemic and 10 (14.9%) were euglycaemic at all times.

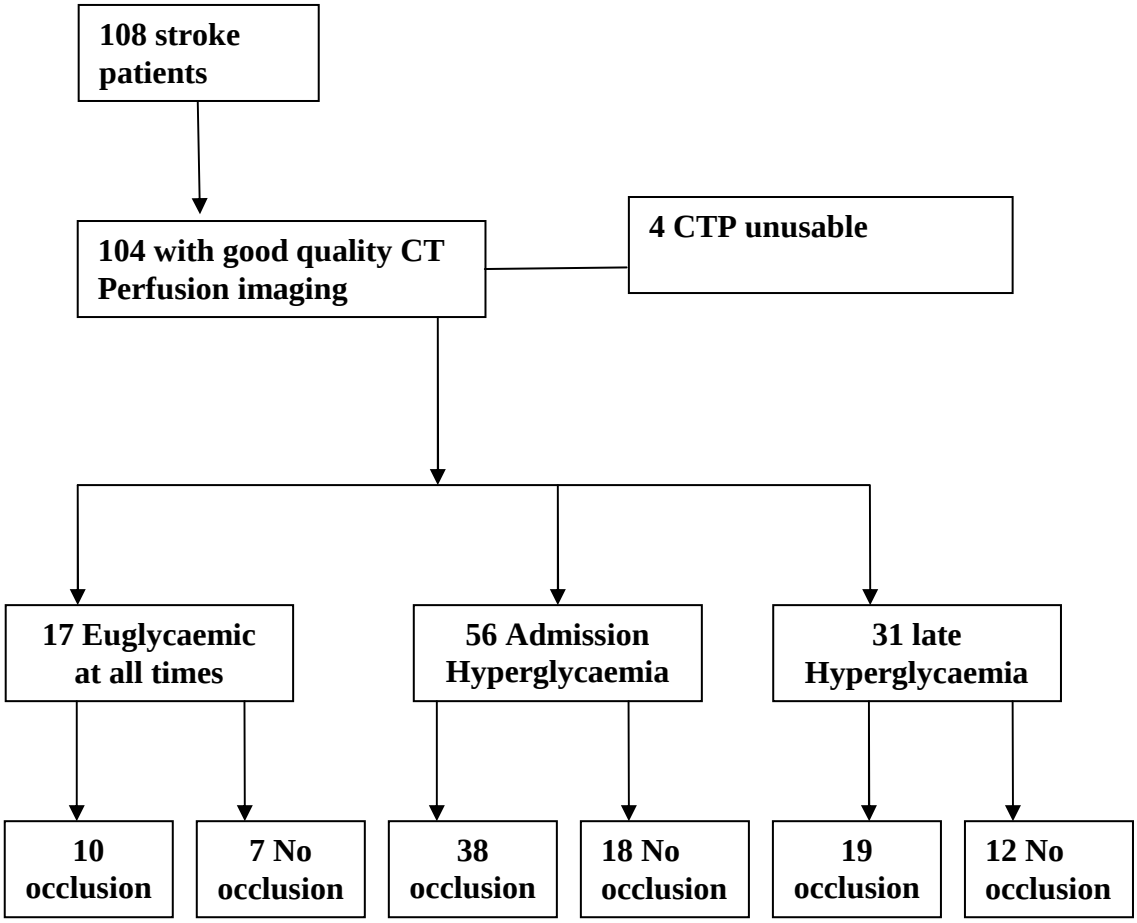

Figure 40 - Flowchart for occlusion status

Table 8-1 Baseline demographics, clinical outcomes and imaging data for patients with occlusions

Group (n)	All (67)	Hyperglycaemia			
		None (10)	Admission (38)	Late (19)	
Male Gender (n/%)	40 (59.7.)	5 (50)	23 (60.5)	12(63.2)	
Age ± SD (mean/years)	71 ± 11.1	62.9 ± 16.4 * ***	71.7 ± 10.5 *	73.8 ± 6.7 ***	p= 0.032
Admission blood glucose ± SD (Mean mmol/L)	7.52 ± 3.36	5.99 ± 0.55 *	8.76 ± 4.05 * **	5.93 ± 0.81 **	p=0.002
Mean capillary blood glucose ± SD (Mean, mmol/L)	6.6 ± 1.29	5.23 ± 0.55 * ***	7.16 ± 1.33 * **	6.22 ± 0.69 ** ***	p<0.001
HbA1c ± SD (Mean)	5.86 ± 1.1	5.51 ± 0.58	6.1 ± 1.4	5.6 ± 0.44	p=0.167
Admission NIHSS (Median/IQR)	15 (8-19)	15 (11-20)	15.5 (8-19)	10 (8-18)	p=0.646
Admission Blood pressure ± SD (mmHg)	150/78 ± 23/15	156/85 ± 23/9	150/76 ± 26/18	148/79 ± 19/10	p=0.721
Diabetes (n/%)	9 (13.4)	0	9 (23.7)	0	p=0.022
Impaired glucose tolerance (n/%)	10 (14.9)	0	9 (23.7)	1 (5.3)	p=0.092
Thrombolytic treatment (n/%)	56 (83.6)	8 (80)	35 (92.1)	13(68.4)	p=0.076
Supra-aortic large artery atherosclerosis (n/%)	22 (32.8)	3 (30)	11 (28.9)	8 (25.8)	
Cardio-aortic embolism (n/%)	24 (35.8)	4 (40)	13 (34.2)	7 (22.6)	
Small artery occlusion (n/%)	3 (4.5)	0	0	3 (9.7)	
Other causes (n/%)	4 (6)	1 (10)	3 (7.9)	0	p=0.795
Undetermined causes (n/%)	26(38.9)	2 (20)	11 (28.9)	13 (42)	
Recanalization (n/%)	37 (55.2)	4 (40)	23 (60.5)	10 (52.6)	p=0.488
CT Perfusion Core volume ±SD (Mean, ml)	26.9 ± 27	27.9 ± 16.4	31 ± 31.3	18.2 ± 20.4	p=0.242
CT Perfusion Penumbra volume ±SD (Mean, ml)	33 ± 23.4	34.9 ± 22.7	32 ± 22.5	33.9 ± 26.6	p=0.925
Total Perfusion Lesion Volume ±SD (Mean, ml)	60 ± 43.8	62.8 ± 25	63 ± 47.5	52.1 ± 44.5	p=0.664
Co-registered 24-48h infarct volume ± SD (Mean, ml)	55.54 ± 57.9	33.5 ± 40.9	69.2 ± 65.8 **	33.4 ± 40 **	p=0.073
24-48h Infarct Volume ±SD (Mean, ml)	89.3 ± 103.2	56 ± 69.9	115 ± 120.6 **	43.1 ± 55 **	p=0.041
Infarct Growth ± SD (Mean, ml)	29.4 ± 41.1	14.6 ± 14.8	39 ± 48.5 **	15.2 ± 24.4 **	p=0.088
Penumbral salvage ± SD (Mean, ml)	4.4 ± 46.5	17.3 ± 40.6	-6.2 ± 53.6	18.7 ± 25.4	p=0.103
Rankin 0-1 Day 30 (n/%)	9 (13.4)	2(20)	5 (13.2)	2(10.5)	
Rankin 2-5 Day 30 (n/%)	46 (68.7)	7(70)	25 (65.8)	14 (73.7)	
Rankin 6 Day 30 (n/%)	12 (17.9)	1(10)	8 (21.1)	3 (15.8)	p= 0.80

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG.

8.3.3 Infarct growth in patients with occlusions

There was a significant difference in 24-48h infarct volume (p= 0.041) that was most pronounced, in post hoc analysis, between patients who were hyperglycaemic on admission and those who became hyperglycemic at a later time (p=0.039).

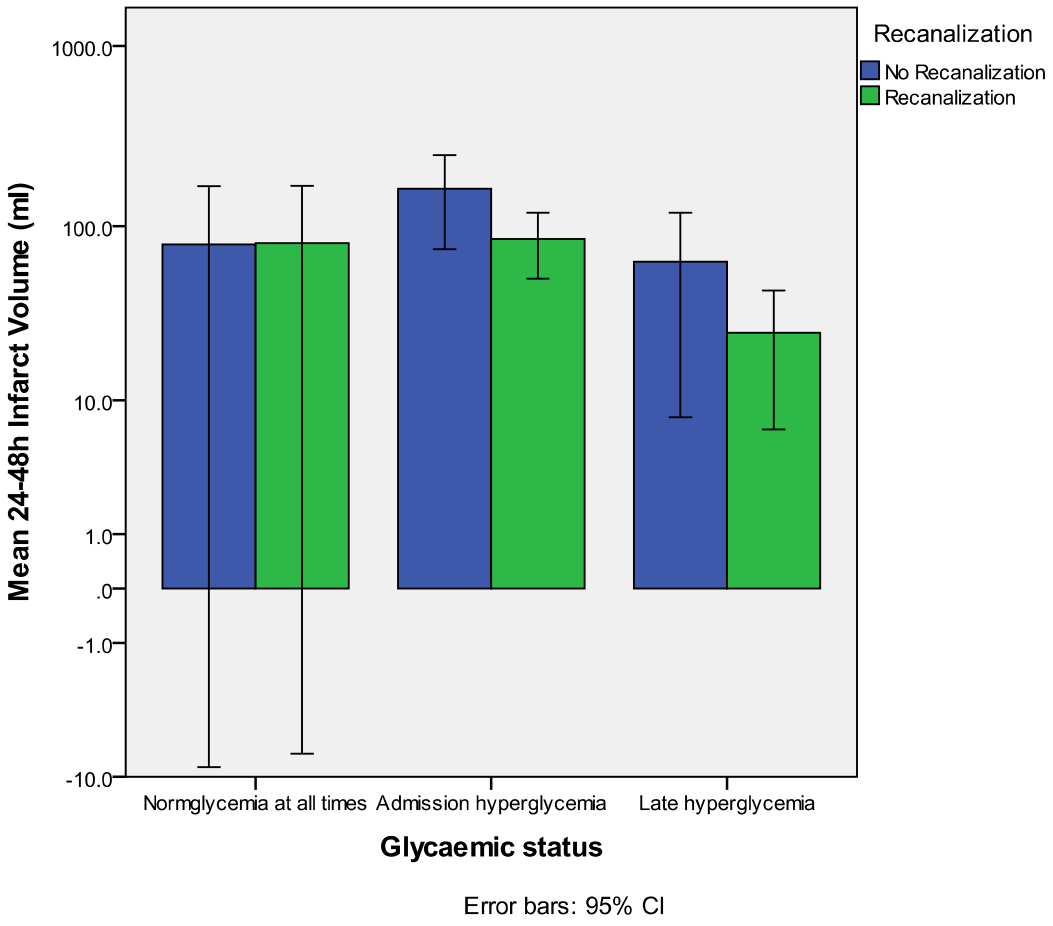

Figure 41 - 24-48h total infarct volume in patients with occlusions subdivided by glycaemic status and recanalization

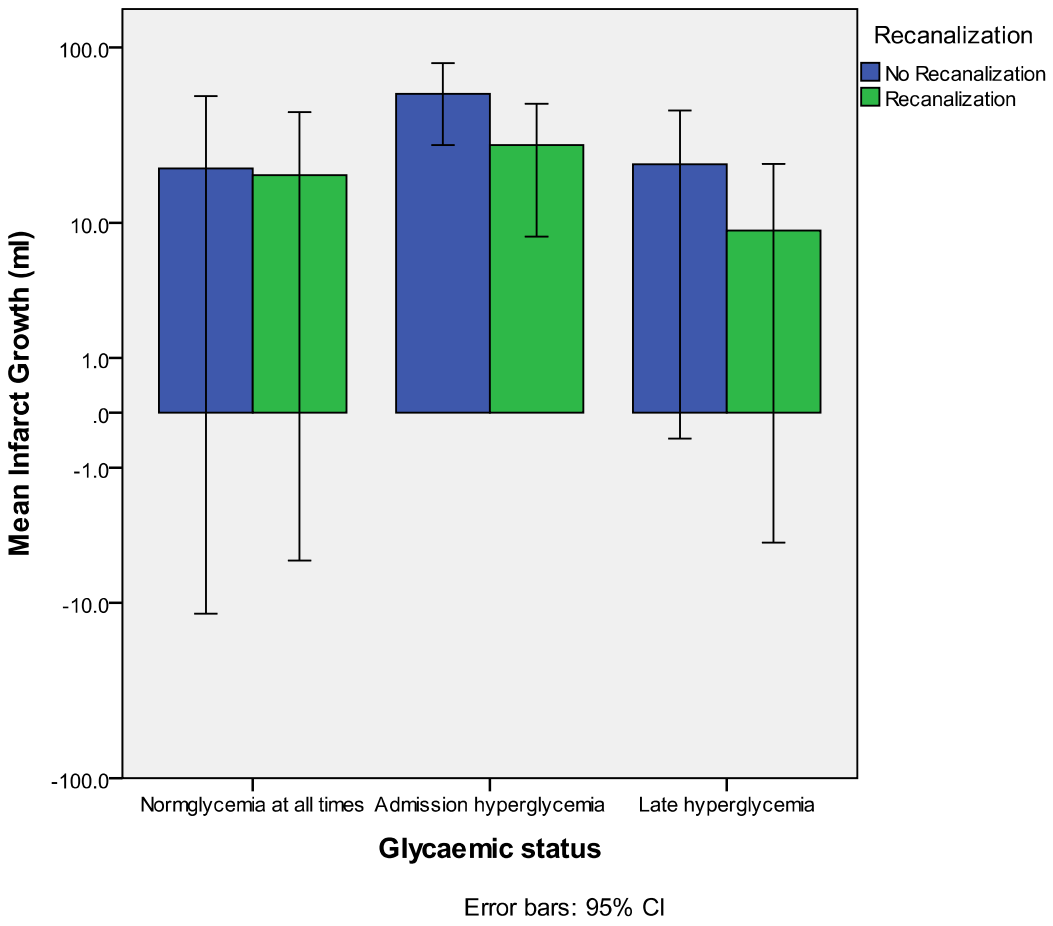

Figure 42 - Infarct growth in patients with arterial occlusions divided by glycaemic status and recanalization

There was no significant difference in recanalization rates between groups (p=0.488) and clinical outcomes were similar. Overall 55.2% of patients recanalized.

Using the Pearson method significant correlations were found between mean capillary blood glucose and infarct growth (p=0.001), co-registered infarct volume (p<0.001), total 24-48h infarct volume (p<0.001) and penumbral salvage (p=0.006).

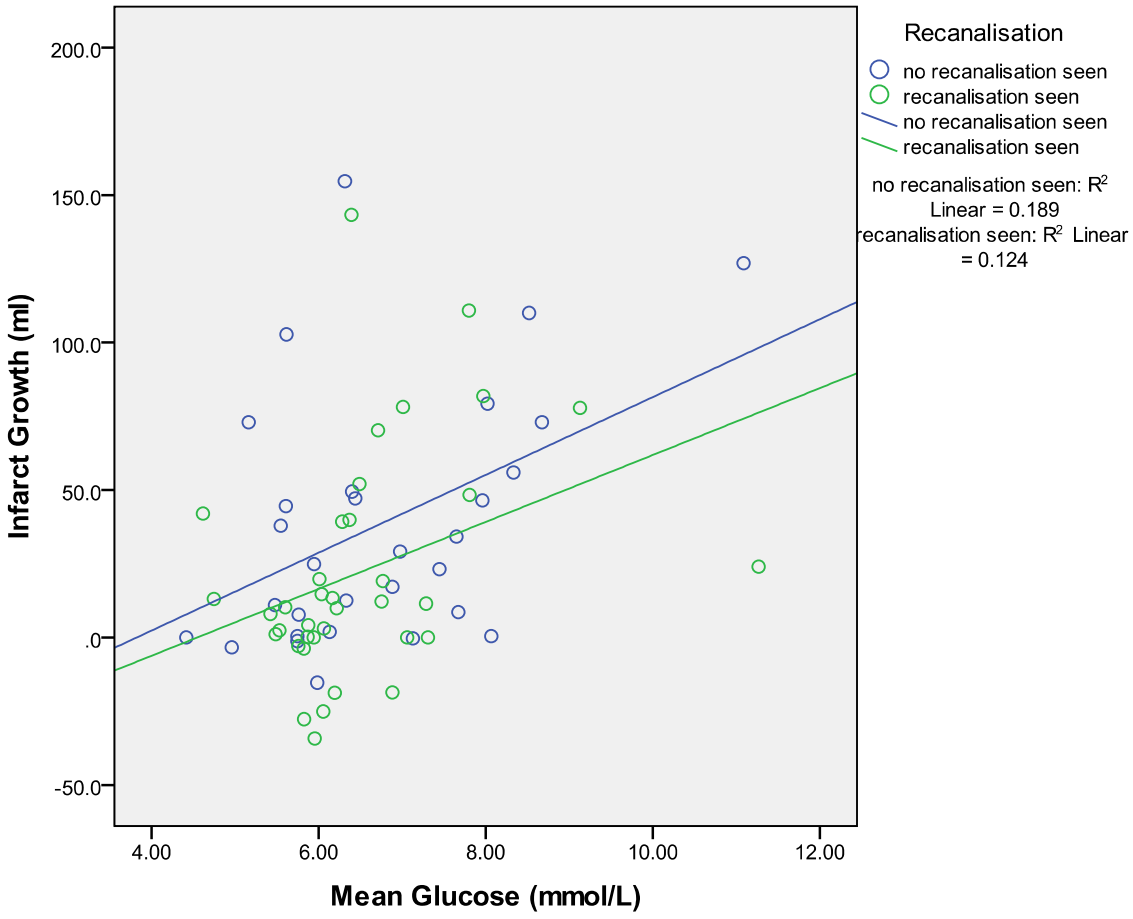

Figure 43 - Mean capillary blood glucose and infarct growth in patients with arterial occlusions

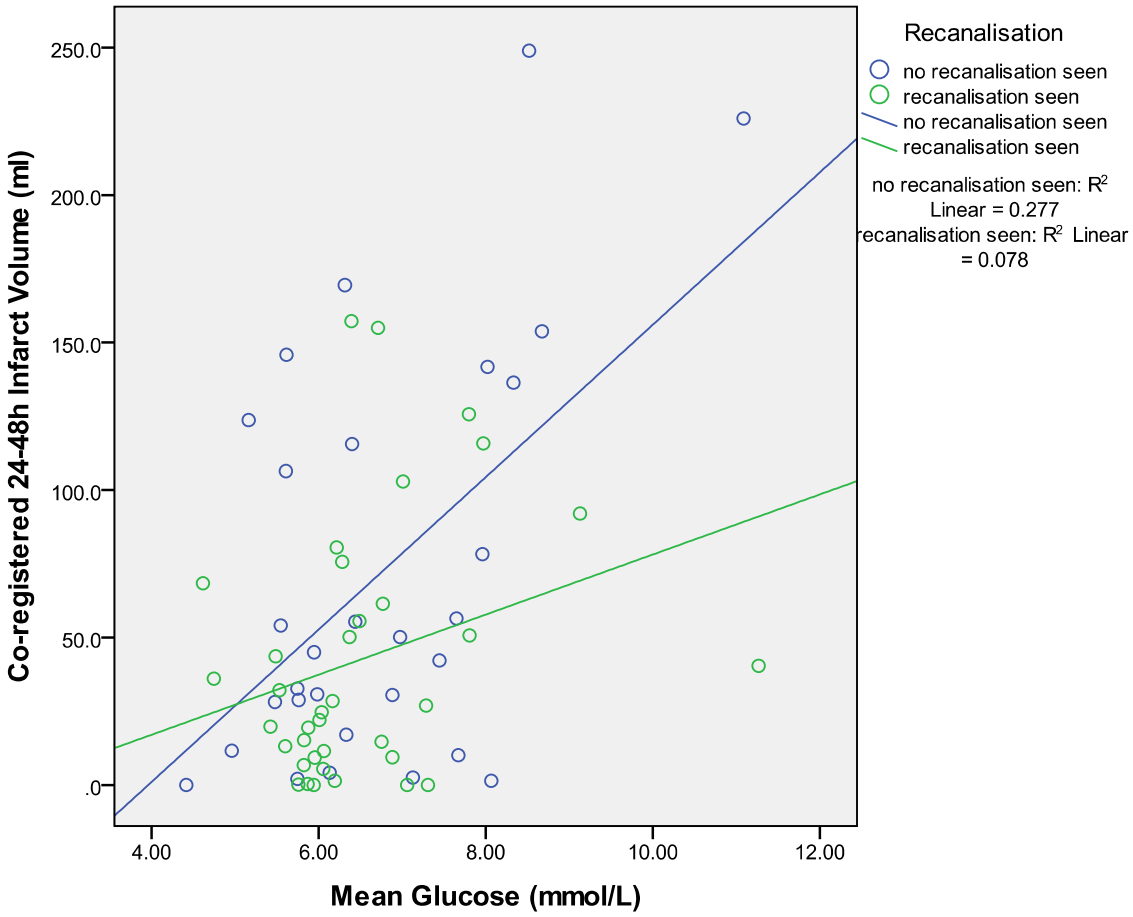

Figure 44 - Co-registered 24-48h infarct volume and mean glucose in patients with occlusions

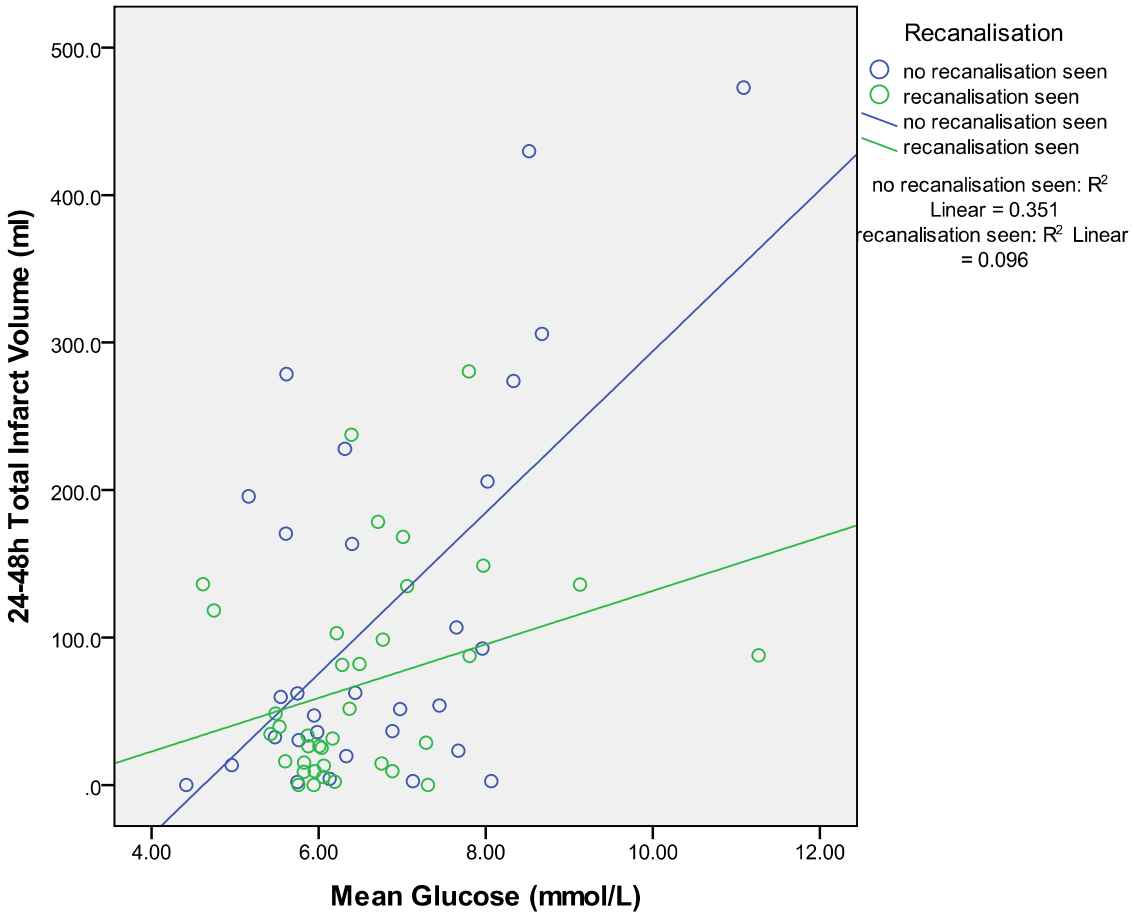

Figure 45 - Total 24-48h infarct volume and mean glucose in patients with occlusions

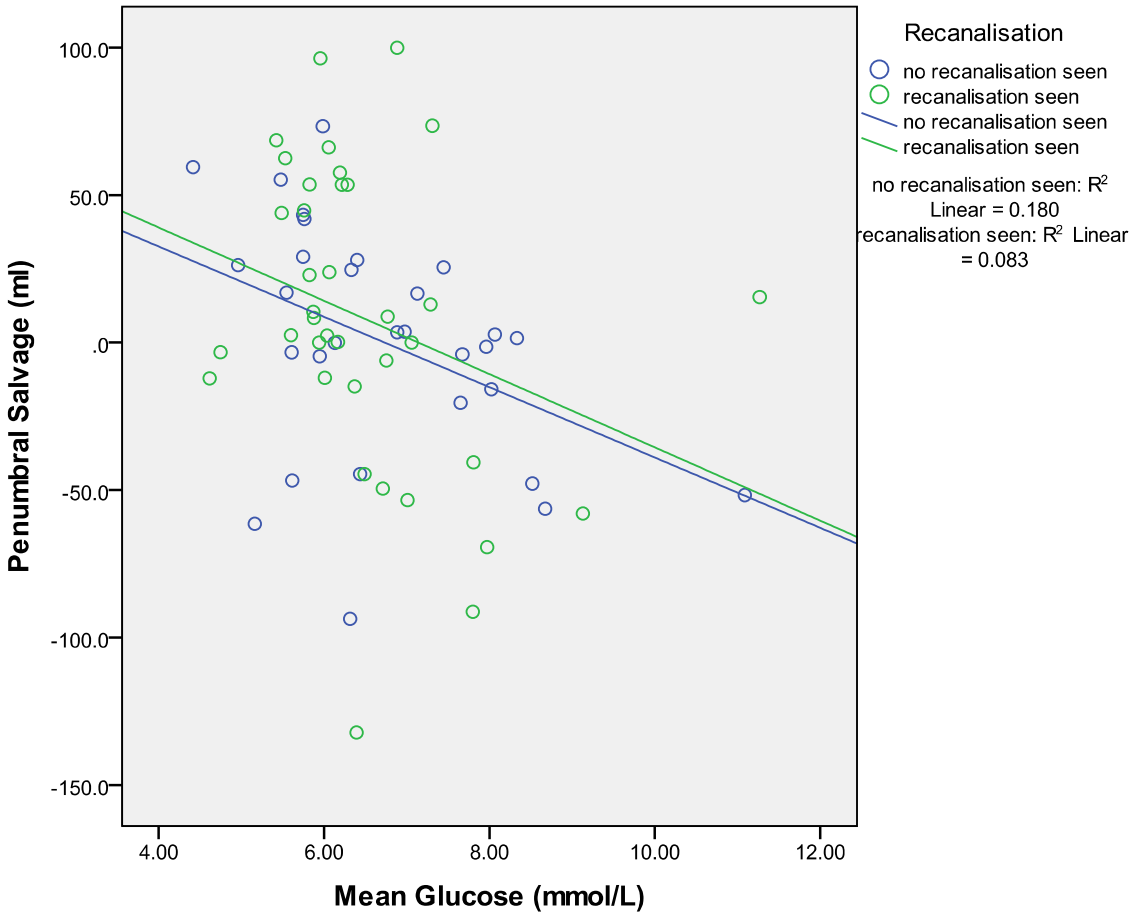

Figure 46 - Scatter plot of penumbral salvage and mean glucose in patients with occlusions

Linear regression for infarct growth suggested a model containing admission NIHSS score (p=0.003, OR 1.95, 95%CI 0.699 to 3.22), mean capillary blood glucose (p=0.003, OR 10.6, 95%CI 3.8 to 17.3) and admission systolic blood pressure (p=0.062, OR 0.36, 95%CI -0.02 to 0.73). This model had an R squared of 0.359.

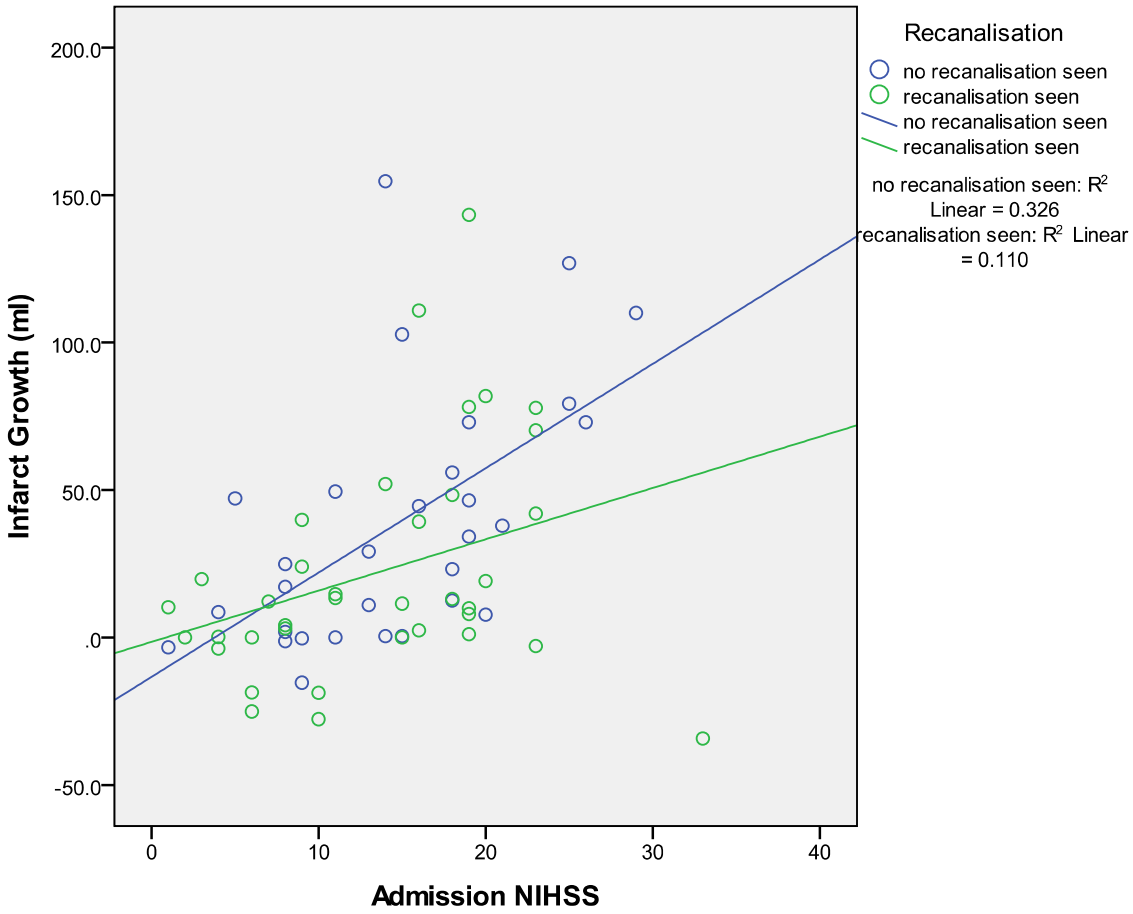

Figure 47 - Scatterplot of admission NIHSS against infarct growth in patients with occlusions

For penumbral salvage linear regression showed that mean capillary blood glucose was inversely associated with tissue salvage with an odds ratio of 11.6 (p=0.006, 95%CI 3.5 to 19.8). The model was moderately predictive with R squared=0.111. Other factors that I tried to fit into the model were not significant.

The linear regression model for 24-48h infarct volume had R squared=0.532 with predictive variables including mean capillary blood glucose (p<0.001, OR 31.1, 95%CI 16.6 to 45.7), admission NIHSS score (p<0.001, OR 6.5, 95%CI 3.8 to 9.2) and admission systolic blood pressure (p=0.021, OR 0.97, 95%CI 0.2 to 1.8).

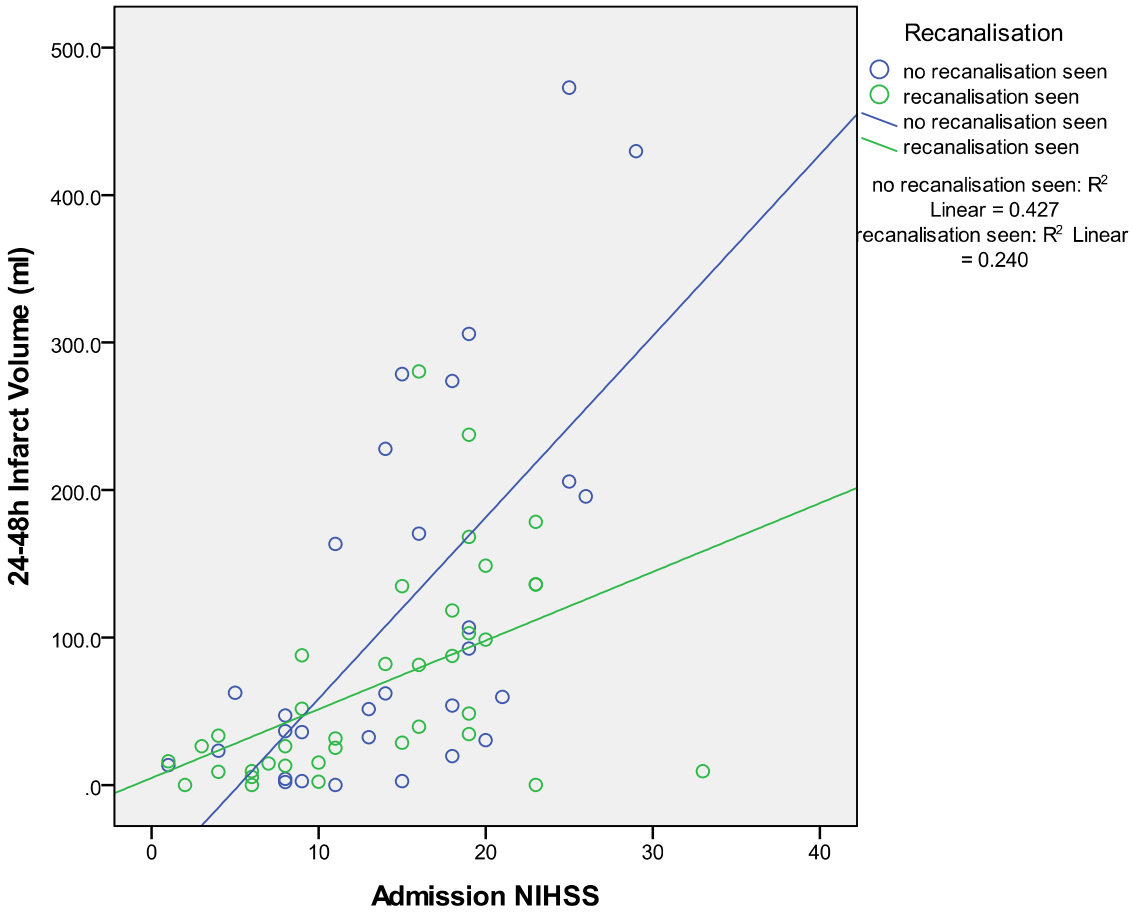

Figure 48 - 24-48h infarct volume against admission NIHSS in patients with occlusions

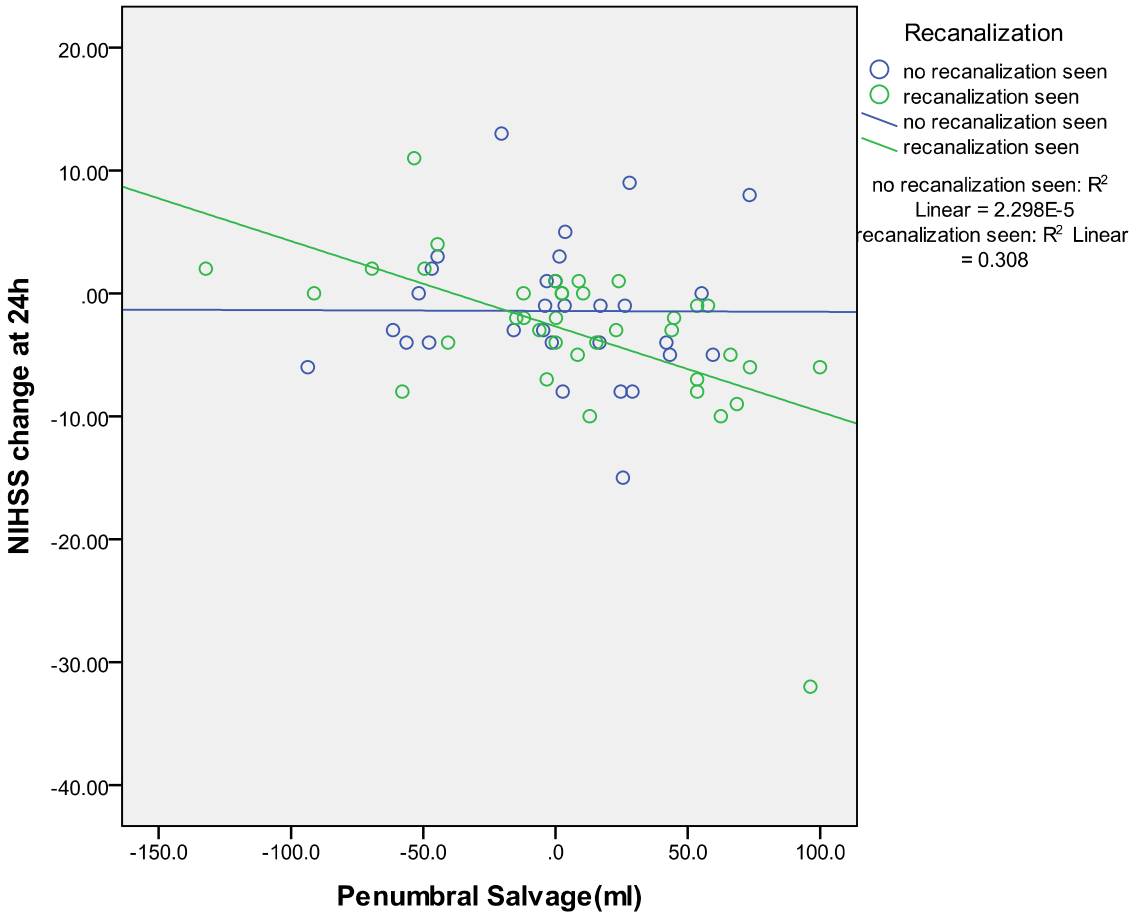

Figure 49 - Change in NIHSS at 24 hours in relation to recanalization status and penumbral salvage

A scatter plot of change in NIHSS at 24 hours (24 hour NIHSS – admission NIHSS) suggests that a fall in NIHSS at 24 hours is associated with increased penumbral salvage.

8.3.4 Recanalization in patients with occlusions

A binary logistic regression model for recanalization correctly predicted arterial patency in 67.7% of cases with a p value of 0.049. The model contained admission blood glucose (p=0.023, OR 1.4, 95%CI 1.05 to 1.89) and HbA1c (p=0.041, OR 0.43, 95%CI 0.19 to 0.97. Thrombolytic therapy was in the model but did not have a significant effect.

8.3.5 Patients without occlusions

Thirty seven patients had strokes without a radiologically documented occlusion. Eighteen of these patients were hyperglycaemic on admission, 12 became hyperglycaemic at a later point and 7 were consistently euglycaemic.

The euglycaemic patients had a significantly lower Hba1c than patients who were hyperglycaemic on admission (p=0.029). Patients who were hyperglycaemic were more likely to have an existing diagnosis of diabetes mellitus (p=0.008) or impaired glucose tolerance (p=0.019). The late hyperglycaemia group had a significantly higher admission blood pressure than the admission hyperglycemia group (p=0.039)

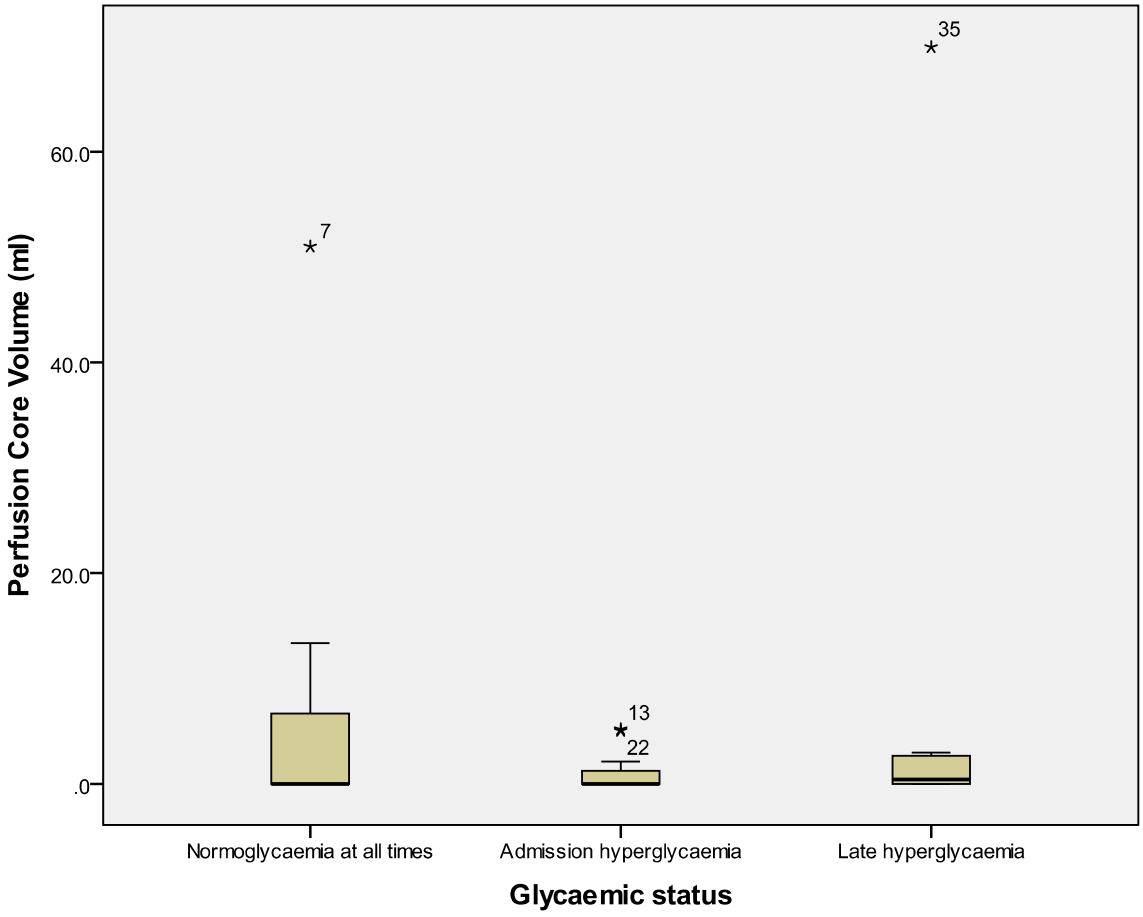

Figure 50 - Perfusion core volumes by glycaemic status in patients without occlusions

There was a non-significant trend towards patients with normoglycaemia having larger core volumes (mean 19.9ml) than those with admission hyperglycaemia (0.9ml) or late hyperglycaemia (6.7ml) in the absence of an occlusion (p=0.331). See Figure 50 above.

There were no significant differences in lesion volumes, lesion growth, stroke aetiology or clinical outcome between glycaemic groups in this sample.

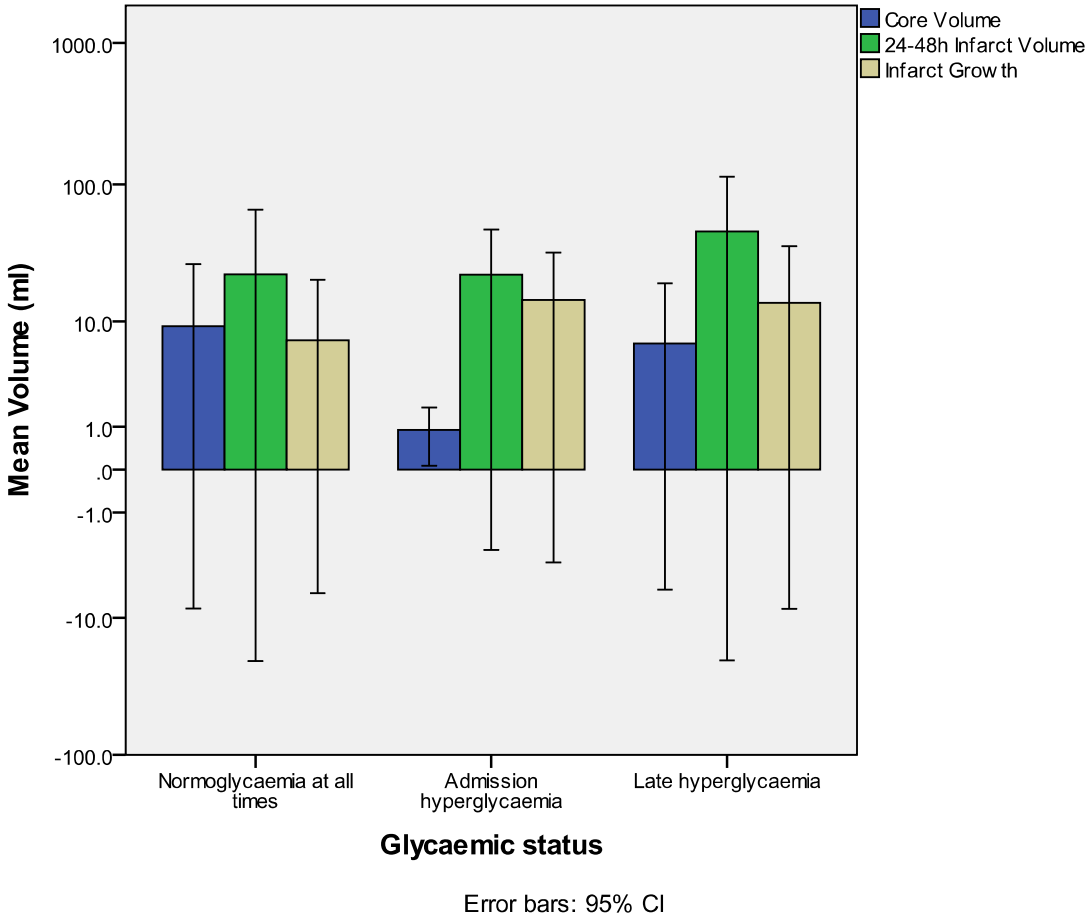

Figure 51 – Mean core volume, 24-48h infarct volume and infarct growth by glycaemic status in patients with no occlusions

While there was no significant difference in 24-48h infarct volumes the bar chart (Figure 51) comparing volumes between glycaemic groups suggests that core volumes were larger in the normoglycaemic group. A scatter plot of mean glucose against outcome infarct volume suggested a trend towards larger infarcts with lower blood glucose levels in patients who do not have occlusions.

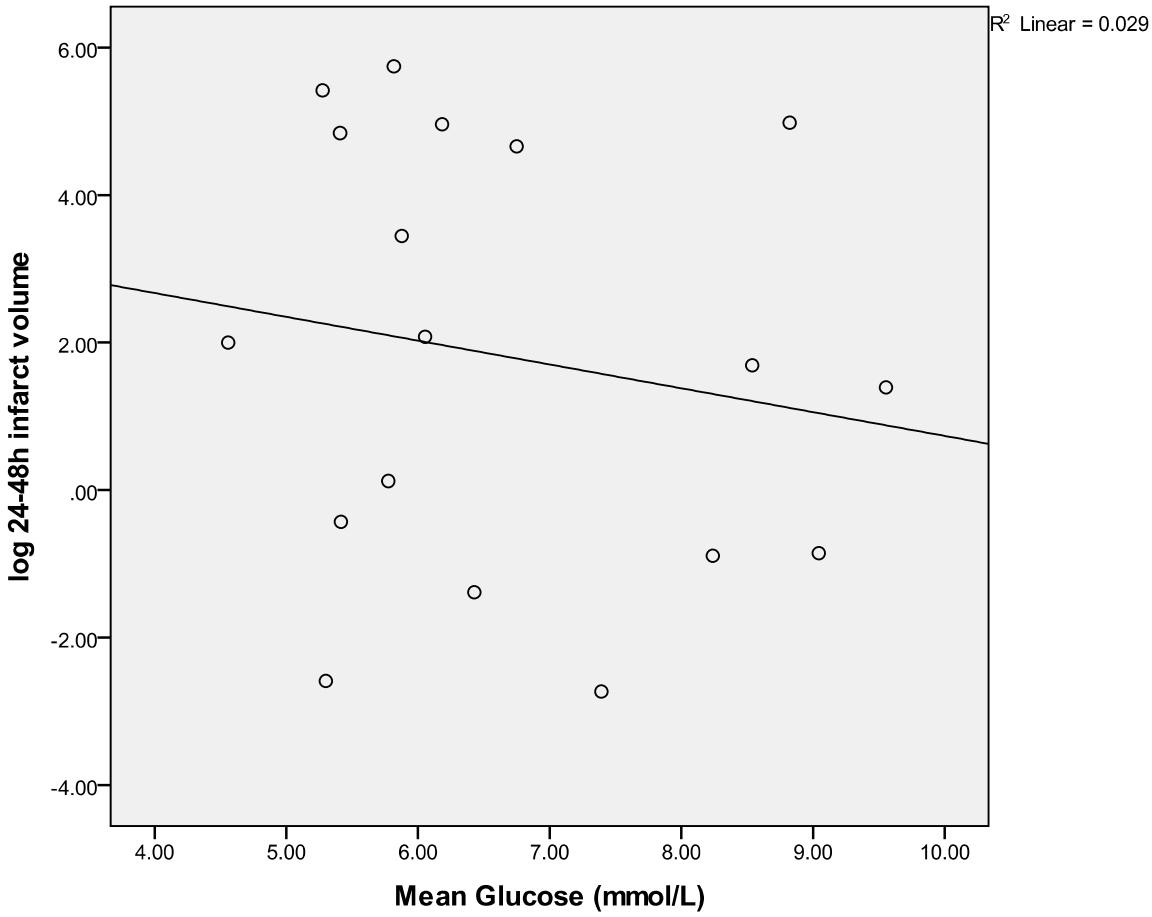

Figure 52 - Scatterplot of mean glucose against 24-48h infarct volume in patients without occlusions

One way ANOVA showed that 24-48h infarct volumes were significantly larger in patients with admission hyperglycaemia and an occlusion that did not recanalize than in patients with recanalization or patients who had no documented occlusion (p=0.001). Post hoc analysis showed significant differences in infarct volume between the no recanalization group and the no occlusion group (p<0.001) and between the no recanalization group and the recanalization group (0.035). The difference in infarct volume was not significant between the no occlusion group and the recanalization group (p=0.056). There were no significant differences in outcome infarct volumes based on occlusion status amongst the euglycaemia patients or the late hyperglycaemia patients.

Table 8-2 Demographics, clinical outcomes and imaging data for patients with no occlusion

Group (n)	All patients	Hyperglycaemia			
		None (7)	Admission (18)	Late (12)	
Male Gender (n/%)	23 (62.1)	5 (71.4)	9 (50)	9 (75)	
Age ± SD (mean/years)	66.8 ±	60.7 ± 11.8	68 ± 10.4	68.7 ± 14.4	p=0.339
Admission blood glucose ± SD (Mean mmol/L)	7.38 ± 3.32	5.58 ± 0.63 *	8.85 ± 3.93 * **	6.22 ± 2.2 **	p=0.025
Mean capillary blood glucose ± SD (Mean, mmol/L)	7 ± 2.1	5.64 ± 0.6*	8.14 ± 2.08* **	6.11 ± 0.96 **	p=0.004
HbA1c ± SD (Mean)	5.9 ± 1.1	5.27 ± 0.38*	6.37 ± 1.3 *	5.8 ± 0.96	p=0.073
Admission NIHSS (Median/IQR)	6 (4-8)	5 (3-9)	6 (4-8)	5.5 (4-8)	p=0.847
Admission Blood pressure ± SD (mmHg)	151/78 ± 25/14	147/69 ± 20/9	143/80 ± 25/17 **	163/81 ± 24/10 **	p=0.105
Diabetes (n/%)	10 (26.5)	0	9 (50)	1 (8.3)	p=0.008
Impaired glucose tolerance (n/%)	9 (24.3)	0	8 (44.4)	1 (8.3)	p=0.019
Thrombolytic treatment (n/%)	17 (46)	2 (28.6)	7 (38.9)	8 (66.7)	p=0.193
Supra-aortic large artery atherosclerosis (n/%)	10	1 (14.3)	5 (27.8)	4 (33.3)	p=0.525
Cardio-aortic embolism (n/%)	26 (70.3)	2 (28.6)	2 (11.1)	0 (0)	
Small artery occlusion (n/%)	9 (24.3)	1 (14.3)	4 (22.2)	3 (25)	
Other causes (n/%)	2 (5.4)	2 (11.8)	0	0	
Undetermined causes (n/%)	15 (40.5)	3 (42.9)	7 (38.9)	5 (41.7)	
CT Perfusion Core volume ± SD (Mean/ml)	4.35 ± 14	9.19 ± 19.1	0.9 ± 1.67	6.7 ± 19.9	p=0.331
CT Perfusion Penumbra volume ± SD (Mean/ml)	5 ± 13.1	12.9 ± 27.8	1.6 ± 2.72	5.4 ± 8.3	p=0.150
Total Perfusion Lesion Volume ± SD (Mean/ml)	9.3 ± 25.8	22.1 ± 46.8	2.5 ± 4.3	12.1 ± 27.5	p=0.213
Co-registered 24-48h infarct volume ±SD (Mean/ml)	17.27 ± 42	16.31± 33.7	15.44 ± 37.6	20.6 ± 54.2	p=0.948
24-48h Infarct Volume ± SD (Mean/ml)	30.2 ± 71.6	22.5 ± 47.3	22.49 ±50.6	46.2 ± 46.2	p=0.654
Infarct Growth ± SD (Mean/ml)	12.9 ± 32.3	7.11 ± 14.6	14.55 ± 36.3	13.9 ± 35.3	p=0.875
Penumbral salvage ± SD (Mean/ml)	-7.9 ± 30.5	5.8 ± 13.2	-12.9 ± 35.1	-8.5 ± 29.8	p=0.395
Rankin 0-1 Day 30 (n/%)	8 (21.6)	3 (42.9)	3 (16.7)	2 (16.7)	
Rankin 2-5 Day 30 (n/%)	29 (78.4)	4 (57.1)	15 (83.3)	10 (83.3)	
Rankin 6 Day 30 (n/%)	0	0	0	0	p=0.317

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG

8.3.6 Baseline characteristics compared with occlusion group

Patients without occlusion have lower initial NIHSS scores that patients with occlusions ($p<0.001$). Patients without occlusion were significantly less likely ($p=0.02$) to have atrial fibrillation (5/37) than patients who had occlusions (23/67). No significant differences were otherwise detected in age, mean capillary blood glucose level, admission blood glucose, HbA1c or blood pressure using ANOVA.

8.3.7 Comparison of day 30 modified Rankin score depending on occlusion status

Generally the patients with no evidence of angiographic occlusion had better day 30 clinical outcomes than those with occlusions. Chi-squared testing showed that patients without occlusion were more likely to have a day 30 Rankin score of 2 or less at 30 days ($p=0.004$) and less likely to be dead at 30 days ($p=0.006$).

The distribution of 30 day Rankin scores is illustrated in Figures 53 and 54 on the following two pages.

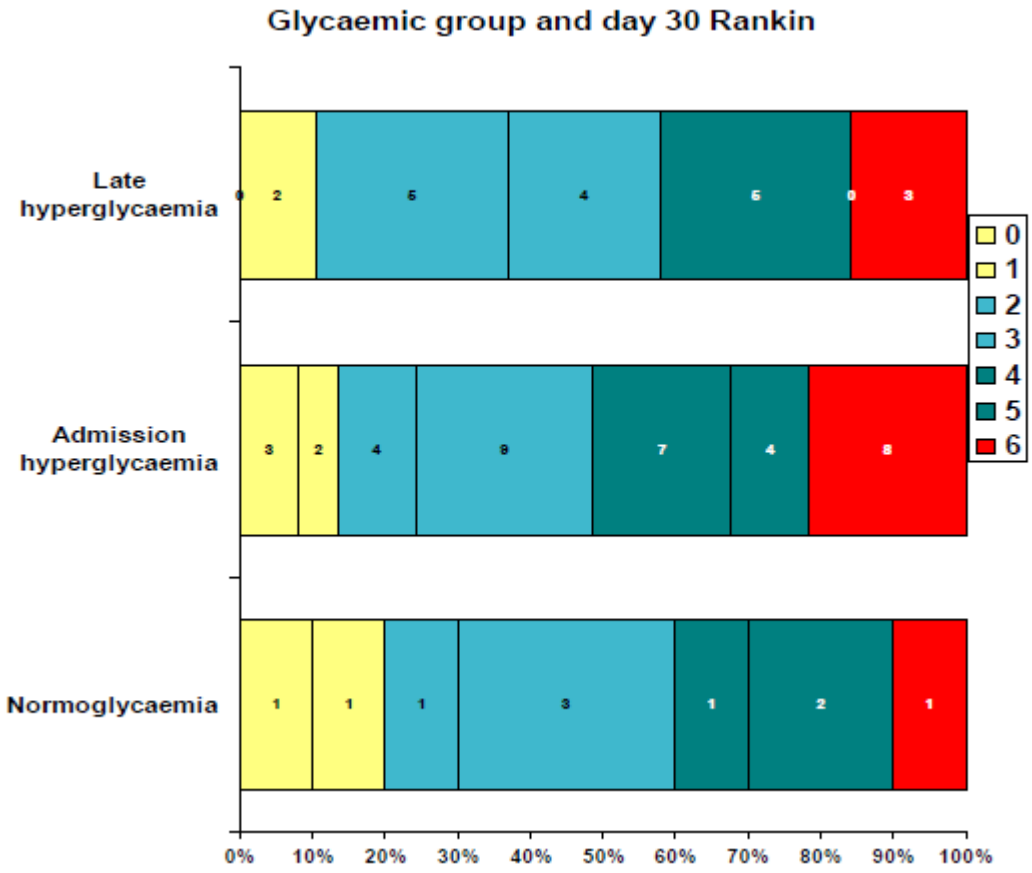

Figure 53 - 30 Day Rankin scores in patients with occlusions

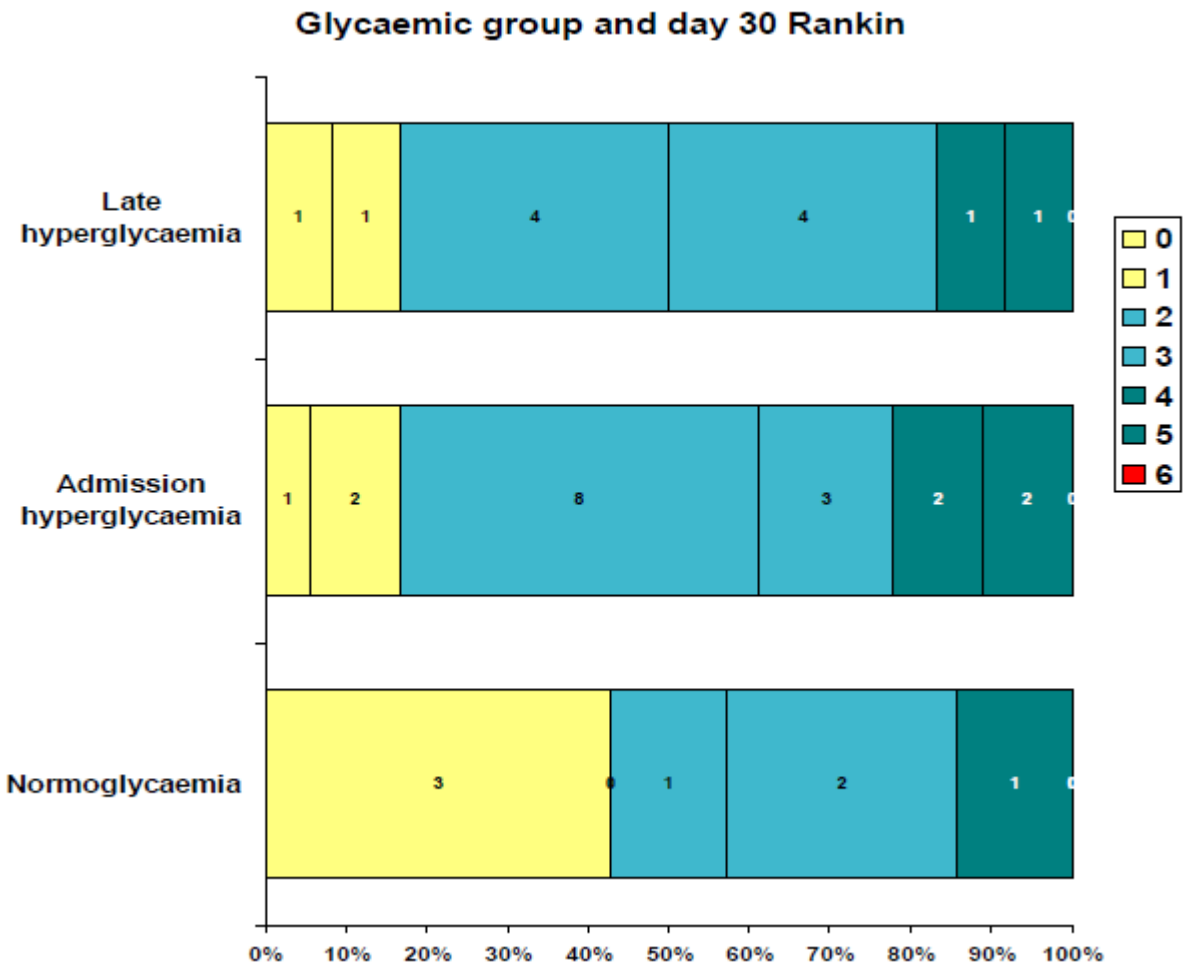

Figure 54 – 30 Day Rankin scores in patients with no occlusion

8.3.8 Patients with evidence of recanalization

Thirty-seven patients had radiological evidence of recanalization. Twenty-three of these patients were hyperglycaemic on admission, 10 became hyperglycaemic later and 4 were consistently euglycaemic. Overall there was no significant difference in admission blood glucose level between these groups although in post-hoc analysis a statistically significant difference in admission blood glucose level between patients who were hyperglycaemic on admission and those who became hyperglycaemic later (p=0.012).

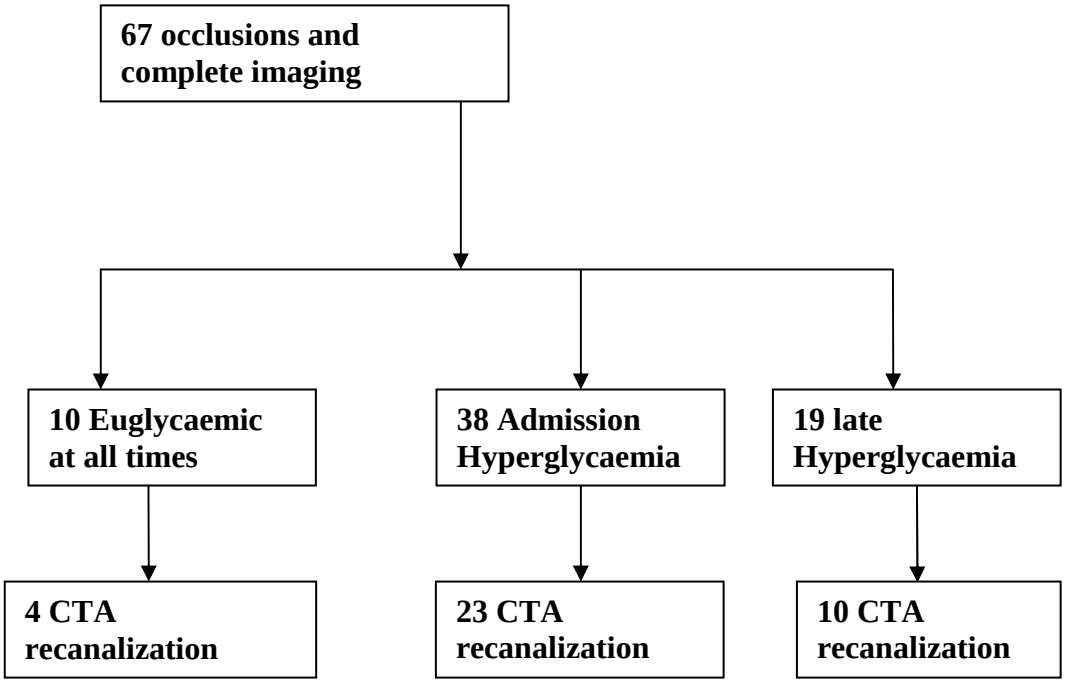

Figure 55 - Flow chart for patients who had evidence of recanalization

In the population as a whole there was no statistically significant difference in core perfusion volumes. Post hoc analysis did suggest that core perfusion lesions were significantly larger in patients who were hyperglycaemic on admission with a mean volume of 26.1ml compared to a mean volume of 10.7ml in patients who became hyperglycaemic later (p=0.035).

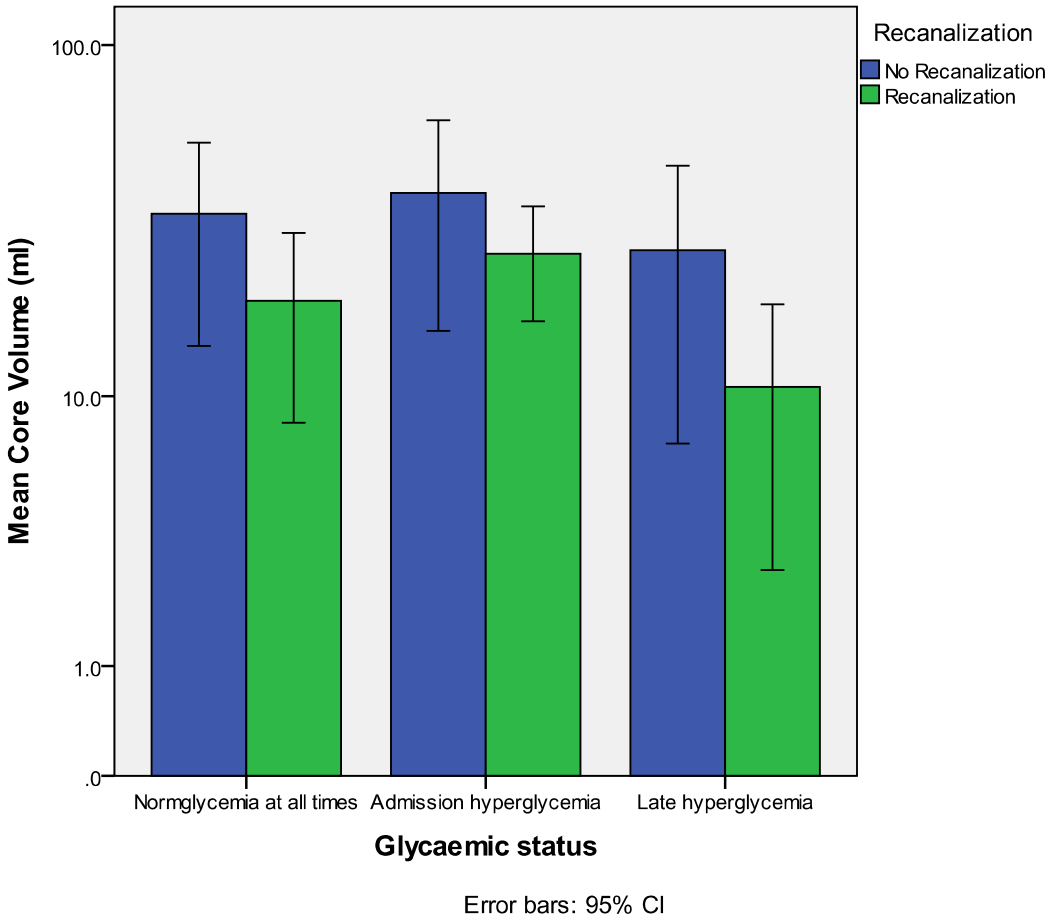

Figure 56 - Core perfusion volumes varies with glycaemic status and final recanalization status

While initial analysis showed no significant difference between groups for co-registered 24-48h infarct volume (p=0.127) and total infarct volume (p=0.072) post hoc analysis suggested that both co-registered 24-48h infarct volume and total 24-48h infarct volume were significantly larger in the admission hyperglycaemia group when compared with the late hyperglycaemia group (p=0.045, p=0.024).

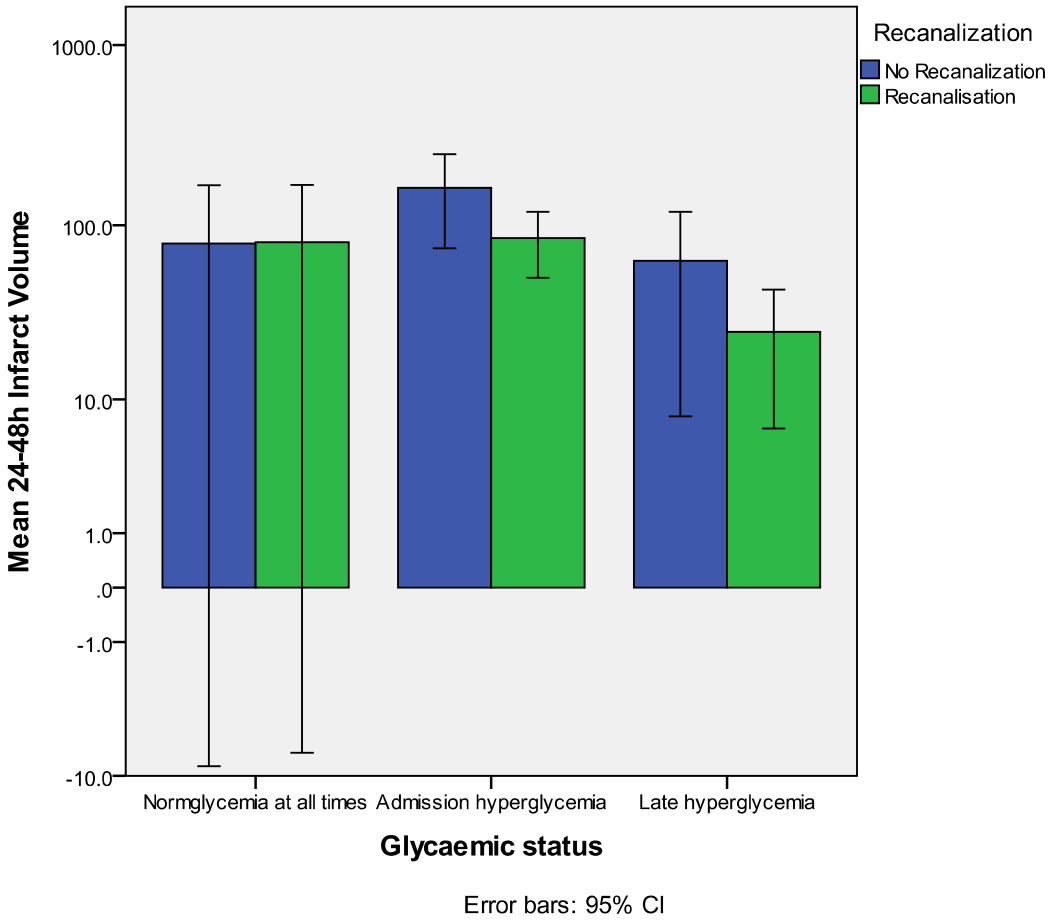

Figure 57 - 24-48h infarct volumes varies with glycaemic status and recanalization

While there was no overall significant difference in 24-48h total infarct volume between groups post hoc analysis suggested a significant difference did exist between patients with admission hyperglycaemia and patients with late hyperglycaemia (p=0.024).

There were no significant differences in clinical outcomes at 30 days between groups.

Table 8-3 Demographics, clinical outcomes and imaging data from recanalization cohort

Group (n)	All patients (37)	Hyperglycaemia None (4)	Hyperglycaemia Admission (23)	Hyperglycaemia Late (10)	
Male Gender (n/%)	24 (64.9)	2 (50)	13 (56.5)	9 (90)	
Age ± SD (mean/years)	66.84 ± 11.9	58.3 ± 18.4 ***	70.4 ± 11. 7	75.8 ± 5.3 ***	p=0.41
Admission blood glucose ± SD (Mean mmol/L)	7.38 ± 4.18	6.3 ± 0.45	9.25 ± 4.98 **	6.01 ± 0.65 **	p=0.084
Mean capillary blood glucose ± SD (Mean, mmol/L)	6.5 ± 1.2	5.23 ± 0.71	6.94 ± 1.29	5.99 ± 1.3	p=0.007
HbA1c ± SD (Mean)	5.9 ± 1.3	5.43 ± 0.8	6.1 ± 1.6	5.4± 1.6	p=0.365
Admission NIHSS (Median/IQR)	6 (4-8)	18.5 (12.75-22)	16 (7-19)	8.5 (3.5-12.25)	p=0.743
Admission Blood pressure ± SD (mmHg)	151/78 ± 21/13	156/91 ± 29/3	142/72 ± 21/12	149/79 ± 19/10	p=0.463
Diabetes (n/%)	10 (27)	0	4 (17.4)	0	p=0.236
Impaired glucose tolerance (n/%)	9 (24.3)	0	4 (17.4)	1 (10)	p=0.655
Thrombolytic treatment (n/%)	32 (86.4)	4 (100)	21 (91.3)	7 (70)	p=0.266
Supra-aortic large artery atherosclerosis (n/%)	11	1 (25)	8 (34.8)	2 (20)	p=0.594
Cardio-aortic embolism (n/%)	13 (35.1)	2 (50)	7 (30.4)	4 (40)	
Small artery occlusion (n/%)	0	0	0	0	
Other causes (n/%)	3 (8.1)	1 (25)	2 (8.7)	0	
Undetermined causes (n/%)	10 (27)	0	6 (26.1)	4 (40	
CT Perfusion Core volume ± SD (Mean, ml)	4.35 ± 19.2	19.1 ± 6.8	26.1 ± 21.7 **	10.7 ± 11.2 **	p=0.103
CT Perfusion Penumbra volume ± SD (Mean, ml)	5 ± 23.5	32.4 ± 31	29.4 ± 22.1	27.8 ± 26.2	p=0.949
Total Perfusion Lesion Volume ± SD (Mean, ml)	9.34 ± 36.4	51.5 ± 27.4	55.4 ± 38.1	38.5 ± 36.1	p=0.482
Co-registered Final infarct volume ± SD (Mean, ml)	17.27 ± 43.9	38.2 ± 21.2	53.2 ± 49.7 **	19.6 ± 25.1 **	p=0.127
Final Infarct Volume ± SD (Mean, ml)	30.2 ± 70.3	80.2 ± 54.9	84.7 ± 79 **	25 ± 25.7 **	p=0.072
Infarct Growth ± SD (Mean, ml)	12.9 ± 38.8	19.1 ± 15.5	28.4 ± 46.6	9 ± 18.4	p=0.424
Penumbral salvage ± SD (Mean, ml)	7.9 ± 51.7	13.3 ± 37.2	2.3 ± 62	18.8 ± 25	p=0.695
Rankin 0-1 Day 30 (n/%)	6 (16.2)	1 (25)	7 (30.4)	6 (60)	
Rankin 2-5 Day 30 (n/%)	27 (73)	3 (75)	14 (60.8)	2 (20)	
Rankin 6 Day 30 (n/%)	4 (10.8)	0	2 (8.7)	2 (20)	p=0.21

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG

8.3.9 Patients with occlusions who did not recanalize

Thirty patients had occlusions but did not recanalize. Fifteen of these patients were hyperglycaemic on admission, 9 became hyperglycaemic at a later point and 6 were never observed to be hyperglycaemic.

Penumbral salvage was significantly different between groups in this population (p=0.036). A significant difference in penumbral salvage was observed in post hoc analysis between the admission hyperglycemia and late hyperglycaemia groups (p=0.016) although there was no significant difference between final infarct sizes in these groups.

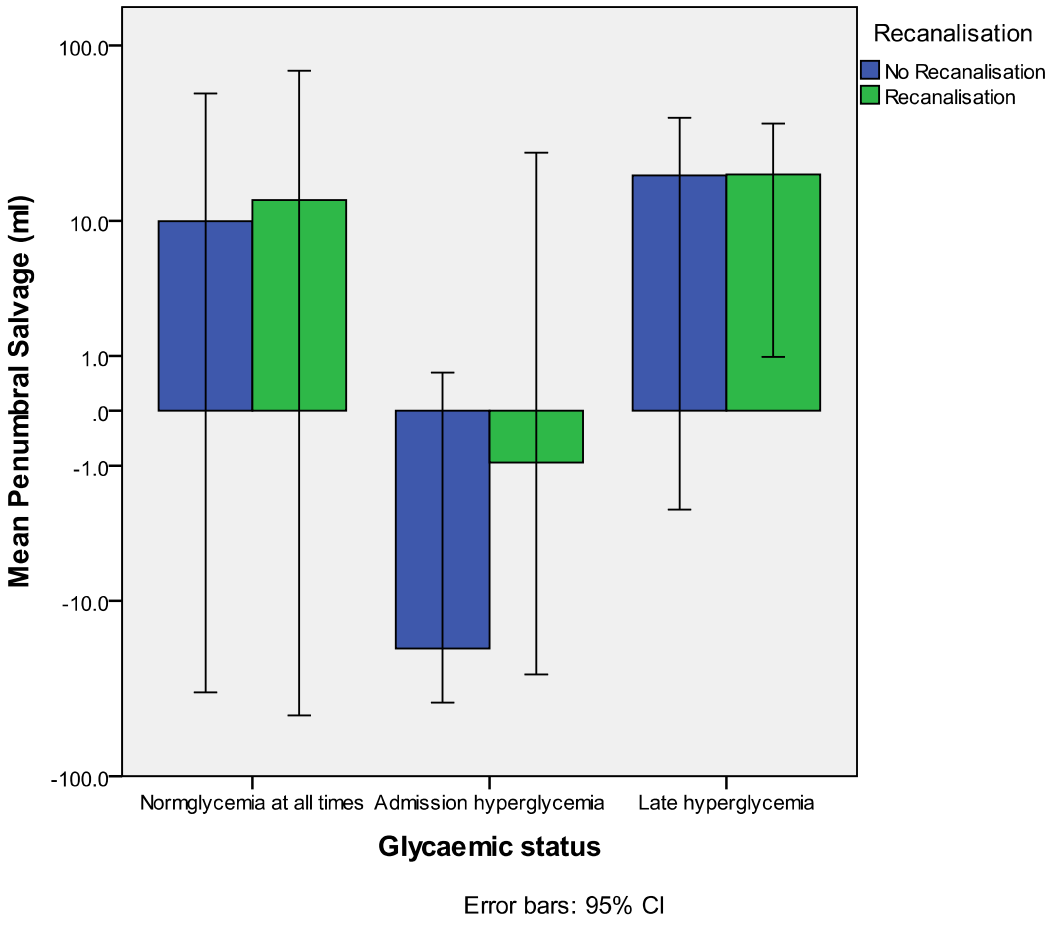

Figure 58 - Penumbral salvage varies with glycaemic status and recanalization

Table 8-4 Baseline demographics, clinical outcomes and imaging data from patients who did not recanalize

Group (n)	All patients (30)	Hyperglycaemia			
		None (6)	Admission (15)	Late (9)	
Male Gender (n/%)	23 (76.6)	3 (50)	10 (66.7)	3 (33.3)	
Age ± SD (mean/years)	66.8 ±10.1	66 ± 16	73.9 ± 8.2	71.67 ± 7.7	p=0.282
Admission blood glucose ± SD (Mean mmol/L)	7.38 ± 1.9	5.78 ± 0.54 *	8.05 ± 2.03 * **	5.85 ± 1 **	p=0.003
Mean capillary blood glucose ± SD (Mean, mmol/L)	6.7 ± 1.4	5.22 ± 0.49	7.5 1 ± 1.4	6.5 ± 0.93	p=0.001
HbA1c ± SD (Mean)	5.9 ± 0.8	5.56 ± 0.5	6.19 ± 1.1	5.84 ± 0.5	p=0.287
Admission NIHSS (Median/IQR)	6 (4-8)	13.5 (8.5-18.5)	15 (8-19)	15 (8.5-20.5)	p=0.575
Admission Blood pressure ± SD (mmHg)	151/78 ± 24/18	156/81 ± 21/10	163/82 ± 30/24	147/78 ± 16/11	p=0.889
Diabetes (n/%)	10 (33.3)	0	4 (33.3)	0	p=0.91
Impaired glucose tolerance (n/%)	9 (30)	0	5 (33.3)	0	p=0.91
Thrombolytic treatment (n/%)	24 (80)	4 (66.7)	14 (93.3)	6 (66.7)	p=0.275
Supra-aortic large artery atherosclerosis (n/%)	7	2 (33.3)	3 (20)	2 (22,2)	
Cardio-aortic embolism (n/%)	11 (36.6)	2 (33.3)	6 (40)	3 (33.3)	
Small artery occlusion (n/%)	0	0	0	0	
Other causes (n/%)	1 (3.33)	0	1 (6.7)	0	
Undetermined causes (n/%)	11 (36.6)	2 (33.3)	5 (33.3)	4 (44.4)	p=1
CT Perfusion Core volume ± SD (Mean, ml)	4.35 ± 33.2	33.8 ± 18.8	38.7±	26.6 ± 25	p=0.706
CT Perfusion Penumbra volume ± SD (Mean, ml)	5 ± 23	36.5 ± 18.8	36 ± 41.7	40.6 ± 26.9	p=0.894
Total Perfusion Lesion Volume ± SD (Mean, ml)	9.34 ± 49.6	70.3 ± 22.4	74.7 ± 23.3	67.2 ± 50	p=0.940
Co-registered Final infarct volume ± SD (Mean, ml)	17.27 ± 69	50.43 ± 51.7	93.86 ± 80.5	48.7 ± 48.9	p=0.216
Final Infarct Volume ± SD (Mean, ml)	30.2 ± 129.7	79 ± 83.6	161.38 ± 157.5	63.2 ± 72	p=0.149
Infarct Growth ± SD (Mean, ml)	12.9 ± 42.7	20.9 ± 31	55.2 ± 48.4	63.2 ± 29.2	p=0.096
Penumbral salvage ± SD (Mean, ml)	-0.03 ± 39.5	19.9 ± 46*	-19.1 ± 35.7 * **	18.5 ± 27.4 **	p=0.024
Rankin 0-1 Day 30 (n/%)	3 (10)	2 (33.3)	1 (6.7)	0	
Rankin 2-5 Day 30 (n/%)	19 (63.3)	3 (50)	8 (53.3)	8 (88.9)	
Rankin 6 Day 30 (n/%)	8 (26.6)	1 (16.7)	6 (40)	1 (11.1)	p=0.363

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG

8.4 Discussion

This chapter focuses on the interaction between arterial patency, glycaemic status and infarct growth. The most interesting finding when focusing on patients with angiographic evidence of arterial occlusion is the statistically significant difference in final infarct volume seen between groups (p=0.041).

As seen in the previous chapter there is a trend for the biggest different in final infarct volumes to be between the patients who are hyperglycaemic within 6 hours of stroke onset and those who become hyperglycaemic at a later time point (p=0.041 in post hoc analysis). Indeed on post hoc analysis statistically significant differences were also seen between these two groups for co-registered final infarct volume (p=0.027), total final infarct volume (p=0.013) and infarct growth (p=0.039).

It is also interesting to note that there is no statistically significant difference in infarct volumes or growth between the euglycaemic group and the other groups. This may be due to the low number of patients in the euglycaemic group as only 10 patients with documented occlusions were recruited. It is possible that data from the euglycaemic group has been skewed by a particularly severe stroke but when I review the raw data I can see that four patients in this group had final infarct volumes of 110ml or greater with initial NIHSS scores of 16 or more suggesting that they were not outliers. These patients all had complete glucose profiles with 12 or more capillary blood glucose measurements within 48 hours. They appear to have been truly euglycaemic with severe strokes.

While early hyperglycaemia does appear to have a negative impact on infarct growth these data presented in this chapter do not support the notion that euglycaemia automatically makes things better. Indeed, if anything a late rise in plasma blood glucose may be a good sign. Certainly Ntaios and colleagues found that hypoglycaemia (a blood glucose below 3.7mmol/L) could be associated with worse outcomes in acute ischaemic stroke.[173] While there were no patients who were found to be consistently hypoglycaemic several had documented glucose levels below 4mmol/L, at least 4 of the 10 patients were documented as being ‘nil by mouth’ at some point while 6/10 are documented as having an impaired swallow. All 10 of the euglycaemic patients were prescribed intravenous normal saline to maintain hydration after their stroke. The five patients with the worst outcomes all had dysarthria documented in their admission NIHSS scores.

Mean capillary blood glucose strongly correlates with final infarct volume and related variables in this population of patients with a documented arterial occlusion.

In the group of patients with arterial occlusions linear regression was able to create a moderately predictive model for infarct growth containing admission NIHSS score, mean capillary blood glucose and admission systolic blood pressure. Several factors that I would have expected to be in this model including thrombolytic treatment and final recanalization did not fit in this model. This could be explained by missing values for some variables, small total sample size (N=67) and the fact that a high proportion of patients (83.6%) received thrombolytic treatment. There may also be a confounding factor that I have been unable to identify. Unusually admission blood glucose was associated with recanalization which also suggests the presence of an unidentified confounding factor. Again, it would be interesting to repeat this analysis in another larger population to see if the model would remain valid and if factors such as thrombolytic treatment or recanalization status would have a more significant effect.

The model for final infarct volume had a larger R squared. This model contained the same factors as before, again omitting thrombolytic treatment and recanalization. A further binary logistic regression model (p=0.049) for angiographic recanalization in this population was made to include thrombolytic therapy although thrombolysis was not a significant variable in the model (p=0.27). The model which also contained admission blood glucose level (p=0.023) and HbA1c (p=0.041) became more significant (p=0.037) when thrombolysis was removed. It is possible that glycaemic status interferes with the fibrinolytic activity of rt-PA and this has been suggested by pre-clinical studies.[349] Ribo and colleagues suggested in their 2005 paper that acute hyperglycaemia may interfere with the fibrinolytic process.[200] The presence of HbA1c as a significant factor in this regression model may hint that chronic dysglycaemia is important too. Certainly clinical studies have suggested that plasminogen activator may be inhibited in Type 2 diabetes mellitus.[365]

Initial analysis of patients who recanalized did not show significant differences for anything other than admission glucose (which had been used to select groups anyway) and mean capillary blood glucose. Post hoc analysis suggested that core perfusion lesions, co-registered final infarct volume and total final infarct volume were all significantly larger in the admission hyperglycaemia group compared to the late hyperglycaemia group. In this subgroup analysis the numbers in the glycaemic subgroups are very small (10 late hyperglycaemia patients and 4 normoglycaemia patients) so it is not surprising that we

have not seen any strongly significant results. The trend suggested by the post hoc analysis would become clearer if this study were to be repeated in a larger population

Basic outcome comparisons between the patients without occlusions and the patients with occlusions suggest that you are more likely to die with an occlusion and less likely to have a good recovery. The only significant baseline differences between the groups are the lower incidence of atrial fibrillation and lower baseline NIHSS score in the no occlusion group.

One interesting trend that was observed in the patients without occlusions was the tendency for normoglycaemia to be associated with larger core perfusion lesions and larger follow-up infarct volumes than patients who were hyperglycaemic on admission or at a later point. This could be in keeping with the observations of Uyttenboogaart and colleagues found a dichotomous relationship between hyperglycemia and clinical outcome when lacunar strokes were compared to non-lacunar strokes.[260] Data from 168 lacunar stroke patients and 1207 non-lacunar patients were available. In their analysis hyperglycemia (defined as a blood glucose above 8mmol/L) was associated with a lower chance of a good outcome (mRs<2) at 3 months in non-lacunar stroke (OR for good outcome 0.60; 95% CI 0.41–0.88, p=0.009). However in lacunar patients hyperglycemia was associated with a good outcome (OR 2.70, 95% CI 1.01- 7.13, p=0.048).

Finally, when looking at the patients who did not recanalize we see a statistically significant difference in penumbral salvage between groups (p=0.024) with post hoc analysis suggesting that this difference is most significant between the admission hyperglycaemia group and the late hyperglycaemia group (p=0.016).

These results, as a whole, suggest that in the presence of arterial occlusion admission hyperglycaemia reduces penumbral survival and increases final infarct volume. The interesting new finding is the suggestion that late hyperglycaemia after 6 hours may be associated with better imaging outcomes and improved penumbral survival.

Arterial patency appears to be a key determinant of the interaction between the ischaemic penumbra and blood glucose. The presence of an occlusion makes admission hyperglycaemia harmful although later hyperglycaemia may be beneficial. The absence of an occlusion may be associated with a beneficial effect from admission hyperglycaemia.

Taken in the context of the results of the SELESTIAL trial,[554] the INSULINFARCT trial,[562] my reassessment of the animal evidence in chapter 3[206] and the systematic review of the clinical trials of insulin for post stroke hyperglycemia presented in chapter 4 there is at present little justification for further human trials of insulin treatment for post-stroke hyperglycaemia after the initial 6 hour window. Any trials of insulin for PSH within 6 hours of stroke onset should also be considered very carefully. Larger scale studies examining the pathophysiology of post stroke hyperglycaemia and its interaction with brain arterial may be of more value in the first instance

9 Interaction between post stroke hyperglycaemia and brain arterial patency in more homogenous subpopulations - M1 occlusions only

9.1 Introduction

Heterogeneity of patients with stroke may obscure biologically relevant interactions due to dilution of the sample. Effects may be evident if a more homogeneous patient population can be identified. In previous studies an effect of recanalization on final infarct volume was only seen in a subgroup analysis looking at M1 occlusions.[579] It has certainly been suggested that neuroprotective stroke trials may have failed due to heterogeneous populations of patients (among other issues).[580] Pathophysiological mechanisms may be obscured in a highly heterogeneous population and in this exploratory chapter I have tried to focus on a more uniform patient group.

The EPITHET study showed that the site of arterial occlusion strongly predicts outcome in treatment with intravenous thrombolysis.[581] In this study ICA occlusions were seen to be associated with poor outcomes while MCA obstructions were associated with better outcomes. A recent pilot study comparing tenecteplase and alteplase for the treatment of acute ischaemic stroke had pre-determined stringent imaging inclusion criteria based on site of occlusion.[582] An earlier pilot study found that differences in occlusion sites between treatment groups prevented accurate comparison of the two drugs.[583]

In order to explore the potential interaction of early and delayed hyperglycaemia with arterial patency and brain perfusion further, this analysis was restricted to those patients with acute occlusion of the proximal segment of the middle cerebral artery (an M1 occlusion). This group represents a more homogeneous population with respect to the expected volumes of ischaemia, clinical severity, response to IV rtPA treatment, and outcomes.

9.2 Methods

9.2.1 Image analysis

Analysis of CTP and plain CT was performed as detailed in the previous chapter. Admission and follow-up scans were again read by two observers (NM and XH) for presence of an arterial occlusion on the admission scan and for recanalization on follow-up imaging. If there was any disparity between observers a final decision was made by an experienced stroke neurologist who could also make reference to neuroradiology reports (KM).

9.2.2 Case selection

In this chapter all analysis was carried out on patients with CTA confirmed M1 occlusion. Initially it looks at all patients with M1 occlusions before comparing patients with pure M1 occlusions against patients with tandem occlusions (M1 plus another arterial occlusion). Finally it focuses on the pure M1 occlusions.

9.2.3 Analysis

Infarct growth was defined as the difference between core perfusion volume lesion and final co-registered infarct volume. Patients were divided into groups based on glycaemic status as described in the previous chapters.

9.2.4 Statistical analysis

Differences between groups were analysed using ANOVA for scale variables and Pearson's chi squared for categorical variables. If a significant difference between groups was discovered using ANOVA it was further analysed using the least significant difference method.

Linear regression analysis and binary logistic regression analysis were carried out as in previous chapters to seek predictors of infarct growth, final infarct volumes and recanalization.

9.3 Results

9.3.1 Patient characteristics

Thirty three patients had CT angiographic evidence of an M1 occlusion on baseline CTA.

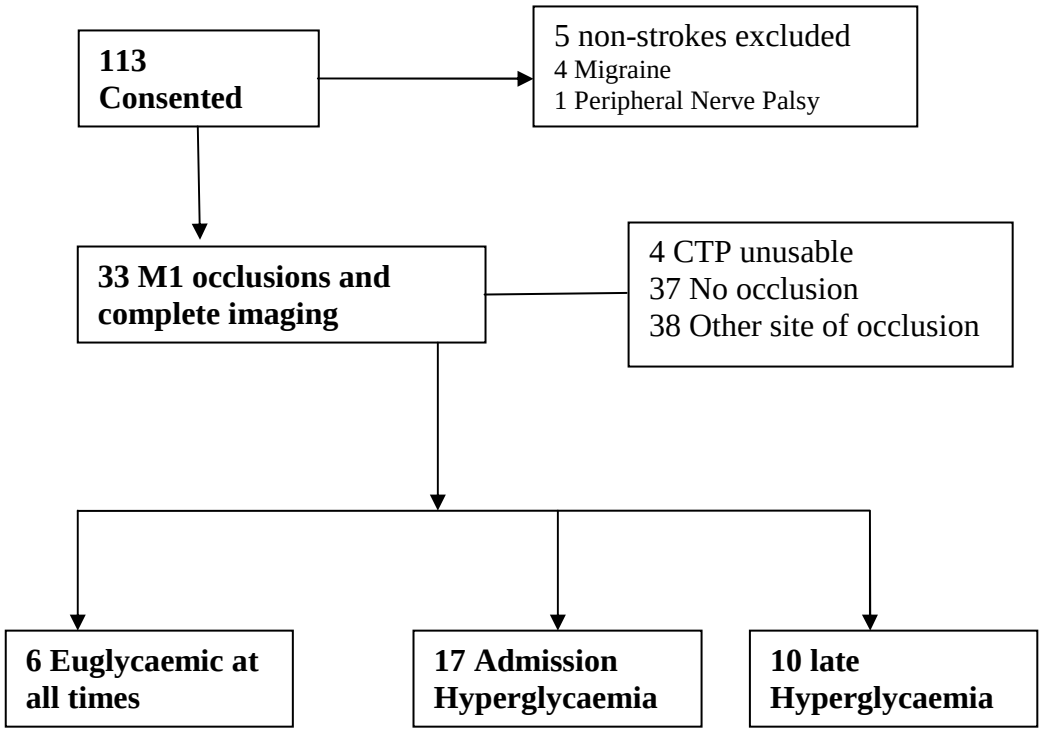

Figure 59 – Recruitment flowchart for patients with an M1 occlusion

Seventeen of these patients were hyperglycaemic on admission, 10 became hyperglycaemic at a later point in the admission and 6 were euglycaemic at all times. Tandem occlusions were present in 16 patients.

In post hoc analysis there was a significant difference in penumbral volume between the patients who were euglycaemic at all times and the group who became hyperglycemic at a later point. The mean penumbral volume was significantly larger in the late hyperglycemia group compared to the euglycaemic group.

There was no significant difference in recanalization rates between groups (p=0.447) and clinical outcomes were not significantly different although a larger proportion of patients died in the hyperglycaemic groups compared to the consistently euglycaemic group. Overall 55% of patients recanalized while 85% received thrombolytic treatment.

There was no significant difference in clinical outcome as graded with the modified Rankin scale at 30 day follow-up.

The data from these patients are summarized in Table 9.1.

Table 9-1 Demographics, clinical outcomes and imaging results with M1 occlusion

Group (n)	All patients (33)	Hyperglycaemia None (6)	Hyperglycaemia Admission (17)	Hyperglycaemia Late (10)	
Male Gender (n/%)	15 (45.4.)	2 (33.3)	7 (41.2)	6 (60)	
Age ± SD (mean/years)	71.5 ±	61.8 ± 15.6 *	75.1 ± 9 *	71.1 ±	p=0.053
Admission blood glucose ± SD (Mean mmol/L)	7.28 ± 11.8	6.18 ± 0.5 *	8.48 ± 1.93 *	5.91 ± 0.91 **	p<0.001
Mean capillary blood glucose ± SD (Mean, mmol/L)	6.57 ± 1.31	5.3 ± 0.58*	7.23 ± 1.38* **	6.21 ± 0.7**	p=0.002
HbA1c ± SD (Mean)	5.7 ± 0.8	5.6 ± 0.7	5.9 ± 0.9	5.44 ± 0.3	P=0.387
Admission NIHSS (Median/IQR)	15 (8-19)	17 (12.5-23.75)	19 (13.5-21.5)	13.5 (9.75-)	p=0.587
Admission Blood pressure ± SD (mmHg)	150/78 ± 26/17	159/87 ± 21/9	146/75 ± 29/21	149/77 ± 22/8	p=0.585
Diabetes (n/%)	3 (9.1)	0	3 (17.6)	0	p=0.212
Impaired glucose tolerance (n/%)	3 (9.1)	0	3 (17.6)	0	p=0.212
Thrombolytic treatment (n/%)	28 (84.8)	5 (83.3)	15 (88.2)	8(80)	p=0.841
Supra-aortic large artery atherosclerosis (n/%)	10 (30.3)	2 (33.3)	4 (23.5)	4 (40)	
Cardio-aortic embolism (n/%)	15 (45.4)	3 (50)	9 (52.9)	3 (30)	p=0.803
Small artery occlusion (n/%)	0	0	0	0	
Other causes (n/%)	0	0	0	0	
Undetermined causes (n/%)	8 (24.2)	1 (16.7)	4 (23.5)	3 (30)	
Recanalization (n/%)	18 (54.5)	3 (50)	11 (64.7)	4 (40)	p=0.447
Tandem occlusions (n/%)	16 (48.5)	4 (66)	6 (35.3)	6 (60)	p=0.447
Internal ICA occlusion (n/%)	12 (36.4)	3 (50)	5 (29.4)	4 (40)	p=0.639
External ICA occlusion (n/%)	9 (23.3)	2 (33)	4 (23.5)	3 (30)	p=0.874
CT Perfusion Core volume ± SD (Mean, ml)	41.1± 30.2	32.4 ± 19.3	47.8 ± 35.6	35.2 ± 24.6	p=0.437
CT Perfusion Penumbra volume ± SD (Mean, ml)	42.1 ± 22.4	28.7 ± 22.1	40.4 ± 21.3	53 ± 21.3 ***	p=0.97
Total Perfusion Lesion Volume ± SD (Mean, ml)	83.2 ± 41.9	61.1 ± 28.8	88.1 ± 47.3	88.1 ± 37.5	p=0.370
Co-registered Final infarct volume ± SD (Mean, ml)	77.5 ± 67.2	65.2 ± 41.7	87 ± 78.1	68.7 ± 62.1	p=0.713
Final Infarct Volume ± SD (Mean, ml)	118.9 ± 120.8	114.1 ± 69	135 ± 145.7	94.3 ± 101.6	p=0.709
Infarct Growth ± SD (Mean, ml)	37.2± 46.6	32.8 ± 24.8	40.9 ± 55.4	33.5 ±	p=0.898
Penumbral salvage ± SD (Mean, ml)	8.8 ± 49.6	-4.1± 37.1	1.2 ± 55.5	31.7 ± 31.2	p=0.263
Rankin 0-1 Day 30 (n/%)	4 (12.1)	0	3 (17.6)	1 (10)	p= 0.70
Rankin 2-5 Day 30 (n/%)	20 (60.6)	5 (83.3)	9 (52.9)	6 (60)	
Rankin 6 Day 30 (n/%)	9 (27.3)	1(16.7)	5 (29.4)	3 (30)	

Using LSD analysis there was a significant difference between the following groups - *AHG vs. EG, ** AHG vs. LHG, *** EG vs. LHG

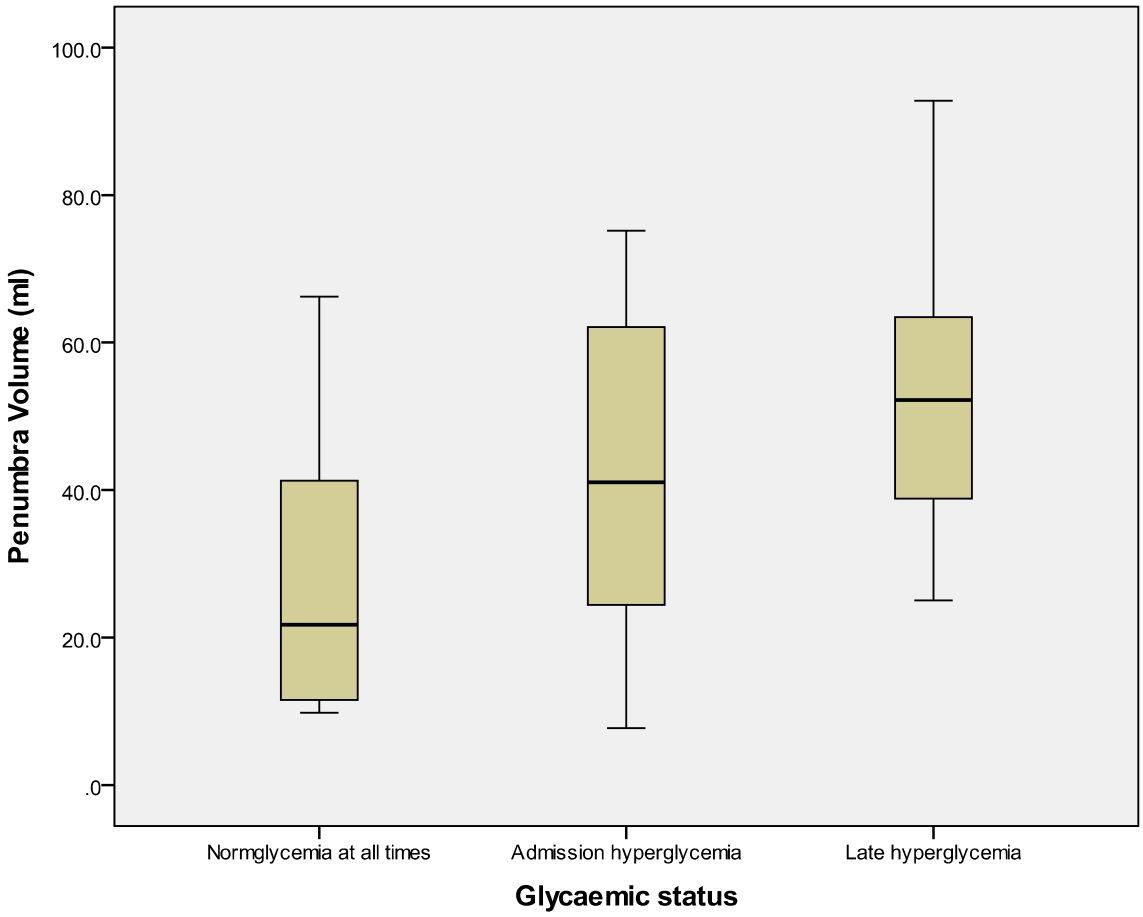

Figure 60 Boxplot of penumbral volume dependant on glycaemic status in patients with M1 occlusions

There was a non-significant trend for patients with normoglycaemia to have smaller volumes of penumbral tissue than patients who had admission hyperglycaemia or late hyperglycaemia.

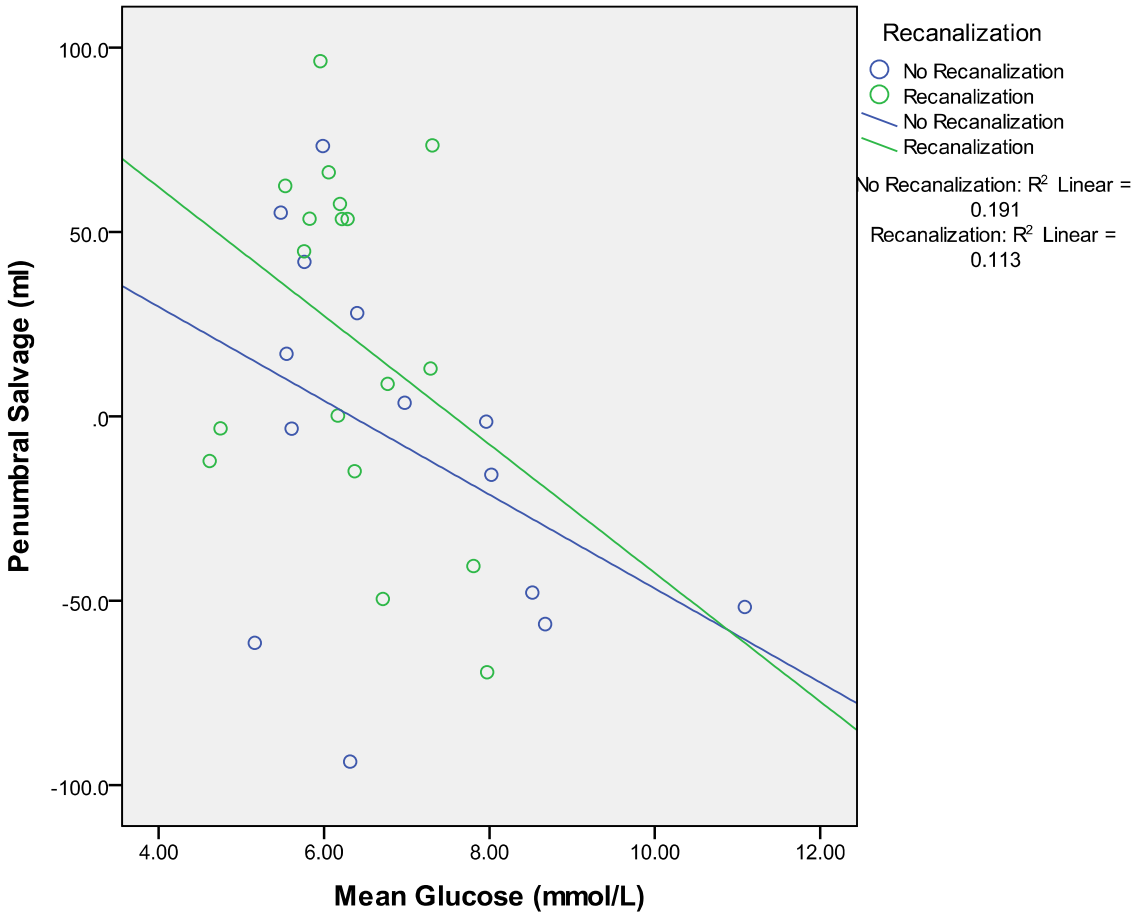

Figure 61 - Relationship between mean glucose and penumbral salvage in patients with M1 occlusions

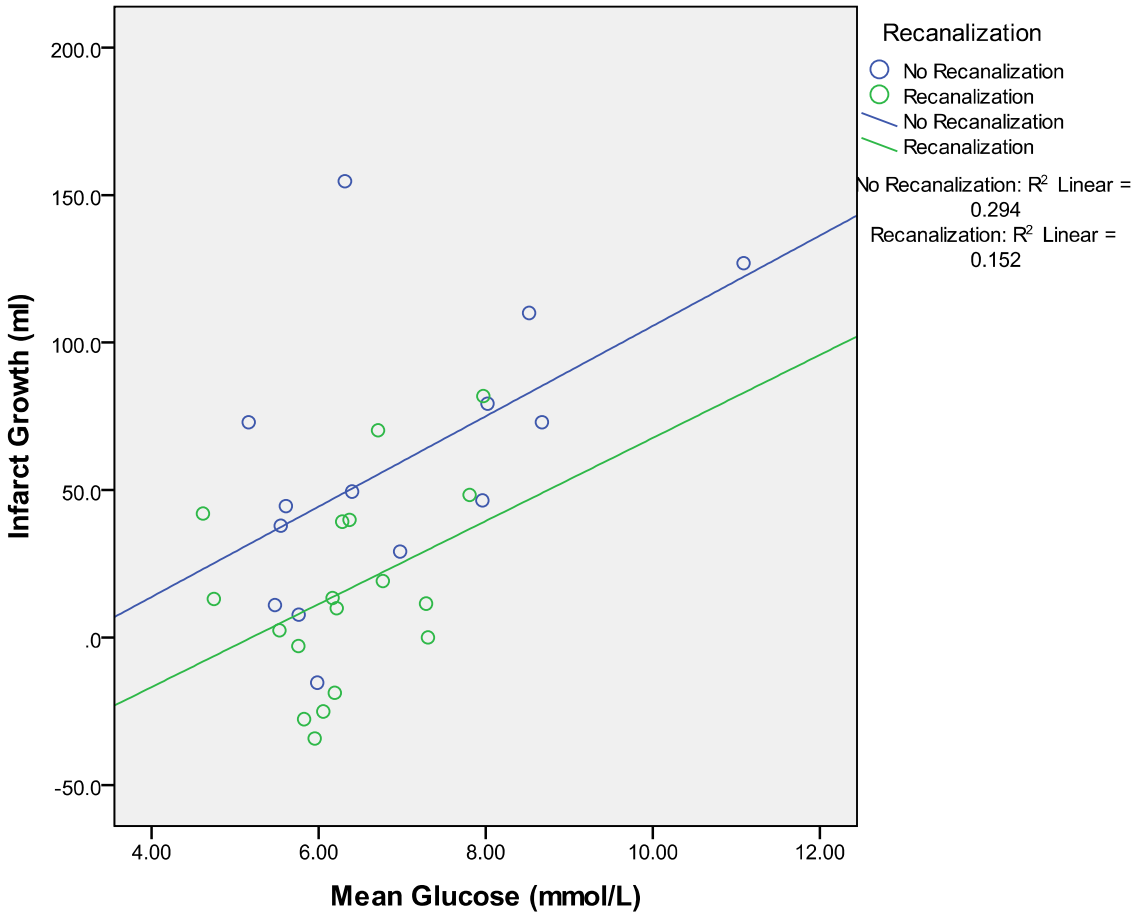

Figure 62 - Relationship between infarct growth and mean glucose in patients with M1 occlusions

A scatter plot suggests that there is a trend towards greater infarct growth with increasing mean glucose levels.

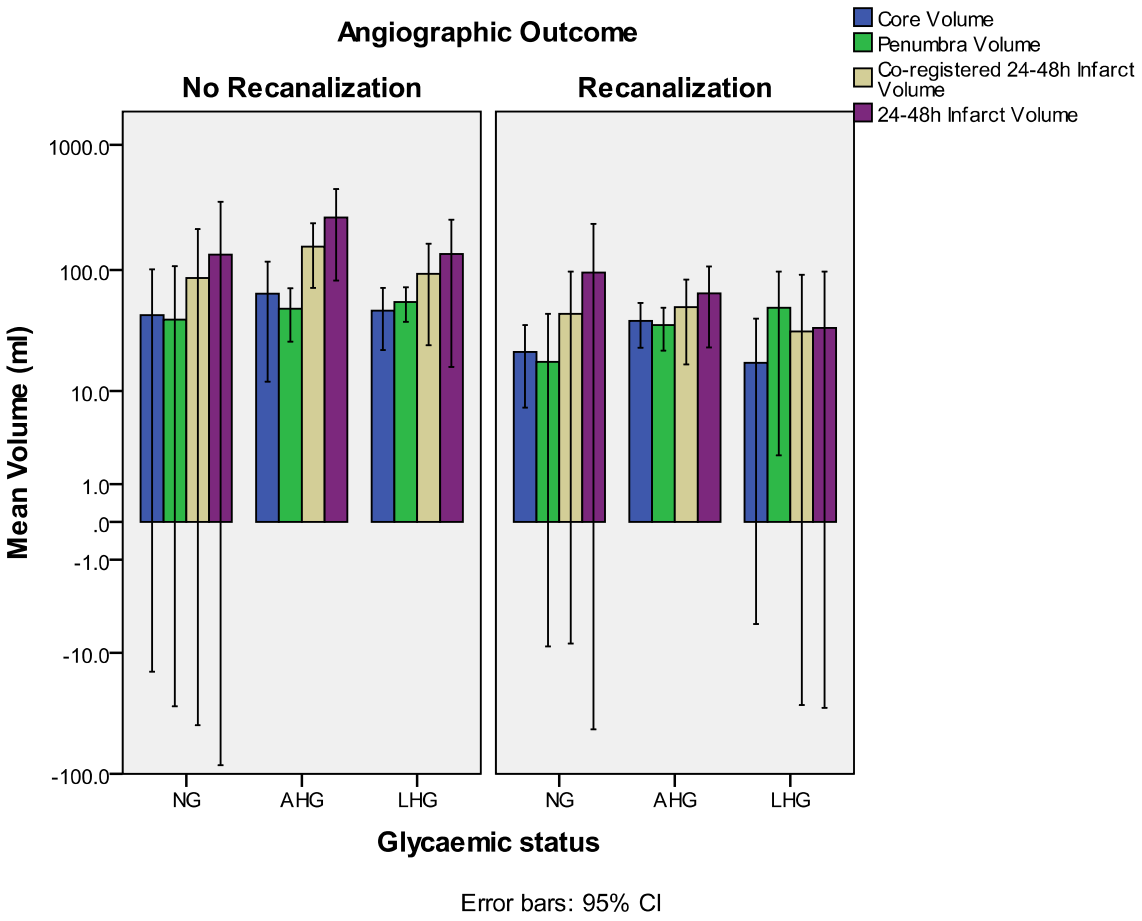

Figure 63 – Imaging results for patients with M1 occlusions vary with recanalization and glycaemic status

9.3.2 Regression analysis for all patients with M1 occlusions

Linear regression analysis suggested that the presence of recanalization was a predictor of final infarct volume (p=0.001, 95% CI -197.9 to -58.5) as was admission blood glucose (p=0.018, 95%CI 4.1 to 40.7).

Binary logistic regression for the prediction of recanalization showed that the presence or absence of a tandem occlusion predicted final recanalization status with p=0.03 (OR 5.2, 95% CI 1.17 to 23). Other factors that were not significant in this model included admission hyperglycaemia, age, HbA1c, thrombolytic treatment, systolic blood pressure, diastolic blood pressure and admission NIHSS.

9.3.3 Tandem occlusions

Overall 16 patients (48.5%) in this group had 2 or more occlusions. There was no significant difference in the presence of tandem occlusions between groups. Patients with tandem occlusions were less likely to recanalize than patients with only one occlusion (p=0.025 using chi squared). Patients with tandem occlusions also tended to have a higher baseline NIHSS although this was non-significant.

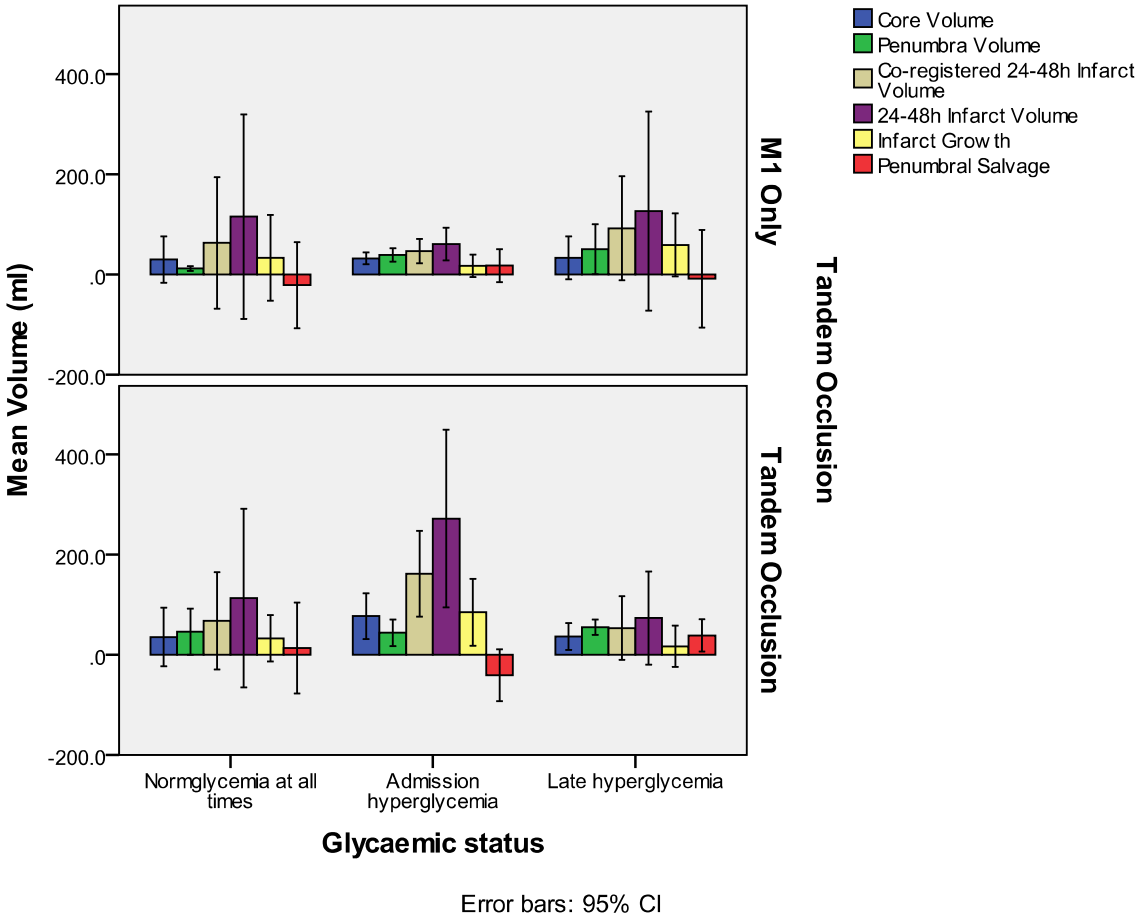

Figure 64 - Perfusion lesion and outcome infarct volumes vary with glycaemic status and presence of tandem occlusion

Outcomes were compared between patients with M1 occlusions only and patients with tandem occlusions. In the admission hyperglycaemia group patients with tandem occlusions had significantly larger core perfusion lesions (p=0.008), total perfusion lesion volumes (p=0.033), co-registered infarct volumes (p=0.001), total infarct volumes (p=0.001) with greater infarct growth (p=0.011) and reduced penumbral salvage (p=0.033). Day 30 clinical outcomes were also worse in the tandem occlusion group (p=0.035).

Glucose variables (admission blood glucose, mean capillary blood glucose, HbA1c) were slightly higher in the tandem occlusion group but not significantly so.

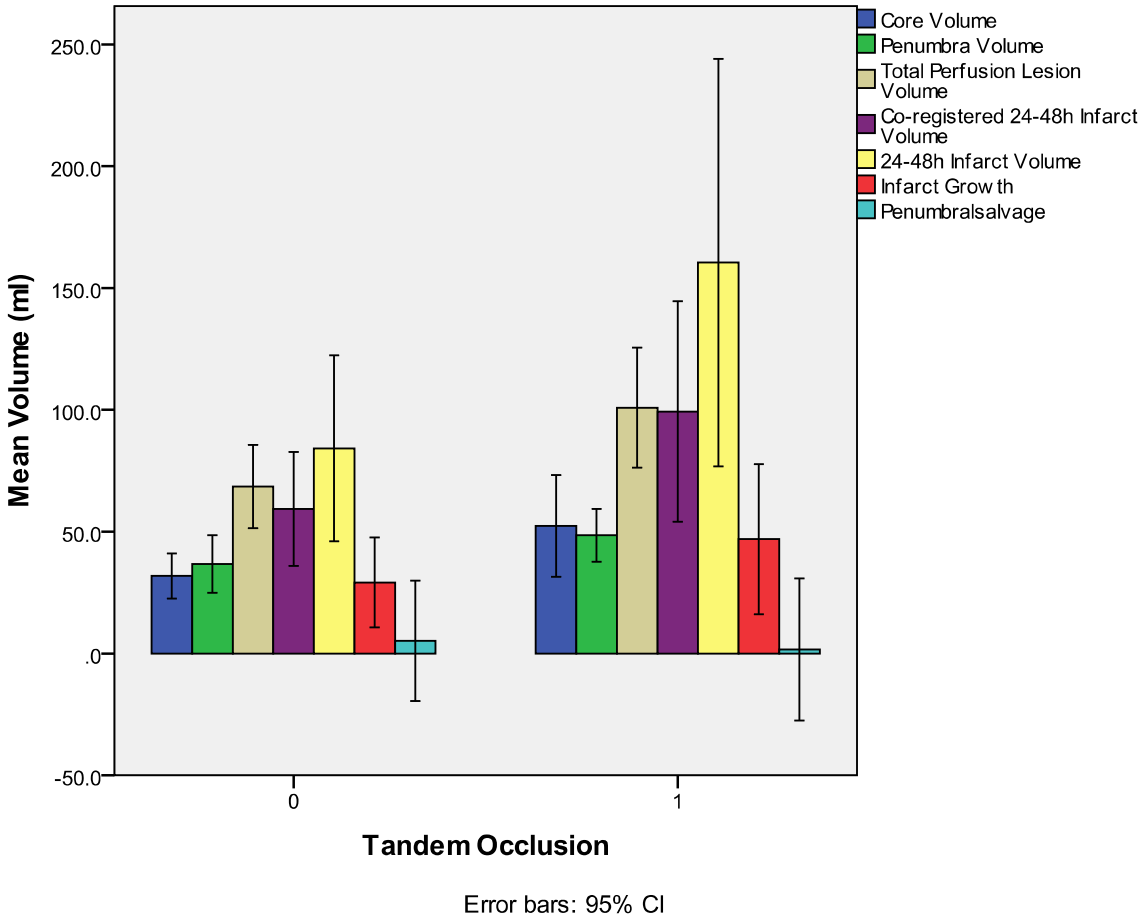

Figure 65 - Imaging findings vary with occlusion type

In the euglycaemic patients smaller perfusion lesions were seen in the M1 occlusion group than the tandem occlusion group (p=0.049) although these groups were both small. No other significant differences were seen.

There were no significant differences in the late hyperglycaemia patients although patients with tandem occlusions tended to have larger infarct volumes with more infarct growth and less penumbral salvage.

There was a significant difference in penumbral salvage between glycaemic groups in the tandem occlusion group (p=0.026).

Table 9-2 Tandem occlusions compared with M1 only in admission hyperglycaemia group

Group (n)	AHG tandem (6)	AHG M1 only (11)	
Age ± SD (mean/years)	75 ± 11	75.2 ± 8.3	P=0.970
Admission blood glucose ± SD (Mean mmol/L)	9.13 ± 1.98	8.12 ± 1.89	P=0.314
Mean capillary blood glucose ± SD (Mean, mmol/L)	7.86 ± 1.97	6.89 ± 0.86	P=0.177
HbA1c ± SD (Mean)	6.3 ± 1.5	5.7 ± 0.6	P=0.336
Admission NIHSS (Median/IQR)	21 (13-26)	18 (13-20)	
Admission Blood pressure ± SD (mmHg)	161/86 ± 42/30	138/69 ± 17/12	P=0.133
Thrombolytic treatment (n/%)	5 (83)	10 (91)	P=0.596
Recanalization (n/%)	2 (33)	9 (82)	P=0.072
CT Perfusion Core volume ± SD (Mean, ml))	76.9 ± 43.3	31.9 ± 17.4	P=0.008
CT Perfusion Penumbra volume ± SD (Mean, ml)	43.6 ± 25.3	38.7 ± 19.8	P=0.662
Total Perfusion Lesion Volume ± SD (Mean, ml)	120.5 ± 54.4	70.6 ± 33.7	P=0.033
Co-registered Final infarct volume ± SD (Mean, ml))	161.4 ± 81.7	46.4 ± 36.1	P=0.001
Final Infarct Volume ± SD (Mean, ml)	271.7 ± 167.1	60.4 ± 48.6	P=0.001
Infarct Growth ± SD (Mean, ml)	84.5± 63.7	17.2 ± 33.4	P=0.011
Penumbral salvage ± SD (Mean, ml)	-40.9 ± 49.36	17.5 ± 48.8	P=0.033
Rankin 0-1 Day 30 (n/%)	0	3 (27.3)	
Rankin 2-5 Day 30 (n/%)	2 (33.3)	7 (63.6)	
Rankin 6 Day 30 (n/%)	4 (66.7)	1 (9.1)	,

Table 9-3 Tandem occlusions compared with M1 only in late hyperglycaemia group

Group (n)	LHG tandem (6)	LHG M1 only (4)	
Age ± SD (mean/years)	75.3 ± 7.3	64.8 ± 14	p=0.151
Admission blood glucose ± SD (Mean mmol/L)	5.87 ± 0.86	5.98 ± 1.12	p=0.866
Mean capillary blood glucose ± SD (Mean, mmol/L)	6.35 ± 0.86	6 ± 0.39	p=0.472
HbA1c ± SD (Mean)	5.5 ± 0.3	5.4 ± 0.4	P=0.770
Admission NIHSS (Median/IQR)	15.5 (9.75-23.5)	13.5 (9.5-19.75)	
Admission Blood pressure ± SD (mmHg)	154/79 ± 10/4	143/73 ± 34/12	p=0.543
Thrombolytic treatment (n/%)	5 (83.3)	3 (75)	p=0.667
Recanalization (n/%)	2 (33)	2 (50)	p=0.548
CT Perfusion Core volume ± SD (Mean, ml))	36.5 ± 25.6	33.2 ± 26.9	p=0.850
CT Perfusion Penumbra volume ± SD (Mean, ml)	54.9 ± 14.5	50.1 ± 31.4	p=0.752
Total Perfusion Lesion Volume ± SD (Mean, ml)	91.4 ± 39.3	83.4 ± 39.9	p=0.761
Co-registered Final infarct volume ± SD (Mean, ml))	92.1 ± 65.1	53.1 ± 60.5	p=0.361
Final Infarct Volume ± SD (Mean, ml)	126.4 ± 124.8	73 ± 88.7	p=0.448
Infarct Growth ± SD (Mean, ml)	58.9± 39.7	16.6 ± 39.3	p=0.136
Penumbral salvage ± SD (Mean, ml)	-8.7 ± 61.25	38.3 ± 30.6	p=0.141
Rankin 0-1 Day 30 (n/%)	0	1 (25)	
Rankin 2-5 Day 30 (n/%)	3 (50)	3 (75)	
Rankin 6 Day 30 (n/%)	3 (50)	0	p= 0.153

Table 9-4 Tandem occlusions compared with M1 occlusion only in euglycaemia group

Group (n)	Euglycaem1a tandem	Euglycaemia M1 only	
Age ± SD (mean/years)	52.5 ± 14.8	60.5 ± 23.3	p=0.901
Admission blood glucose ± SD (Mean	6 ± 0.5	6.55 ± 0.35	p=0.244
Mean capillary blood glucose ± SD (Mean,	5.22 ± 0.5	5.46 ± 1	p=0.682
HbA1c ± SD (Mean)	5.9 ± 0.7	5.1± 0.6	P=0.211
Admission NIHSS (Median)	19.5 (13.75 – 25.25)	14.5	
Admission Blood pressure ± SD (mmHg)	161/86 ± 17/10	157/91 ± 35/1	p=0.673
Thrombolytic treatment (n/%)	3 (75)	2 (100)	p=0.667
Recanalization (n/%)	1 (25)	0	p=0.2
CT Perfusion Core volume ± SD (Mean, ml))	39 ± 20.8	19 ± 5.6	p=0.274
CT Perfusion Penumbra volume ± SD (Mean,	37.2 ± 22.9	11.7 ± 2.7	p=0.212
Total Perfusion Lesion Volume ± SD (Mean,	76.2 ± 21.4	30.7 ± 2.9	p=0.047
Co-registered Final infarct volume ± SD	81.7 ± 42.5	32.3 ± 5.4	p=0.197
Final Infarct Volume ± SD (Mean, ml)	133.7 ± 71.8	75 ± 61.4	p=0.383
Infarct Growth ± SD (Mean, ml)	22.6 ± 25.3	13.3 ± 0.2	p=0.197
Penumbral salvage ± SD (Mean, ml)	-5.4 ± 47.8	-1.6 ± 2.5	p=0.920
Rankin 0-1 Day 30 (n/%)	0	0	
Rankin 2-5 Day 30 (n/%)	3 (75)	2 (100)	
Rankin 6 Day 30 (n/%)	1 (25)	0	p=0.439

9.3.4 Pure M1 occlusions

Seventeen patients had ‘pure’ M1 MCA occlusions with no evidence of occlusion of another blood vessel. Eleven of these patients had admission hyperglycaemia, 4 had late hyperglycaemia and 2 were consistently euglycaemic.

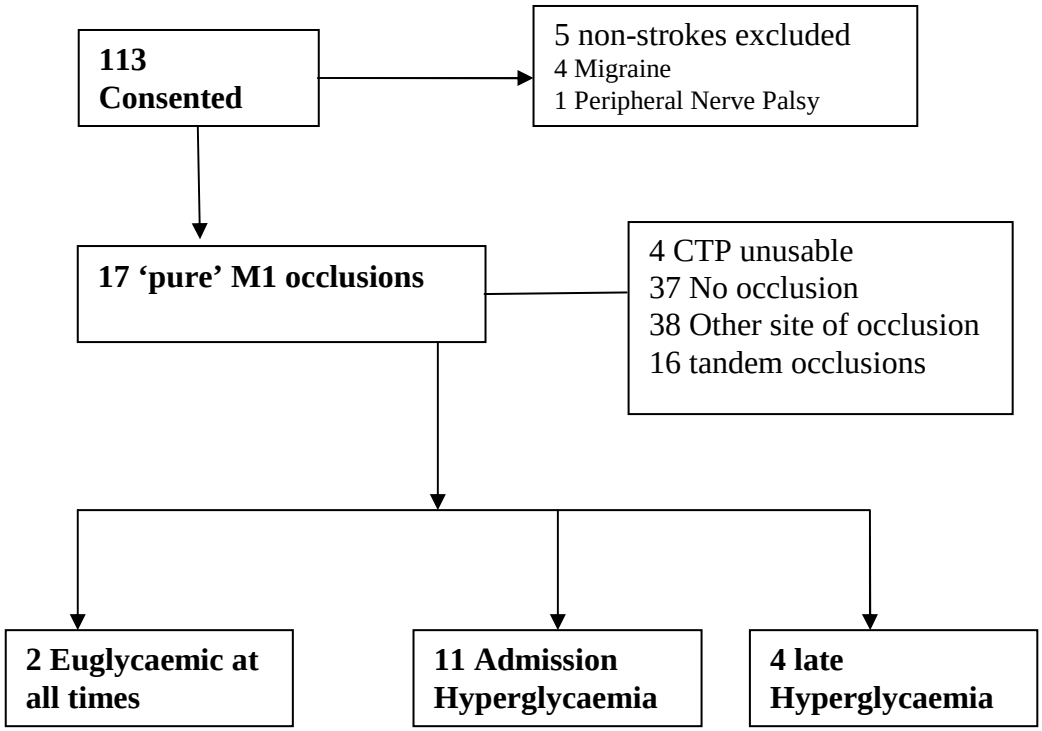

Figure 66 - Flowchart for 'pure' M1 occlusions

There was no significant difference in age although there was a trend for the admission hyperglycaemia patients to be older. Admission blood glucose was not significantly different between groups although there were significant differences for mean blood glucose.

The perfusion lesions in the euglycaemic group were smaller than those in the other groups although this was not significant. There was no significant difference between infarct volumes in the follow-up imaging.

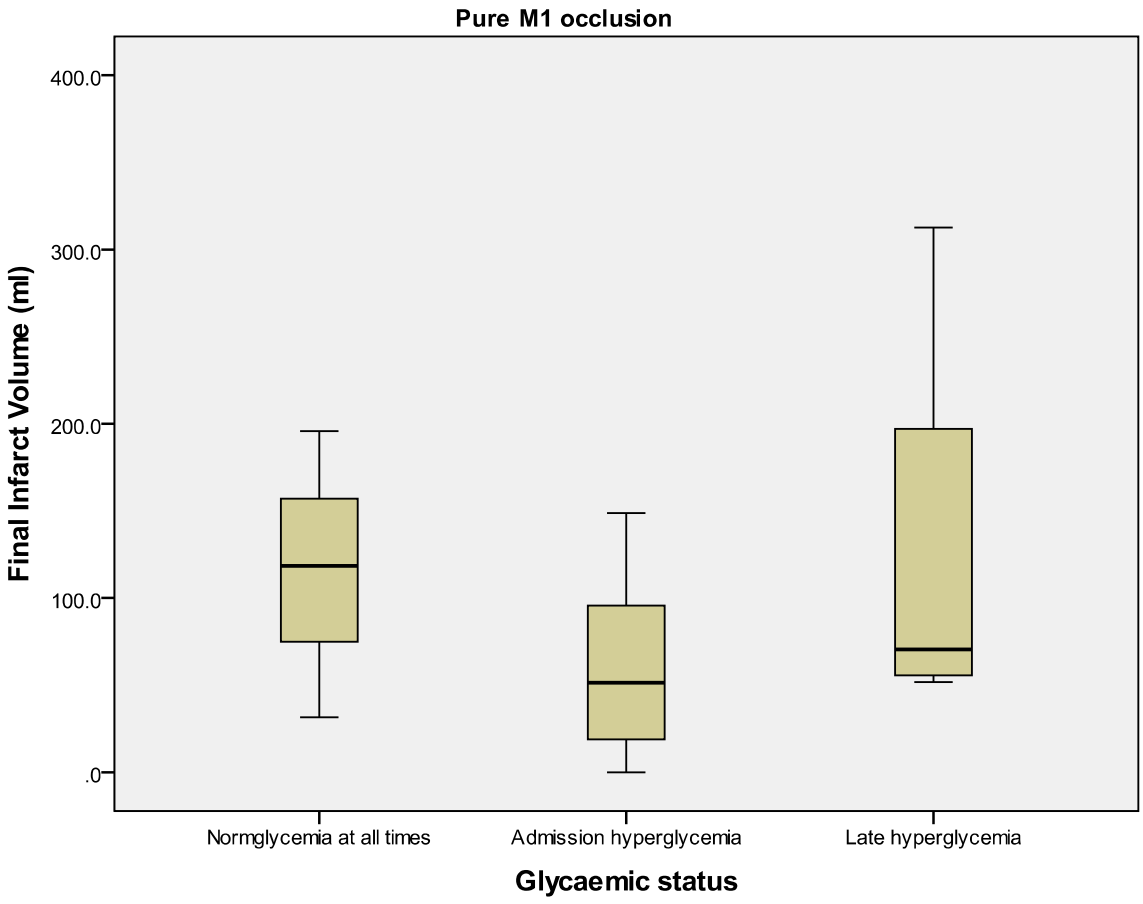

Figure 67 - 24-48 hour infarct volumes in patients with 'pure' M1 occlusions

Table 9-5 Outcomes for patients with pure M1 occlusions

Group (n)	All patients (17)	Hyperglycaemia			
		None(2)	Admission (11)	Late (4)	
Male Gender (n/%)	8 (47)	2 (100)	4 (36)	2 (50)	
Age ± SD (mean/years)	71 ± 12.2	60.5 ± 23.3	75.2 ± 8.3	64.8 ± 14	p=0.149
Admission blood glucose ± SD (Mean mmol/L)	7.43 ± 1.85	6.55 ± 0.35	8.12 ± 1.89	5.98 ± 1.12	p=0.103
Mean capillary blood glucose ± SD (Mean, mmol/L)	6.52 ± 0.93	5.46 ± 1	6.89 ± 0.86	6 ± 0.39	p=0.047
HbA1c ± SD (Mean)	5.6 ± 0.6	5.1± 0.6	5.7 ± 0.6	5.4 ± 0.4	P=0.297
Admission NIHSS (Median/IQR)	16 (11-19.5)	14.5	18 (13-20)	13.5 (9.5-19.75)	p=0.477
Admission Blood pressure ± SD (mmHg)	142/73 ± 22/13	157/91 ± 35/1	138/69 ± 17/12	143/73 ± 34/12	p=0.578
Thrombolytic treatment (n/%)	15 (88)	2 (100)	10 (91)	3 (75)	p=0.601
Recanalization (n/%)	11 (65)	0	9 (82)	2 (50)	p=0.309
CT Perfusion Core volume ± SD (Mean, ml))	30.7± 18.6	19 ± 5.6	31.9 ± 17.4	33.2 ± 26.9	p=0.665
CT Perfusion Penumbra volume ± SD (Mean, ml)	38.2 ± 23.5	11.7 ± 2.7	38.7 ± 19.8	50.1 ± 31.4	p=0.171
Total Perfusion Lesion Volume ± SD (Mean, ml)	68.9 ± 35.3	30.7 ± 2.9	70.6 ± 33.7	83.4 ± 39.9	p=0.229
Co-registered 24-48h infarct volume ± SD (Mean, ml))	55.5 ± 45.5	32.3 ± 5.4	46.4 ± 36.1	53.1 ± 60.5	p=0.174
24-48h Infarct Volume ± SD (Mean, ml)	77.7 ± 73.7	75 ± 61.4	60.4 ± 48.6	73 ± 88.7	p=0.329
Infarct Growth ± SD (Mean, ml)	26.6± 36.6	13.3 ± 0.2	17.2 ± 33.4	16.6 ± 39.3	p=0.126
Penumbral salvage ± SD (Mean, ml)	15.1 ± 47.3	-21.5 ± 34.6	24.2 ± 51.1	18.5 ± 34.2	p=0.352
Rankin 0-2 Day 30 (n/%)	4 (24)	0	3 (27.3)	1 (25)	
Rankin 3-5 Day 30 (n/%)	12 (71)	2 (100)	7 (63.6)	3 (75)	
Rankin 6 Day 30 (n/%)	1 (6)	0	1 (9.1)	0	P=0.841

9.4 Discussion

The exploratory analyses presented in this chapter are limited by the small number of patients with evidence of a proximal M1 middle cerebral artery occlusion and imaging that was adequate for full analysis. Of the 113 patients initially recruited into this study only

33 met the criteria for analysis in this section. If we exclude patients with tandem occlusions (M1 occlusion with at least one other occlusion) we are left with 17 patients..

In these data follow-up infarct volume was predicted by recanalization and admission blood glucose. The relationship between recanalization and final infarct size is interesting and in keeping with what we know of the pathophysiology of stroke. It is unusual that thrombolytic treatment did not emerge as a significant predictor of infarct size but the sample size is small and a large proportion of the patients were treated with alteplase. The relationship between admission glucose and final infarct volume is also of interest although we saw no significant relationship between glycaemic status and final infarct volume.

The trend to larger infarct volumes may have been partially masked by the small number of euglycaemic patients with M1 occlusions and the large proportion of these patients who had tandem occlusions. It is also interesting to note that only one of the four euglycaemic patients recanalized. Larger proportions of patients recanalized in the other groups which may again skew the trend. While none of these patients received insulin treatment their low mean blood glucose levels and larger infarct sizes may reflect the situation seen in the SELESTIAL and InsulInfarct studies where larger infarct volumes were seen after insulin treatment in patients who did not recanalize.[554, 562]

It is recognised that tandem occlusions are associated with poor outcome after stroke thrombolysis.[584-586] Recanalization rates are often reduced in patients with tandem occlusions.[587] Ipsilateral internal carotid artery occlusion has also been associated with early arterial re-occlusion after treatment with alteplase.[588] Case reports have described good results with intra-arterial intervention but there is no good evidence based treatment strategy for these patients at present.[589]

In patients with admission hyperglycaemia the presence of a tandem occlusion significantly influences both baseline imaging findings and outcome infarct volumes. The patients with tandem occlusions also had significantly worse outcomes as graded by Rankin score. This significant effect was not seen in the late hyperglycaemia group or the euglycaemia group although there was a trend towards a similar pattern. The small numbers in the late hyperglycaemia and euglycaemia groups mean that our results are slightly limited.

It is also interesting to note that euglycaemic patients with M1 occlusions are rare. Only 2/17 of patients with proven M1 occlusions were hyperglycaemic. Overall only 16% of the patients we recruited were euglycaemic for the 48 hours after their strokes. This may reflect the poor health and bad dietary habits of the West of Scotland population although it may also suggest that a degree of hyperglycaemia in the days after acute ischaemic stroke is very common.

In the population of all 33 patients with M1 occlusions (including those with tandem occlusions) the difference in penumbral volumes between the late hyperglycemia group and the euglycaemic group may indicate that larger volumes of tissue at risk could create a demand for glucose as time goes on.. Post stroke hyperglycaemia may be the body trying to attain physiological homeostasis by getting glucose to dead or dying brain tissue. We may be witnessing a failing protective mechanism as opposed to a pathogenic process.

In Table 9.1 (all M1 occlusions) post hoc analysis of penumbra volumes suggested that euglycaemic patients may have smaller volumes of penumbra compared to the late hyperglycaemic group. One hypothesis could be that the late hyperglycaemic group is stimulated to mount a hyperglycaemic response to salvage penumbra while the euglycaemic group with the smaller volume of tissue at risk does not.

These data presented in this chapter show us how heterogeneous the pathophysiology of acute ischaemic stroke is. A wide variety of arterial occlusions can cause a stroke on a background of variable prior glycaemic states. The hyperglycaemic response that the body will mount after a stroke is also highly variable and is influenced by factors such as dysphagia, glucose metabolism and medical intervention with drugs and IV fluids. The number of permutations of factors that may influence the growth of an infarct is vast. With advanced imaging techniques and close observation of physiological variables we are only beginning to understand these interactions. To allow large studies of more homogenous populations of stroke victims we must recruit even larger numbers of patients. We will reach a more complete understanding of the pathophysiology of post stroke hyperglycaemia and its interaction with brain arterial patency through such studies.

10 Post-Stroke Hyperglycemia and brain arterial patency – Discussion

10.1 Anecdotal background to thesis

Before I started working on this thesis in 2008 I knew very little about the importance of hyperglycaemia in stroke. As I have worked on this thesis I have learned a lot about the relationship between blood glucose levels and stroke. When I consider the results of my research I realise that there is still much that we do not understand about this relationship.

I had certain preconceived notions before I started as a research fellow. I thought that post stroke hyperglycaemia was implicitly a bad thing and that insulin treatment would therefore be a good thing. The stroke physician that I worked for before I started the project told me that there was nothing else to know about post stroke hyperglycaemia and that insulin didn't work! He was joking but there main have been a grain of truth in his joke.

10.2 Scientific background to thesis

For my introduction I reviewed the literature on hyperglycaemia and stroke. There have been over 400 clinical papers published on this relationship and I have a spreadsheet detailing 424 of them.

To make sense of the volume of papers I broke them down into two broad groups. The first group dealt with pre-stroke hyperglycaemia; hyperglycaemia, impaired glucose tolerance, insulin resistance and diabetes as risk factors for ischaemic stroke. There is certainly strong evidence suggesting that these risk factors are very real and if abnormal glucose metabolism is a risk factor for stroke we should not discuss post-stroke hyperglycaemia without at least being aware of pre-stroke hyperglycaemia.

I then wrote about post-stroke hyperglycaemia starting with the earliest references that I could find in the literature of the 1960's and 1970's.[71, 148, 151] A correlation between PSH and poor outcomes was well established and clearly delineated in the meta-analysis published by Capes *et al* in 2001.[146] However the mechanisms by which PSH worsens outcome from stroke (if indeed it really does worsen outcome) are still at least partially unclear.

Indeed dissenting voices have suggested that PSH may actually be a protective mechanism and that therapeutic intervention to achieve normoglycaemia is inappropriate until we know more about the phenomenon.[287] Uyttenboogaart and colleagues have suggested that PSH may be associated with better outcomes after lacunar stroke.[260]

We are not even sure what PSH is. It has been suggested that PSH is a stress response[153, 218, 221] PSH may represent undiagnosed diabetes mellitus.[130, 231, 233, 236, 237] It seems less likely that PSH is related to specific neuroanatomical lesions.[226] My own theory is that PSH is partly unrecognised abnormal glucose metabolism and partly a stress response. The significant difference I observed in the HbA1c levels between the admission hyperglycaemia group and the euglycaemic group in the POSH cohort would support this hypothesis (Chapter 6).

We are learning more about the pathophysiology of post-stroke hyperglycaemia. This has been partly as a side-effect of advances in the acute treatment of ischaemic stroke with thrombolytic therapy. PSH has been found to predict a poor outcome after thrombolysis [175, 176, 179-181, 183-185] although the association is not always clear cut.[186] Intracerebral haemorrhage after thrombolytic treatment is associated with hyperglycaemia.[34, 182, 189, 191] A similar relationship has also been noted after intra-arterial procedures for stroke.[193, 194, 196]

A more interesting finding for me (or at least one that is more directly relevant to my thesis) is the suggestion that hyperglycaemia accelerates the deterioration of ischaemic penumbra into infarcted core after rt-PA treatment for acute stroke.[160, 176, 202, 590] This effect may mediated by hyperglycaemia reducing the recanalization rate after alteplase treatment.[180, 198, 200, 201]

Interventions to control hyperglycaemia in acute stroke have not attenuated infarct growth and indeed insulin appears to have accelerated infarct growth in some patients.[554, 562] Indeed one study that purports to be an observational study claiming to demonstrate that hyperglycaemia quickens penumbral loss is confounded by the fact that patients were treated with an insulin sliding scale.[197]

It has also been hypothesised that alteplase have a reduced effect on penumbral salvage in diabetic patients due to a impaired collateral circulation.[590] This theory is in keeping with an observational study published by Toni and colleagues in 1994.[204] Although I have not

had time to look at this in POSH cohort we do have good data on collateral circulation and glycaemic status so it is something that we may be able to revisit in the future.

Another interesting concept is the possibility that hyperglycaemia is not a negative prognostic factor in TIA patients[175, 207]. This observation may be because non-diabetic patients have been transiently hyperglycaemic as a physiological response to their TIA while diabetic patient have gone on to tissue infarction and stroke.[209-212] My hypothesis is that diabetic patients may be more likely to have a stroke than a TIA due to a lack of collateral circulation in their cerebral vasculature.

10.3 Conclusions of thesis

In Chapter 3 I systematically reviewed the existing animal data on models of stroke and hyperglycaemia. I had started this review expecting to find good evidence of a strong relationship between hyperglycemia and infarct growth in animal models of stroke. I also expected to find clear evidence of the utility of insulin in preventing infarct growth. However when I looked at things systematically I found that the available evidence did not fully support my preconceived ideas. The animal models for hyperglycaemia did not really match the human patient with post-stroke hyperglycaemia and the evidence for the use of insulin was very limited.

I subsequently undertook a systematic review on the clinical use of insulin for post-stroke hyperglycaemia (Chapter 4). The available clinical trials examining the use of insulin for post-stroke hyperglycaemia show no benefit from intervention. Indeed, insulin may be harmful for many patients. I presented these data at the European Stroke Conference in Hamburg in 2011 and a Cochrane Review with very similar findings has subsequently been published by another group.[591]

Chapter 5 was a retrospective study looking at patients who had been treated with alteplase and entered into the SITS database in the Southern General Hospital. I was able to 48 hour obtain glucose profiles for a subgroup of this population and we were able to look at the relationship between different patterns of hyperglycaemia and clinical outcomes.

We found that, in this population, patients with a mean capillary blood glucose (MCBG) of above 7mmol/L had poorer outcomes than those who had an admission glucose level of above 7mmol/L or those who had two isolated glucose levels above 7mmol/L. This retrospective study had been partially inspired by the GLIAS paper[161] which suggested that

a ‘cut-off ‘ glucose level of 8.6mmol/L was associated with the poorest outcomes after stroke so we also used this value in our analysis and found it to be less predictive that the 48 hour mean capillary blood glucose.

In Chapter 6 I established the demographics of the patients in the different glycaemic categories. We divided patients into 3 groups. The first group was labelled as having admission hyperglycaemia with a glucose level of 7mmol/L or more within 6 hours of stroke onset. This group of patients would have been hyperglycaemic while a potentially salvageable ischaemic penumbra existed.

The second group became hyperglycaemic 6 or more hours after stroke onset. This late hyperglycaemia group were less likely to have been hyperglycaemic whilst the ischaemic penumbra was in existence.

The third group of normoglycaemic patients had no evidence of a blood glucose level of 7mmol/L during the first 48 hours after stroke onset.

The normoglycaemic patients were significantly younger than the other patients and were less likely to have a history of diabetes. All glycaemic parameters (HbA1c, admission blood glucose, mean capillary blood glucose) were significantly different between groups. Only 15.7% of patients were normoglycaemic for the entire study period. I suspect that the younger patients may be more likely to be normoglycaemic with a lower HbA1c as the incidence of diabetes increases with age.[575]

In Chapter 7 I looked at the interaction between glycaemic status and infarct growth. We defined infarct growth as the difference between the volume of infracted ‘core’ tissue seen on perfusion CT and the volume of co-registered infarct seen in a follow-up CT scan at 24-48 hours.

In this population there was a trend towards larger 24-48 hour infarct volumes in the admission hyperglycaemia patients when compared to patients with late hyperglycaemia or normoglycaemia. The trend was more apparent when I focused on patients who had a core perfusion lesion of more than 10ml.

There was also a trend towards reduced survival of penumbra in the patients with admission hyperglycaemia. Post hoc analysis suggested that there was significantly more

penumbral survival in the late hyperglycaemia group than the admission hyperglycaemia group.

Interestingly the patients with late hyperglycaemia had smaller mean core perfusion lesions and smaller follow-up mean infarct volumes than the normoglycaemic patients although this difference was non-significant.

The population in this chapter is still very heterogeneous as the underlying vascular status of the patients has not been defined. Arterial patency was clearly defined in chapter 8 and some interesting patterns began to appear. I've illustrated these patterns in Table 10.1 on the next page.

Table 10-1 - Summary table of interactions between arterial patency, blood glucose kinetics and infarct volume

		Mean Infarct volume ± SD (ml)		
		Admission Hyperglycaemia	**Normoglycaemia**	**Late Hyperglycaemia**
Occlusion	**Recanalization**	infarct volume * (84.7 ± 79)	infarct volume (80.2 ± 54.9)	**infarct volume (25 ± 25.7)**
	No recanalization	**infarct volume * (156.4.38 ± 153.4)**	infarct volume (79 ± 83.6)	infarct volume (63.2 ± 72)
No occlusion		**infarct volume * (22.49 ±50.6)**	infarct volume (46.2 ± 46.2)	**infarct volume (22.5 ± 47.3)**

* Significant difference ($p<0.001$)

In Chapter 8 I initially looked at the subgroup of patients with arterial occlusions in the POSH study. In the subgroup of patients with arterial occlusions admission hyperglycaemia reduces penumbral survival and increases final infarct volume. The interesting new finding is the suggestion that late hyperglycaemia after 6 hours may be associated with better imaging outcomes and improved penumbral survival.

These findings are somewhat in conflict with those published in a prospective observational study by Dziedzic and colleagues who found that patients with late hyperglycaemia had an increased risk of mortality.[247] However Dziedzic and colleagues did not have the extensive imaging data that we have used to characterise the pathological progression of acute stroke. They also had less measurement of glucose levels in the first 48 hours and they may have missed physiological fluctuations in a critical time period.

I subsequently looked at the patients with no evidence of arterial occlusion. In these patients the relationship between 24-48 hour infarct volume and glucose appears to be different. There was a trend towards normoglycaemic patients having larger infarcts than patients with hyperglycaemia. This trend may be in keeping with the suggestion made by Uyttenboogaart that hyperglycaemia may be associated with better outcomes in lacunar stroke.[260] A degree of hyperglycaemia may support tissue at risk of infarction in the absence of visible cerebral artery occlusion.

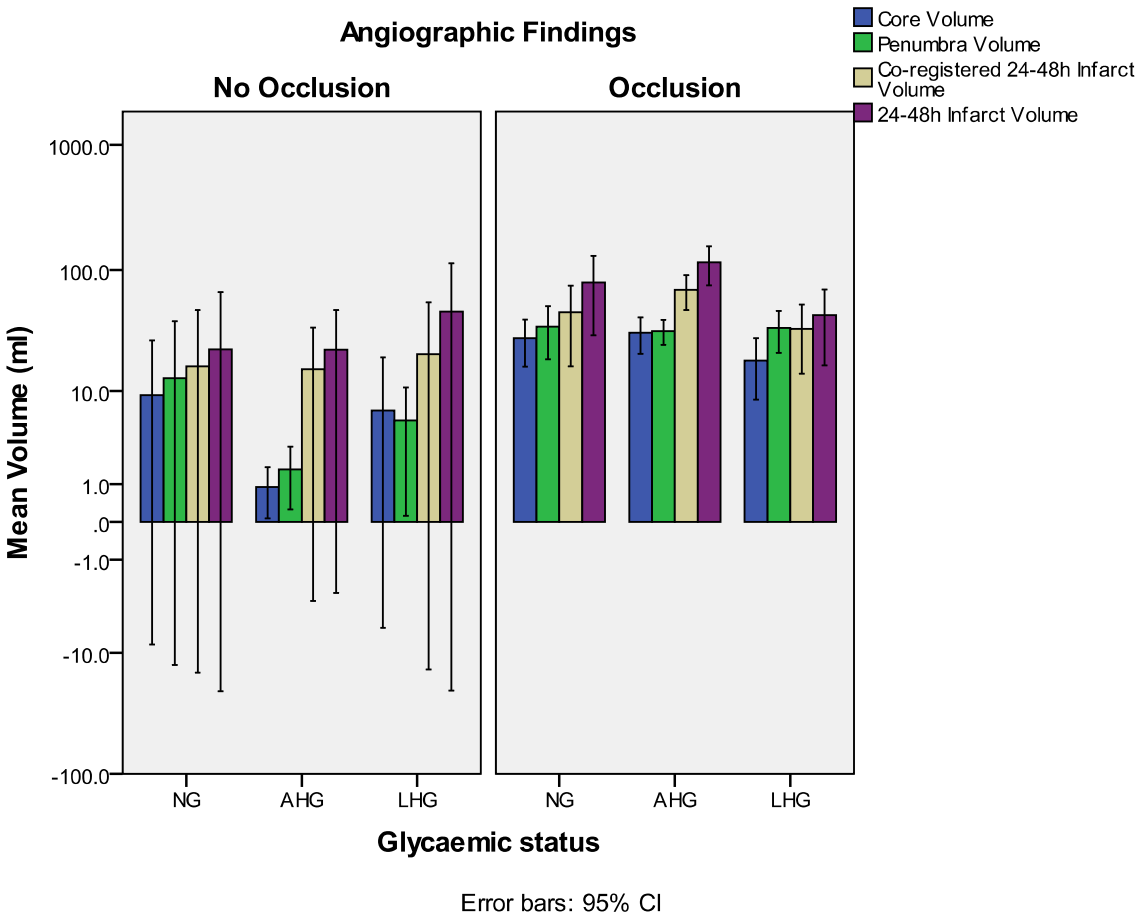

Figure 68 - Final infarct volume is influenced by the presence of arterial occlusion and blood glucose profile

In Chapter 9 I tried to delineate a very homogeneous population based on subtype of arterial occlusion. Initially I looked at all patients with proximal middle cerebral artery occlusions (M1 occlusions) before excluding patients with any other occlusions (tandem occlusions) to obtain a population of 'pure' M1 occlusions.

The presence of a tandem occlusion is associated with larger infarct volumes and worse clinical outcomes, in keeping with previous observations.[584, 585] In patients with admission hyperglycaemia follow up infarct volumes were 4 times larger in the presence of more than one occlusion. In the late hyperglycaemia and normoglycaemia groups tandem occlusions were associated with infarcts of approximately double the volume of those seen in 'pure' M1 occlusions. There were no significant differences in admission glucose, HbA1c or mean capillary blood glucose when patients with tandem occlusions were compared to 'pure' M1 occlusions or the POSH cohort as a whole.

Patients with late hyperglycaemia tended to have larger penumbral volumes than patients with normoglycaemia when we looked at all M1 occlusions (including tandem occlusions). This observation was only significant on post-hoc analysis. I would hypothesise that a larger penumbra may stimulate a physiological increase in blood glucose levels in an attempt to save dying brain tissue. The euglycaemic patients may fail to mount this response for some reason. Patients with late hyperglycaemia also tended to salvage more penumbra.

10.4 The findings of this thesis in the context of future stroke research

Taken in the context of the results of the SELESTIAL trial,[554] the INSULINFARCT trial,[562] my reassessment of the animal evidence in chapter 3[206] and the systematic review of the clinical trials of insulin for post stroke hyperglycemia presented in chapter 4 there is at present little justification for further human trials of insulin treatment for post-stroke hyperglycaemia after the initial 6 hour window. Trials of insulin for post stroke hyperglycaemia have failed to take account of the heterogeneous and acutely evolving pathophysiology of ischaemic stroke. Future 'blind' trials are unlikely to be helpful.

Any trials of insulin for hyperglycaemia within 6 hours of stroke onset should also be considered very carefully. There may even be a role for inducing hyperglycaemia in patients with no evidence of arterial occlusion.

The presence of 'tandem' occlusions is associated with very poor outcomes and these outcomes are worsened by admission hyperglycaemia. The identification of multiple occlusions of the cerebral vasculature is important for prognostication and appropriate treatment strategies need to be developed.

Larger scale studies examining the pathophysiology of post stroke hyperglycaemia and its interaction with brain arterial patency may be of more value in the first instance. Potential therapeutic interventions must be based on a solid understanding of the underlying pathophysiological mechanisms.

Appendices

Appendix 1 – Literature search strategy

exp *Stroke/ or cerebrovascular.mp. or exp Cerebrovascular Disorders/ or Thrombosis/ or Intracranial Thrombosis/ or "Intracranial Embolism and Thrombosis"/ or Brain Ischemia/ or Brain/ or Brain Infarction/ or Infarction, Posterior Cerebral Artery/ or Cerebral Infarction/ or Infarction, Middle Cerebral Artery/ or Middle Cerebral Artery/ or Infarction, Anterior Cerebral Artery/ or Brain Ischemia/ or "Intracranial Embolism and Thrombosis"/ or Middle Cerebral Artery/ or Brain Ischemia/ or Ischemia/ or isch?emia.mp. or Intracranial Embolism/ or Embolism/ or "Intracranial Embolism and Thrombosis"/ or neuron.mp. or exp *Neurons/or Nervous System/ or Central Nervous System/ or neuronal.mp. or exp *Brain Ischemia/ or exp *Ischemia/ or ischemia.mp. or exp *Ischemic Attack, Transient/ or exp *Ischemia/ or ischaemia.mp. or exp *Infarction, Posterior Cerebral Artery/ or exp *Cerebral Infarction/ or exp *Infarction, Anterior Cerebral Artery/ or exp *Brain Infarction/ or exp *Infarction/ or infarction.mp. or exp *Infarction, Middle Cerebral Artery/ or exp *Cerebrovascular Disorders/ or cva.mp. and exp *Blood Glucose/ or glucose.mp. or exp *Glucose/ or *Hyperglycemia/ or *Insulin/

Author	Year	Peer reviewed publication	Monitoring of BP	Temperature control	Random allocation to treatment or control	Blinded induction of ischemia	Blinded assessment of outcome	Anaesthetic agent	Appropriate animal model (aged, diabetic, hypertensive)	Sample size calculation	Compliance with animal welfare standards	Conflict of interest	Total
Araki	1992	1	1	1	0	0	1	Halothane	0	0	0	0	4
Berger	1989	1	0	1	0	0	0	Halothane	0	0	0	0	2
Bomont	1995	1	1	1	0	0	0	Isoflurane	0	0	0	0	3
Combs	1990	1	1	1	0	0	0	Ketamine, Xylazine	0	0	0	0	3
De Courten-Myers	1989	1	1	1	0	0	0	Phenobarbitol	0	0	0	0	3
De Courten-Myers	1994	1	1	1	0	0	0	Phenobarbitol	0	0	0	0	3
De Courten-Myers	1988	1	1	1	0	0	1	Phenobarbitol	0	0	0	0	4
Duverger	1988	1	0	0	0	0	0	Halothane	1	0	0	0	2
Hamilton	1995	1	1	1	1	0	1	Halothane	0	0	0	0	5
Huang	1996	1	1	1	0	0	0	Halothane	0	0	1	0	4
Izumi	1992	1	1	1	1	0	0	Halothane	0	0	0	0	4
Kittaka	1996	1	1	1	0	0	0	Metofane, Phenobarbital, Methonitrate	0	0	1	0	4
Kraft	1990	1	1	1	1	0	1	Halothane	0	0	1	0	6
Li	2004	1	0	0	0	0	0	Ketamine, Xylazine	0	0	1	0	2
Liu	2007	1	0	1	0	0	0	Chloral hydrate	0	0	1	0	3
Martin	2006	1	1	1	1	0	0	Ketamine	0	0	1	0	5
Nedergaard	1987	1	1	1	0	0	0	Halothane	0	0	0	0	3
Nedergaard & Diemer	1987	1	1	1	0	0	0	Halothane	0	0	0	0	3
Quast	1997	1	0	0	0	0	0	Halothane	0	0	0	0	1
Slivka	1991	1	1	1	0	0	1	Halothane	0	0	0	0	4
Wei	1997	1	1	1	0	0	0	Halothane	0	0	0	0	3
Wei	1998	1	1	1	0	0	0	Halothane	0	0	0	0	3
Wei	2003	1	1	1	0	0	0	Isoflurane	0	0	1	0	4
Zasslow	1989	1	1	1	1	0	1	Ketamine, Halothane	0	0	1	0	6
Zhu	2004	1	1	1	1	0	1	Halothane	0	0	0	0	5

Appendix 2 – Quality Scores for Included Papers

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Post-script

The soundtrack to this thesis included Philip Glass, Neutral Milk Hotel, Fugazi, Mogwai, the Yeah Yeah Yeahs, Sonic Youth and 12 Crass Songs by Jeffery Lewis. Initial parts of this thesis were written in August 2008 and this last section is being typed on Sunday 28th of July 2013, two days after I have received confirmation that the minor corrections to this thesis have been completed. I am currently listening to instrumental music by Black Flag.

This thesis contains work that took up 5 years of my life. It was hard work, but mostly enjoyable and mostly worthwhile. If any reader of this thesis has not undertaken a period of academic research I would urge the reader to do so as it expands your worldview. I believe that this work has made me a better clinician and a better person. Research, like most things in life is 99% perspiration and 1% inspiration.

If anyone is looking for stuff to read I urge them to check out the work of Alan Moore, Grant Morrison, Will Eisner, Kurt Vonnegut, Oliver Sachs, Iain (M) Banks and Hunter S. Thiompson. These writers may also expand your worldview.

Thanks for picking up this book.

Best wishes,

Niall