



**AN INVESTIGATION INTO CENTRAL NERVOUS SYSTEM INVOLVEMENT IN DISTAL
SYMMETRICAL DIABETIC NEUROPATHY IN**

TYPE 1 DIABETES MELLITUS

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“In saecula saeculorum”

To,
Amma and Appa

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Synopsis

Diabetes is a leading cause of peripheral neuropathy. It is the main initiating factor for foot ulceration and amputation resulting in considerable morbidity and remarkable consumption of scarce medical resources. Relatively little is known about the pathophysiology underlying DPN. Research into DPN has focused mainly on the peripheral nervous system (PNS) with central nervous system (CNS) involvement relatively overlooked. The studies undertaken have been designed to investigate CNS involvement in DPN.

1. Before embarking on spinal cord studies, I reviewed and modified the techniques employed in the pilot study to improve the precision and accuracy of cord cross sectional area measurements. These modifications were patiented to quality control studies, which are reported in Chapter 2.
2. I performed in-vivo cross-sectional magnetic resonance imaging of the cervical spine and reported evidence of spinal cord shrinkage (atrophy) in Painless DPN (Chapter 3). This study showed spinal cord atrophy to be an early phenomenon, present even in subclinical DPN. As the spinal cord is the caudal portion of the CNS, its involvement made us question whether the brain too may be involved.
3. Using MR spectroscopy I examined thalamic involvement in Painless DPN (Chapter 4). This deep brain nucleus is considered the gateway to all somatosensory information entering the brain, and responsible for modulation of sensory information prior to presentation to the cerebral cortex. I demonstrated thalamic biochemical abnormalities consistent with possible neuronal dysfunction in patients with Painless DPN.
4. The demonstration of thalamic neuronal dysfunction in DPN suggests that CNS involvement is not limited to the spinal cord but other important areas, responsible for somatosensory perception, may also be involved. Although the pathogenesis of thalamic involvement is unknown, it is likely that both vascular and metabolic factors that have been implicated in the pathogenesis of DPN are involved. In Chapter 4, I examined the possible

role of metabolic factors in the pathogenesis of thalamic neuronal dysfunction in DPN. Using MR spectroscopy, I demonstrated a significant elevation in thalamic glutamine/glutamate in patients with diabetes. Glutamate is the most abundant excitatory neurotransmitter and implicated in various models of neuronal cell death. Astrocytes, which play an important role in glutamate/glutamine metabolism, were impaired in the thalamus of diabetic patients in this study. The combination of elevated glutamate and impaired thalamic astrocytes may provide a pathophysiological explanation for thalamic dysfunction in DPN.

5. In Chapter 5, an alternative hypothesis for thalamic neuronal dysfunction in DPN was tested. Using dynamic contrast enhanced MR perfusion imaging, I demonstrated that Painful DPN is associated with unique thalamic perfusion abnormalities. Intriguingly, these abnormalities were present in patients with Painful but not Painless DPN.

6. Finally, in Chapter 6, I conducted a randomised, double blind and placebo-control trial (RCT) comparing the efficacy and tolerability of sativex, a cannabis based medicinal extract (CBME), with placebo in the symptomatic treatment of painful DPN. This is the first ever RCT using a CBME in painful DPN. We report no significant difference in the primary outcome measure due to a massive placebo effect and that depression is a potential major confounder in such clinical trials.

Author's Declaration

The studies that form the basis of this thesis are partly the result of collaborative work. My contributions were the following:

1. The primary role in the conception and design of each of the studies.
2. Recruitment, clinical and neurophysiological assessment of all patients.
3. Organisation of MR imaging including overseeing arrival and departure of patients. Prof I D Wilkinson designed MR protocols. Imaging was performed by departmental radiographers and qualitatively assessed by a neuroradiologist.
4. Processing, quantification and analysis of all data from quality control studies. CJE assisted with inter-observer variability assessments.
5. Processing, quantification and analysis of all spinal cord area measurements.
6. Processing, quantification and analysis of all spectroscopic data.
7. Processing, quantification and analysis of all perfusion data. Pharmacokinetic modelling was performed by Prof A Rostami-Hodjegan, University of Sheffield, UK.
8. Processing, quantification and analysis of all data from CBME RCT.
9. Collation of all data and subsequent statistical analysis. Additional statistical advice was provided by Dr D Witte, University College London, UK.

Dr Dinesh Selvarajah

Table of Contents

I	Index Of Figures	14
II	Index Of Tables	17
III	Abbreviations	19
IV	Units	22
1	INTRODUCTION	22
1.1	Diabetes Mellitus and Nervous System Complications	22
1.1.1	Diabetic Neuropathy	22
1.1.2	Epidemiology	22
1.1.3	Pathogenesis of DPN	23
1.1.4	Classification	24
1.1.5	Sub-clinical neuropathy	26
1.1.6	Distal symmetrical sensorimotor polyneuropathy (DPN)	26
1.1.7	Diabetic Autonomic Neuropathy	34
1.1.8	Painful Diabetic Neuropathies	34
1.2	Central Nervous System Involvement in Diabetes Mellitus	42
1.2.1	Central Nervous System Involvement in Diabetic Neuropathy	42
1.3	Summary	48
1.4	Aims of Thesis	49
2	QUALITY ASSESSMENTS OF METHODOLOGICAL TECHNIQUES	50
2.1	Introduction	50
2.2	Phantom Studies	51
2.2.1	Materials and Methods	51

2.2.2	Test tube phantoms	51
2.2.3	MR imaging protocol	53
2.2.4	Cross sectional area measurement	53
2.2.5	Errors of cross-sectional area measurement	57
2.3	Human Studies	59
2.3.1	Patients and methods	59
2.3.2	Inter-patient variation in cord CSA	59
2.4	Data Analysis	60
2.4.1	Study end points	60
2.4.2	Statistical Analysis	61
2.5	Results	62
2.5.1	Test Tube Phantom Studies	62
2.5.2	Human Studies	63
2.6	Discussion	68
3	EARLY INVOLVEMENT OF THE SPINAL CORD IN DIABETIC DISTAL SYMMETRICAL POLYNEUROPATHY	72
3.1	Introduction	72
3.2	Methods	73
3.2.1	Patients	73
3.2.2	Assessment of neuropathy	74
3.2.3	Magnetic resonance imaging protocol	75
3.3	Data analysis	75
3.3.1	Spinal cord area measurements	75
3.3.2	Statistical analysis	77
3.3.3	Reproducibility	77

3.4	Results	79
3.4.1	Demographics	79
3.4.2	Reproducibility	82
3.4.3	Spinal cord area assessments	82
3.5	Discussion	87
4	THALAMIC NEURONAL DYSFUNCTION IN PATIENTS WITH TYPE I DIABETES MELLITUS AND CHRONIC SENSORIMOTOR DISTAL SYMMETRICAL POLYNEUROPATHY	91
4.1	Introduction	91
4.2	Methods	94
4.2.1	Patients	94
4.2.2	Assessment of neuropathy	94
4.2.3	Magnetic resonance spectroscopy protocol	95
4.3	Data analysis	97
4.3.1	Statistical method	97
4.4	Results	98
4.4.1	Subgroup Demographics	98
4.4.2	MR spectroscopy assessments	99
4.4.3	Spectroscopy and neurophysiological correlations	99
4.5	Discussion	102
5	RAISED LEVELS OF GLUTAMATE/GLUTAMINE IN DIABETES, A CLUE TO THE PATHOGENESIS OF THALAMIC NEURONAL DYSFUNCTION IN DISTAL SYMMETRICAL DIABETIC POLYNEUROPATHY	105
5.1	Introduction	105
5.2	Methods	106

5.2.1	Patients	106
5.2.2	Assessment of Neuropathy	106
5.2.3	Magnetic Resonance Spectroscopy Protocol	107
5.3	Data Analysis	108
5.3.1	Statistical Method	108
5.4	Results	109
5.4.1	Subgroup Demographics	109
5.4.2	MR Spectroscopy Assessments	110
5.5	Discussion	112
6	CEREBROVASCULAR PERFUSION ABNORMALITIES IN DISTAL SYMMETRICAL DIABETIC POLYNEUROPATHY AND TYPE 1 DIABETES MELLITUS	115
6.1	Introduction	115
6.2	Methods	116
6.2.1	Patients	116
6.2.2	Assessment of Neuropathy	117
6.2.3	Magnetic Resonance Perfusion Protocol	118
6.3	Data analysis	119
6.3.1	Modelling of gadolinium concentration-time curves	120
6.3.2	Statistical analysis	130
6.4	Results	130
6.4.1	Comparisons between Healthy Volunteers and patients with diabetes: regional perfusion of major vascular territories	130
6.4.2	Comparison between Healthy Volunteers and diabetic patients divided into neuropathy subgroups: thalamic perfusion	132
6.5	Discussion	138

7	A RANDOMISED DOUBLE BLIND PLACEBO CONTROLLED TRIAL OF CANNABIS BASED MEDICINAL PRODUCT (SATIVEX) IN PAINFUL DPN: IS DEPRESSION A MAJOR CONFOUNDING FACTOR?	142
7.1	Introduction	142
7.2	Methods	143
7.2.1	Patients	143
7.2.2	Study Design	143
7.2.3	Assessment of Neuropathy	146
7.2.4	Concomitant Medication Use	146
7.2.5	Primary Outcome Measure	147
7.2.6	Secondary Outcome Measure	147
7.2.7	Study Medication	147
7.2.8	Data Analysis and Statistical Methods	148
7.3	Results	149
7.3.1	Baseline Characteristics	149
7.3.2	Withdrawals	152
7.3.3	Primary outcome measure	154
7.3.4	Secondary outcome measures	154
7.3.5	Neurophysiological assessments	157
7.3.6	Depression post hoc analysis	158
7.4	Discussion	160
8	FINAL DISCUSSION AND FURTHER WORK	164
	APPENDIX 1: METABOLIC AND VASCULAR CONSEQUENCES OF DIABETES ON THE CENTRAL NERVOUS SYSTEM.	168
	APPENDIX 2: STANDARD OPERATING PROCEDURES	183

9	RESEARCH OUTPUT	218
10	REFERENCES	220

I. INDEX OF FIGURES

Figure 2.1:	(a) Test tube phantom, (b) cross sectional images of test tube phantoms acquired following from magnetic resonance imaging, (c) magnetic resonance image of the cervical spine.	52
Figure 2.2:	Test tube phantoms.	52
Figure 2.3:	Magnetic resonance image of test tube phantoms with three different inner test tube cross sectional areas.	54
Figure 2.4:	Cross sectional magnetic resonance images of the test tube phantom demonstrating measurement of inner test tube cross sectional area using the manual outlining technique.	54
Figure 2.5:	Cross sectional magnetic resonance images of the test tube phantoms demonstrating measurement of inner test tube cross sectional area using the semi automated computer assisted technique.	56
Figure 2.6:	Differences in inner test tube cross-sectional area measurement caused by variation in patient positioning.	57
Figure 2.7:	Errors in inner test tube cross sectional area that can result from altering the slice angle.	58
Figure 2.8:	Variations in cord cross-sectional area in two patients over time.	60
Figure 2.9:	Linear Fit of Cross Sectional Area Measurements vs Reference Control.	66

Figure 2.10: Precision and Bland-Altman Plots of Cross Sectional Area measurements.	67
Figure 2.11: Measurement and sampling errors.	71
Figure 3.1: T_2^* -weighted magnetic resonance imaging of the cervical spine.	78
Figure 3.2: Group spinal cord area measurements.	86
Figure 4.1: Somatotopic representation of tactile receptors of the body surface in the thalamus.	92
Figure 4.2: Axial section of the brain with voxel positioned to encompass the ventroposterior thalamic subnucleus.	96
Figure 4.3: Example spectra obtained at long echo time using a point-resolved acquisition technique.	96
Figure 4.4: Example spectra obtained at short echo time using a stimulated-echo acquisition technique.	96
Figure 4.5: Mean N-Acetyl Aspartate/Choline and N-Acetyl Aspartate/Creatine ratios in Healthy Volunteers, diabetic patients with neuropathy and no neuropathy.	101
Figure 6.1: Cross sectional image of the cerebral cortex at the level of the thalamus.	119
Figure 6.2: White circles are regions of interests within the three major vascular territories.	120
Figure 6.3: Relative concentration-time profile of gadolinium passage through the thalamus in a healthy volunteer.	122
Figure 6.4: First pass relative concentration time profile of gadolinium through the thalamus in a healthy volunteer.	123

Figure 6.5: Relative concentration time profile of gadolinium DTPA tracer bolus as it passes through the left thalamus in a healthy volunteer and a patient with painless DPN.	128
Figure 6.6: Relative concentration time profile of the passage of exogenous contrast agent through the thalamus in each subgroup.	136
Figure 7.1: Treatment Phases and Study Visits.	145
Figure 7.2: Participant Flow Plan.	153
Figure 7.3: Mean weekly total pain scores of patients categorised according to Hospital Anxiety and Depression Scores.	159

II. INDEX OF TABLES

Table 1.1 : Treatments aimed at pathogenetic mechanisms of distal symmetrical neuropathy.	32
Table 1.2: Varieties of focal and multifocal neuropathies.	41
Table 2.1: Accuracy assessment of the semi-automated and manual outlining methods.	64
Table 2.2: Assessment of the semi-automated and manual outlining cross sectional area measurements.	65
Table 3.1: Subgroup Demographics.	79
Table 3.2: Subgroup Neurophysiological Assessments.	80
Table 3.3: Subgroup Neurophysiological Assessments and Cervical Cord Cross-sectional Area Measurement.	81
Table 3.4: Relation Between Actual Spinal Cord Area and Clinical and Neurophysiological Assessments Diabetic Patients.	84
Table 3.5: Relation Between Normalised Spinal Cord Area and Clinical and Neurophysiological Assessments Diabetic Patients.	85
Table 4.1: Subgroup Demographics.	98
Table 4.2: H-MRS subgroup metabolite ratios (acquired at long echo time) and metabolite areas relative to that of unsuppressed water (acquired at short echo time).	100
Table 5.1: Subgroup Demographics.	109

Table 5.2: H-MRS patient group metabolite areas relative to that of unsuppressed water.	111
Table 6.1: Demographics of Healthy Volunteers and Patients With Diabetes.	131
Table 6.2: Regional Cerebral Perfusion Characteristics of Healthy Volunteers and Patients with Diabetes.	132
Table 6.3: Demographics of Healthy Volunteers and Diabetic Neuropathy Subgroups.	133
Table 6.4: Regional Cerebral Perfusion Characteristics of Healthy Volunteers and Diabetic Patients Divided into Neuropathy Subgroups.	134
Table 6.5: Thalamic Perfusion Biomarkers of Healthy Volunteers and Patients with Diabetes.	137
Table 7.1: Baseline Characteristics of Study Participants.	150
Table 7.2: Baseline and Endpoint Neurophysiological Assessments.	151
Table 7.3: Concomitant Analgesic Medication.	152
Table 7.4: Primary Outcome Measures.	155
Table 7.5: Secondary Outcome Measures (Part 1).	156
Table 7.6: Secondary Outcome Measures (Part 2).	157

III. ABBREVIATIONS

95% CI	Ninety Five Percent Confidence Interval
ACA	Anterior Cerebral Artery
ADA	American Diabetic Association
AI	Area Inner
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AO	Area Outer
AP	Antero-posterior
BMI	Body Mass Index
CASE IV	Computer Assisted Sensory Evaluation IV
CBD	Cannabidiol
CBF	Cerebral Blood Flow
CBME	Cannabis Based Medicinal Product
Cho	Choline
Cho:Cr	Choline to Creatine Ratio
CN	Charcot Neuroarthropathy
CNS	Central Nervous System
Cr	Creatine
CSA	Cross Sectional Area
CSF	Cerebrospinal Fluid
CV	Coefficient of Variation
DCCT	Diabetes Control and Complications Trial
DPN	Distal Symmetrical Diabetic Sensorymotor Polyneuropathy
DUK	Diabetes UK

EQ-5D	EuroQOL Questionnaire
ETL	
FMRI	Functional Magnetic Resonance Imaging
GI_x	Glutamate/glutamine
HADS	Hospital Anxiety and Depression Questionnaire
HbA1c	Glycosylated Haemoglobin
H-MRS	Proton Magnetic Resonance Spectroscopy
HRDB	Heart Rate Deep Breathing
ITT	Intention To Treat
MCA	Middle Cerebral Artery
McGill	McGill Pain and Quality of Life Questionnaire
MI	Myoinositol
MR	Magnetic Resonance
Na:Cho	N-acetyl Aspartate to Choline Ratio
Na:Cr	N-acetyl Aspartate to Creatine Ratio
NAA	N-acetyl Aspartate
NAA	N-acetyl Aspartate
NCS	Neuropathy Composite Score
NIS	Neuropathy Impairment Score
NIS(LL)	Neuropathy Impairment Score of the Lower Limbs
NIS(LL) +7	Neuropathy Impairment Score of the Lower Limbs Plus Seven Tests
No DPN	Diabetic Patients with No Neuropathy
NPS	Neuropathic Pain Scale
NSS	Neuropathy Symptom Score
NTSS-6	Neuropathy Total Symptom Score 6
Painful DPN	Diabetic Patients with Painful Neuropathy
Painless DPN	Diabetic Patients with Painless Neuropathy

PCA	Posterior Cerebral Artery
PET	Positron Emission Tomography
PNS	Peripheral Nervous System
PPI	Present Pain Intensity
PPM	Parts Per Million
PRESS	Point Resolved
QC	Quality Control
rCBF	Relative Cerebral Blood Flow
rCBV	Relative Cerebral Blood Volume
ROI	Region of Interest
SD	Standard Deviation
SEM	Standard Error of Mean
SEP	Somatosensory Evoked Potentials
SF-36	Short Form 36 Health Survey Questionnaire
SI	Signal Intensity
SPECT	Single Proton Emission Computer Tomography
SPSS	Statistical Package for the Social Sciences
STEAM	Stimulated Echo Acquisition Mode
Subclinical DPN	Diabetic Patients with Early Subclinical Neuropathy
TE	Echo Time
TENs	Transcutaneous Electric Nerve Stimulator
THC	Tetrahydrocannabinol
t_{lag}	Bolus Arrival Time
TR	Resonance Time
TT_{FM}	First Moment Transit Time
TTP	Test Tube Phantoms
UK	United Kingdom
USA	United States of America

VAS	Visual Analogue Scale
VPI	Ventro Posterior Lateral
VPm	Ventro Posterior Medial

IV. UNITS

°C	Degree Celsius
G	Gram
Hz	Hertz
Kg	Kilogram
Kg/m²	Kilogram per Metre square
M	Metre
m/s	Metre per second
Mcl	Mill centiliter
Mg	Milligram
mg/d	Milligram per day
Min	Minutes
Mm²	Millimeter square
Ms	Millisecond
μV	Microvolt
mV	Millivolt
T	Tesla

1 INTRODUCTION

1.1 Diabetes Mellitus and Nervous System Complications

The worldwide prevalence of diabetes is increasing at an alarming rate. The number of cases worldwide in 2000 among adults over 20 years of age was estimated to be about 171 million (Wild, Roglic et al. 2004). With increasing population growth, aging, urbanisation and increased prevalence of obesity and physical inactivity, conservative estimates predict that the number of people with diabetes will more than double. The human and economic costs of this growing public health burden are enormous (Ward 1995). This increasing prevalence will inevitably result in increased mortality and morbidity from associated complications of diabetes. Microvascular complications of neuropathy, retinopathy and nephropathy occur in patients with type 1 and 2 diabetes.

1.1.1 Diabetic Neuropathy

Diabetes mellitus is one of the commonest diseases to affect the nervous system. Distal symmetrical diabetic sensorymotor polyneuropathy (DPN) is the main initiating factor for foot ulceration and is the commonest cause of non-traumatic lower limb amputation in the western world (Hollingshead 1991). It can severely affect an individual's social functioning and occupational productivity.

1.1.2 Epidemiology

Epidemiological reports on the prevalence of DPN have been confounded by the lack of a consensus over diagnostic criteria, the wide variety of measurement techniques used and the patient selection for study (Shaw and Zimmet 2000). As a result the prevalence of DPN has been shown in clinical and population based studies to vary from 8% to 63% depending on the criteria used to diagnose neuropathy (Shaw, Hodge et al. 1998) (Dyck, Kratz et al. 1993). The EURODIAB complications study showed the prevalence in 16 European countries to be 28% (Tesfaye, Stevens et al. 1996). In this study, DPN was diagnosed if

there were more than two abnormalities amongst symptoms, signs, vibration perception threshold and autonomic function tests. This prevalence was related to age, duration of diabetes and the quality of metabolic control. The presence of severe neuropathy is often accompanied by the other microvascular diabetic complications of retinopathy and nephropathy (Pirart, Lauvaux et al. 1978). It has also been shown more recently that traditional cardiovascular risk factors of smoking, hyperlipidaemia and hypertension are also independent risk factors for the development of DPN (Tesfaye, Chaturvedi et al. 2005). Patients with type 1 and 2 diabetes of similar disease duration have equal prevalence of DPN (Shaw, Hodge et al. 1998).

As mentioned above DPN plays a central role in the pathophysiological pathway leading to foot ulceration and amputation. In fact the presence of DPN increases the risk of amputation 1.7 fold, 12 fold if there is deformity and 36 fold if there is a history of previous foot ulceration (Armstrong, Lavery et al. 1998).

1.1.3 Pathogenesis of DPN

As early as 1890, Charcot opined that vascular factors could be of significance in the development of neurological complications in diabetes. Woltman and Wilder in 1929 were the first to describe marked thickening of the intraneural blood vessels in three autopsy cases and seven amputated lower limbs from patients with diabetes (Woltman H.W 1929). But it was not until 1959 when Fagerberg first observed the microvascular changes in an autopsy study of cases exhibiting clinical signs of neuropathy (Fagerberg 1959). He demonstrated thickening and occlusion of intraneural vessel walls by materials staining positive with Periodic Acid Schiff. Other morphological changes such as closed capillaries, microvascular thrombosis, basement membrane thickening, endothelial cell reduplication and multifocal loss of myelinated fibres have also been described in DPN (Malik, Tesfaye et al. 1994; Theriault, Dort et al. 1997). Tesfaye et al. demonstrated impairment of blood flow and the presence of epineural arterio-venous shunts in human DPN using sural nerve

photography and fluorescein angiography (Tesfaye, Harris et al. 1993). Newrick et al have shown that sural nerve oxygen tension measured by microelectrode polarography, is reduced in diabetic patients with neuropathy (Newrick, Wilson et al. 1986). Similarly reduced microvascular oxygen saturation and raised fluorescein rise time, which are suggestive of ischaemia, have been shown in the sural nerve by Ibrahim et al, in patients with DPN (Ibrahim, Harris et al. 1999). Morphometric studies on human sural nerve have demonstrated increased perineurial capillary endothelial cell hyperplasia and reduced luminal area. A significant relationship was observed between endothelial cell hyperplasia and measures of neuropathic severity, providing further insight into the pathogenesis of DPN (Malik, Tesfaye et al. 1994).

The Diabetes Control and Complications Trial (DCCT), a large scale prospective study, showed a causal relationship between hyperglycaemia and the microvascular complications of diabetes mellitus (DCCT research group 1993). However, other factors are likely to contribute to an individual's susceptibility to hyperglycaemia and these may be governed by genetic factors such as gene polymorphisms.

It is clear that a complex cascade of vascular and metabolic factors are involved eventually resulting in end-organ damage. The ultimate challenge is to identify the crucial initiating or perpetuating factors; particularly those that may have the potential for manipulation and therapeutic gain.

1.1.4 Classification

Peripheral nerve damage in diabetes mellitus can be broadly separated into its effects on the somatic and autonomic nervous systems, although the two frequently co-exist. Clinical classification of the various syndromes of diabetic somatic neuropathy has proved very difficult. The variation and overlap in aetiology, clinical features, natural history and prognosis have meant that most classifications are necessarily oversimplified and none has proved capable of accounting for all of these factors. In 1998, during the American Diabetes

Association (ADA) Scientific Congress in San Antonio (Texas, USA), a working group consisting of representatives from both the ADA and the American Academy of Neurology was convened to classify DPN. Based on this consensus, there are four main groups of neurological disturbance in diabetes (ADA 1988).

These were:

1. Subclinical neuropathy

This group consists of asymptomatic individuals with abnormalities on formal testing of their nervous system with quantitative sensory testing and nerve conduction studies.

2. Distal symmetrical sensorymotor polyneuropathy (DPN)

This is the most common form of diabetic somatic neuropathy. It is characterised by a length dependent, distal distribution involvement of sensory and motor nerves (otherwise known as distal symmetrical polyneuropathy) and autonomic involvement, which together form part of this clinical complex.

3. Focal and multifocal neuropathies

This sub-group of neuropathies often occur in the context of DPN. Both vascular and compressive aetiologies can be involved. They can present either with a rapid, self-limiting onset or a slowly progressive persistent (without intervention) nature

4. Hyperglycaemic neuropathy

Pain, paraesthesia and hyperalgesia predominantly in the feet associated with poor glycaemic control occur in this subset of diabetic somatic neuropathy. Symptoms and signs are rapidly reversed with improvement of glycaemic control.

1.1.5 Sub-clinical neuropathy

As mentioned above, patients in this category are frequently asymptomatic with the presence of neuropathy diagnosed based on quantitative sensory testing and nerve conduction studies. Nerve conduction velocities of the most distal sensory nerves (plantar and sural), typically provide the first evidence of neuropathy. As the disease progresses, nerve conduction studies show loss of distal nerve sensory and motor amplitudes. This is a reflection of axonal loss, which is the hallmark of this form of DPN.

Abnormal quantitative sensory testing for vibration, cooling and thermal perception thresholds assessed using specialised equipment (CASE IV, Minnesota, USA) is also seen. Quantitative autonomic function tests also show abnormalities with heart rate variation with deep breathing, valsalva manoeuvre and postural testing. The early identification of the neuropathic process offers the patient an opportunity to alter its course by correcting or at least improving the risk factors ultimately reducing the morbidity associated with neuropathy (Boulton 1998).

1.1.6 Distal symmetrical sensorimotor polyneuropathy (DPN)

This is the most common and widely recognised form of diabetic somatic neuropathy. There is often long duration of diabetes, which precedes its diagnosis. If present at the diagnosis of type 2 diabetes, it suggests a long period of pre-existing abnormal glucose metabolism, usually of many years duration. Often the onset is insidious and it is not uncommon for DPN or even diabetes mellitus itself, to present initially as foot ulceration (Ward 1982). As foot ulcerations are wholly preventable this represents a failure somewhere along the pathway of care. This length-dependent, "glove and stocking," neurological deficit affects both sensory and motor nerves. Sensory loss predominate the early stages, starting at the toes and gradually progressing into the feet and legs. The fingers and hands become involved when the neuropathy has extended above the mid calf. In the worst cases the deficit may extend into the buttocks and trunk, although spreading beyond this is exceptionally rare. Some

patients, however, have “positive neuropathic symptoms” which can range from mild paraesthesia and numbness to more severe burning, allodynia and lancinating pains. These symptoms are often but not always symmetrical in distribution and nocturnal exacerbation is a common feature.

Motor nerve involvement is usually sub-clinical although it is easily detectable on neurophysiological testing (Andersen 1999). Non-invasive imaging techniques have revealed atrophy of the musculature of the foot and this may be visible clinically in severe cases (Andreassen, Jakobsen et al. 2009). However, early and predominantly motor involvement should raise the possibility of alternative diagnoses.

1.1.6.1 Clinical signs

On inspection of the feet, deformities or calluses should be noted. The presence of any one of these in the context of DPN highlights regions in the feet that are patient to abnormal pressure loading and prone to developing foot ulceration. One should inspect for fissures and ulcers in obvious pressure bearing regions (e.g. heels and toes). Frequently there is wasting of the small muscles of the feet resulting in clawing of the toes. A shiny, dry appearance of the foot (decreased sweating) with prominent veins (arterio-venous shunting) suggests accompanying autonomic neuropathy. While a dusky appearance with venous guttering and atrophic skin and nails is the result of peripheral vascular disease.

The methods available for the detection and quantification of DPN are numerous and varied. The clinical setting usually governs the technique used to assess DPN (Eaton and Tesfaye 2000). In a busy annual review diabetic clinic, where the main concern is identifying individuals at risk for foot ulceration, a careful clinical examination, inspection of footwear and simple clinical instruments such as the 10g monofilament and a pinprick will suffice (Boulton 1998). For epidemiological studies, techniques such as vibration perception threshold (VPT) are usually a minimum requirement. On the other hand, when greater diagnostic accuracy is required (e.g. randomised control trials) expensive, computer-assisted

quantitative sensory testing and laboratory based techniques (e.g. nerve conduction studies) are employed.

Testing of all sensory modalities will reveal abnormalities in a “glove and stocking” distribution. Individual modalities tested will provide clues to the type of nerve fibres affected. For example impairment of vibration perception (128Hz tuning fork) and joint position sense reflect large fibre neuropathy. Whereas, defective warm and cold thermal discrimination is in keeping with small nerve fibre involvement. The use of ankle reflexes and vibration testing has been advocated in guidelines for screening for DPN (ADA 2001). There is, however, greater evidence in the literature for the use of the Semmes-Weinstein 5.07 (10g) monofilament (Perkins, Olaleye et al. 2001). It is a cheap, simple, rapid, reproducible screening tool for DPN, which can be used in a busy diabetic clinic (Perkins, Olaleye et al. 2001). An abnormal monofilament test is associated with a three year relative risk as high as 15 for the development of ulceration or amputation. The 10g monofilament is applied to a non-callused site on the dorsum of the first toe just proximal to the nail bed. This is repeated four times on both feet in an arrhythmic manner. Five or greater insensate stimuli is associated with a very high probability of DPN (Perkins, Ngo et al. 2002).

If there is upper limb sensory loss in a glove distribution then the level of impairment in the legs has to have reached mid thigh. If not, look for another explanation for the upper limb sensory loss. Motor weakness is usually mild and confined to the feet. As the disease progresses severe distal weakness limits a patient’s ability to stand/walk on the heels or toes.

1.1.6.2 Complications and Sequelae

- Foot ulceration

The most important consequence of DPN is the development of foot ulceration which affects 15% of diabetic patients in their lifetime (Mayfield, Reiber et al. 1998). Foot ulcers are the

commonest reason for admission to hospital in the diabetic patient and precede 80% of non-traumatic lower limb amputations, exerting a massive economic burden on health services (Ramsey, Newton et al. 1999). In addition and perhaps more importantly, this condition has devastating consequence on a social and personal level to the patient (Gries 2003). High levels of depression and social isolation are associated with chronic foot ulceration and lower limb amputation (Carrington, Mawdsley et al. 1996), (Price and Harding 2000). Furthermore the economic consequences of time off work or early retirement are significant, particularly in younger patients.

Even in the presence of DPN, foot ulceration is wholly preventable. Simple, but effective interventions, such as education, podiatry, footwear and orthoses are capable of reducing ulceration rates by up to 80% (Boulton 1995).

- Charcot Neuroarthropathy

Charcot neuroarthropathy (CN) is a chronic and progressive disease of the bone and joints, characterised by painful bone and joint destruction in limbs that have lost sensory innervation (Edmonds 1999). Affected joints exhibit synovitis, instability, subluxation and destruction. Trauma is thought to be an important initiating factor which is often not recalled. (Rajbhandari, Jenkins et al. 2002). The typical acute presentation is often undiagnosed due to a lack of recognition and the often asymptomatic nature of the condition. As a result the true prevalence of CN in patients with diabetes mellitus is not known. The main cause of CN in the developed world is now DPN with the joints of the foot being most commonly affected (Frykberg and Kozak 1978).

A complete understanding of the pathogenesis of CN remains elusive although a variety of simultaneous occurring factors are likely to be involved (Rajbhandari, Jenkins et al. 2002). Diabetic peripheral neuropathy is present in all cases and may contribute by permitting minor repetitive traumatic events, which trigger a reparative response within the bone. In the presence of autonomic neuropathy, blood flow increases dramatically and

causes over compensated osteoclastic activity and bone resorption. This in turn may exacerbate the trauma site, cause fracture and subluxation, and ultimately leads to the gross deformity characteristically seen. The presence of foot deformity leads to an increase in the risk of foot ulceration, which can be very difficult to prevent and treat. Hence, the main aim of treatment should be the early identification and treatment in the acute phase. With the advent of non-invasive techniques, such as isotope bone scans and magnetic resonance (MR) imaging, and the use of non-weight bearing treatments, and bisphosphonates, this may become a more realistic aim (Rajbhandari, Jenkins et al. 2002).

- Mortality and premature death

There is an increase in mortality associated with DPN, mainly from cardiovascular causes, especially in the presence of cardiac autonomic neuropathy (Boyko, Ahroni et al. 1996; Forsblom, Sane et al. 1998; Faglia, Favales et al. 2001). As mentioned previously, traditional atherosclerotic risk factors play an important role in the pathogenesis of DPN. Hence, it is likely that a combination of the nerve damage itself and associated atherosclerotic co-morbidities explain this increase in mortality and premature death. Thus, the management of DPN should not only focus on cardiovascular risk factors but also strategies for its prevention.

1.1.6.3 Management of DPN

The mainstay of treatment for neuropathy currently is prevention, mainly by delaying its onset. Once DPN is established, it is usually irreversible and slowly progressive. Aims of treatment should then be directed at reducing symptoms and slowing the progression of neuropathy.

- Correction of the risk factors for neuropathy

There is now overwhelming evidence that strict glycaemic control from the time of diagnosis of diabetes reduces the prevalence rates of DPN. In type 1 diabetes, the DCCT

showed that intensive insulin treatment reduced the prevalence rates for DPN by as much as 50% in both the primary and secondary prevention cohorts (DCCT research group 1995). In type 2 diabetes, strict control of blood glucose was associated with an improvement in vibration perception threshold (UK Prospective Diabetes Study group 1998; 2002). This benefit was however, far less than that observed in the DCCT (DCCT research group 2002) . A possible explanation for this is proposed by the outcome of the STENO 2 study. In this study, patients with type 2 diabetes were divided into two treatment cohorts (Gaede, Lund-Andersen et al. 2008). One cohort received conventional treatment and the other intensive treatment to tackle multiple other risk factors for DPN. This included antihypertensive drugs e.g. angiotensin converting enzyme inhibitors and calcium channel antagonist, hypoglycaemic agents, aspirin, lipid lowering agents and anti-oxidants. There was a 0.32 reduction in the odds ratio for developing autonomic neuropathy as a result. Notably, this study did not show a reduction in the risk of developing DPN.

- Treatments aimed at pathogenetic mechanisms of DPN

Numerous treatments directed at the pathogenetic mechanisms of DPN have been through rigours clinical trials. Despite early promising results many of these compounds have failed to make sufficient impact and hence are not widely prescribed. There are however, a few treatments that have been granted licenses for use in certain countries (e.g. α -lipoic acid in Germany and the aldose reductase inhibitor (epalrestat in Japan) and this provides us with more experience of their use. A summary of these compounds can be found in the Table 1.1 below.

Table 1.1 : Treatments aimed at pathogenetic mechanisms of distal symmetrical neuropathy.

Aldose reductase inhibitors

Method of action

Reduces glucose flux through the polyol pathway, Lowers accumulation of toxic sorbitol, Prevents reduction of redox potentials

Clinical Trials

19 trials, testing four different aldose reductase inhibitors between 4 – 208 weeks duration (median 24 weeks) were patiented to a meta-analysis (Airey, Bennett et al. 2000). The reviewers concluded that although aldose reductase inhibitors treatment diminished the reduction in motor nerve conduction velocity, the clinical relevance of such a change in this outcome measure is uncertain. There was no effect in terms of this outcome measure in small sensory nerve fibres, degeneration of which is primarily responsible for DPN.

Aminoguanidine

Method of action

Inhibitor of the formation of advanced glycation end products

Clinical Trials

Administration in diabetic animals prevents the development of microvascular complications (Miyauchi, Shikama et al. 1996).

Human trials discontinued because of toxicity.

α -lipoic acid

Method of action

Alpha-lipoic acid is an endogenous, sulphur-containing, free radical scavenger found in mitochondria. Free radicals can reduce nitric-oxide-mediated vasodilation and damage to the vascular endothelium (Cameron and Cotter 1997). Alpha-lipoic acid also recycles other antioxidants, such as vitamins E and C.

Clinical Trials

Treatment with α -lipoic acid improves nerve blood flow and distal nerve conduction and increases endoneurial glucose uptake and energy metabolism in animals.

A systemic review of 15 randomised clinical trials concluded that α -lipoic should be considered as a treatment option for diabetic patients with mild to moderate neuropathy. It has a limited side effect profile and patients should be warned of possible side effects which include headache, skin rash, stomach upset at high doses (>600 mg/d) and possible hypoglycaemia (Halat and Dennehy 2003).

Nerve Growth Factor

Method of action

Have trophic effects on sensory neurons in the dorsal root ganglion and sympathetic postganglionic neurons. Nerve injury impairs release.

Clinical Trials

Two large worldwide multicentre studies showed that recombinant human nerve growth factor had no beneficial effects over placebo (Pittenger and Vinik 2003).

1.1.7 Diabetic Autonomic Neuropathy

Although clinically significant autonomic neuropathy is uncommon, formal autonomic function testing will be abnormal in 90% of patients with DPN (Vinik 1999). When present the variety of symptoms reflects the diversity of organ systems involved. Mortality associated with autonomic neuropathy is placed at between 20-50% within five to ten years (Rathmann, Ziegler et al. 1993). The major manifestations are cardiovascular, gastrointestinal and genitourinary dysfunction. Frequent complaints include symptomatic postural hypotension, gustatory sweating, nocturnal diarrhoea, incontinence, heat intolerance, erectile dysfunction and poor hypoglycaemic awareness. Methods of testing the autonomic nervous system range from simple blood pressure estimation with postural change to monitoring R-R variation (using ECG monitoring software) with deep breathing, valsalva manoeuvre and postural changes. There is however a wide variability with repeat testing, which can be minimised by standardizing the methodology and using a central testing facility (Low 1993). In addition it is not clear whether these are useful indicators of autonomic problems elsewhere.

1.1.8 Painful Diabetic Neuropathies

In some patients pain is an unwanted component of diabetic neuropathy. It can accompany DPN or other comparatively less common varieties of acute or chronic diabetic neurologic syndromes. Pain is also a feature of certain focal and multifocal neuropathies, which will be discussed later in this chapter. The underlying pathogenesis of painful neuropathy remains poorly understood and as a result there are currently not effective, rational treatments (Thomas 1999).

1.1.8.1 Chronic Painful Diabetic Neuropathy

The prevalence of chronic painful DPN is not clear. In one study 11% of insulin treated patients under 60 years of age had painful symptoms (Boulton, Knight et al. 1985). Another

study based in a hospital diabetic clinic population put the prevalence at 25% compared with 15% in a control population (Chan, Mac Farlane et al. 1990). Painful symptoms can be variable but are typical of neuropathic pain. There is a slow but progressive build up of neuropathic symptoms starting in the feet marching up the leg to involve the thighs in severe cases. Progress occurs over many years and eventually in some cases the upper limbs are involved. Typical features of neuropathic pain are paraesthesia (tingling, pins and needles), burning, lancinating (shooting), deep aching, allodynia (pain sensation when affected limbs are touched by normally non painful stimuli e.g. bed sheets or clothes) and hyperaesthesia. Often patients describe a continuous background dull aching or cramping sensation affecting the legs and feet with periodic sharp shooting exacerbations, which are superficial. Pain accompanies walking which is likened to walking on stones or hot coals. Nocturnal exacerbations are commonly experienced but are not unique to DPN. Sleep quality and pattern are also frequently affected. Not surprisingly quality of life and social functioning is impaired, often leading to depression and unfortunately suicide. Requests for amputations of the affect limbs are not uncommon from sufferers. Paradoxically, sensory loss can be complete, such that the individual is unable to feel pain of pressure, trauma or temperature, whilst continuing to experience constant neuropathic pain.

Chronic painful symptoms may last for many years. Eventually a large proportion of patients experience a reduction in symptoms, which in one study occurred after a mean follow up of 3.6 years (Mayne 1968; Bischoff 1980; Boulton, Armstrong et al. 1983; Benbow, Chan et al. 1994). This was associated with deterioration in peripheral nerve function (Benbow, Chan et al. 1994). The reason why some get it and others do not is still unclear (Benbow, Cossins et al. 1999).

The aetiology of painful neuropathy is likely multifactorial with vascular, autoimmune and biochemical mechanisms playing important roles (Thomas 2003). Limited work has explored metabolic or molecular mechanisms of painful diabetic neuropathy. However, Llewellyn et al (Llewellyn, Gilbey et al. 1991) and Britland et al (Britland, Young et al. 1992)

were unable to detect any correlation between painful neuropathy and morphologic indicators of regeneration in sural nerve biopsies.

- Management

The treatment options we have available for this condition are often unsatisfactory, as they are poorly tolerated and frequently ineffective (Tesfaye 1997; Benbow, Cossins et al. 1999; Thomas 1999). The first step in managing painful DPN should be optimising glycaemic control, with insulin in type 2 diabetes if necessary (Boulton, Drury et al. 1982).

Tricyclic antidepressants

These compounds are the first line treatment option for neuropathic pain. Numerous crossover and parallel placebo control trials have shown the effectiveness of these compounds in reducing both background pain (dull ache) and shooting pains (Davis, Lewis et al. 1977; Kvinesdal, Molin et al. 1984; Gomez-Perez, Rull et al. 1985; Mendel, Klein et al. 1986; Sindrup, Ejlertsen et al. 1989; Max, Lynch et al. 1992). Amitriptyline and imipramine are the common drugs of choice from this class. They are usually prescribed at low doses taken at bedtime, which is gradually titrated if necessary to overcome painful symptoms at the same time keeping a watchful eye out for their side effects. In fact optimum use of these drugs are limited by their troublesome side effects. These include drowsiness, dizziness, dry mouth and urinary retention. Particular caution is advised when prescribing these drugs to elderly patients. If the side effects limit the use of amitriptyline, imipramine could be tried and vice versa.

Anticonvulsants

These compounds have a role as second line agents in treating painful DPN. The anticonvulsants carbamazepine (Rull, Quibrera et al. 1969; Wilton 1974; Badran, Aly et al. 1975), phenytoin (Ellenberg 1968; Saudek, Werns et al. 1977), clonazepam, gabapentin (Backonja, Beydoun et al. 1998; Gorson, Schott et al. 1999; Hemstreet and Lapointe 2001)

and more recently pregabalin (Rosenstock, Tuchman et al. 2004) have been evaluated in various diabetic painful neuropathy trials. Uses of these compounds are also limited by their side effect profiles which include drowsiness, ataxia and dizziness. Treatment should be started at a low dose and gradually increases to an appropriate maintenance dose for these drugs.

Capsaicin

Derived from capsicum peppers, this topically applied compound works by depleting substance P from nociceptors at the end of unmyelinated C fibres (Buck and Burks 1986). Nociceptive nerve transmissions is blocked as a result. It is applied sparingly 3-4 times a day to affected areas with a warning that neuropathic symptoms could worsen in the first 2-4 weeks before relief occurs (Halat and Dennehy 2003).

Local anaesthetic agents

Both intravenous lignocaine (Boas, Covino et al. 1982; Kastrup, Petersen et al. 1987) and oral mexiletine (Dejgard, Petersen et al. 1988; Oskarsson, Ljunggren et al. 1997) (a lignocaine analogue) are used to treat patients with severe pain not responding to the agents above. Intravenous lignocaine has to be administered slowly over 30 minutes with continuous cardiac monitoring. If successful, treatment can be continued with oral mexiletine. A review of clinical trials evaluating these agents is beyond the scope of this chapter and can be found in the following reference (Ziegler 2008).

Pain clinics

The management of chronic pain is full of challenges which specialised pain clinics are well adapted to address. The availability of different therapeutic options and accompanying expertise available in these clinics, help patients cope better with their pain through their holistic approach. Among the therapeutic options available include psychological counselling, alternative therapies (e.g. acupuncture), spinal blocks or even spinal cord

stimulation. In a limited number of patients with severe painful neuropathy, unresponsive to other treatments, electrical spinal cord stimulation has been used with some benefit (Tesfaye, Watt et al. 1996).

1.1.8.2 Acute painful neuropathy

Acute painful neuropathy is an uncommon painful syndrome associated with diabetes that should be distinguished from chronic painful neuropathy described above. It is characterised by a rapid onset of severe pain in a glove and stocking distribution similar to that of chronic painful neuropathy. Despite the intensity of accompanying symptoms, there is often little objective abnormality found on neurophysiological assessments. Clinical tests of sensory function are often normal and nerve conduction velocities are usually only mildly reduced. The most consistent abnormality is loss of small fibre function with reduced temperature discrimination on quantitative sensory testing. Fortunately patients can be reassured that their symptoms will resolve within ten months. However this can still be an utterly disabling condition for some patients. This form of neuropathy can be categorised into two different clinical syndromes, differentiated on whether they present in the context of poor glycaemic control or periods of rapid improvement of glycaemic control. In the former scenario, there is accompanying precipitous weight loss and was initially described by Ellenberg as "neuropathic cachexia" (Ellenberg 1974). A further description of the natural history of this syndrome illustrated that pain improved as tight glycaemic control was initiated and weight started to increase (Archer, Watkins et al. 1983). Paradoxically, acute painful neuropathy has also been described following rapid improvements in glycaemic control. This was often in the context of insulin use and was originally called "insulin neuritis". This term is misleading, however, as acute painful neuropathy has also been reported after improvements in control associated with oral hypoglycaemic agents (Ellenberg 1958).

1.1.8.3 Proximal Motor Neuropathy

Initially described by Bruns (Bruns 1890) and then by Garland (Garland 1955), this syndrome has been reported under many names including diabetic amyotrophy, femoral neuropathy and proximal diabetic neuropathy. This entity of diabetic neuropathy tends to affect type 2 diabetic patients more frequently than type 1 diabetes (Sander and Chokroverty 1996). Males are also more frequently affected compared to females. Pain, typically burning and aching in nature, affects anterior thighs, lower back and buttocks with nocturnal exacerbations. Symptoms usually begin unilaterally and then progress to become bilateral. After a period of a few days weakness of the affected muscle groups develops and progresses slowly over several weeks. This weakness manifests as difficulty climbing stairs or rising from a low chair and examination reveals wasting of the quadriceps muscle. Occasionally other muscle groups may be involved, including some not supplied by the femoral nerve. There may be associated weight loss of between 10-20 kg over the course of this disease. Prognosis is reasonable, with recovery commencing with stabilisation of body weight and optimising glycaemic control. Muscle strength improves slowly over many months, but a number of patients never regain normal lower limb strength (Sander and Chokroverty 1996). Agents used to treat painful DPN can be used for symptom control in diabetic amyotrophy.

The cause of proximal motor neuropathy is unknown although clinical and neurophysiological evidence would suggest femoral nerve dysfunction (Chopra and Hurwitz 1968; Lamontagne and Buchthal 1970; Subramony and Wilbourn 1982). Histological assessment of a cutaneous branch of the femoral nerve has revealed changes of microvasculitis (Said, Goulon-Goeau et al. 1994).

1.1.8.4 Thoracolumbar Radiculopathy

This is another rare manifestation of an acute, painful neuropathic syndrome. A dermatomal pattern of pain and sensory loss is experienced, often with muscle weakness causing bulging of the abdominal wall (Ellenberg 1978; Boulton, Angus et al. 1984; Chaudhuri, Wren et al. 1997). It may again be associated with weight loss and recovery is usually complete within 3 – 12 months.

1.1.8.5 Focal and Multifocal Neuropathies

Focal and multifocal neuropathies are part of the clinical spectrum of diabetic neuropathy (Malik 2002). These tend to occur among older individuals and DPN is invariably present. A list of this subset of neuropathy can be found in Table 1.2.

1.1.8.6 Cranial Nerve Palsies

With an underlying vascular aetiology, cranial nerve palsies often have a rapid onset and a good prognosis for recovery. Sixth nerve palsy is the most common variety. Full recovery occurs in the vast majority over 3-5 months. Third nerve palsy not as common, are occasionally associated with retro orbital pain. Traditionally the pupil is spared as the parasympathetic fibres supplying the pupil run on the outer layers of the fascicle and ischaemia tends to affect its centre. Nonetheless, a magnetic resonance scan is indicated to exclude compression from a posterior communicating artery aneurysm is recommended. While waiting for resolution, troublesome diplopia can be corrected with prisms. Resolution is expected to take 3-6 months.

1.1.8.1 Compression Neuropathies

Diabetic nerves are more susceptible to damage from compression. The treatment of entrapment neuropathies should follow the same management guidelines as in a non-diabetic individual. Surgical decompression is indicated when pain is intractable and

especially when there is associated muscle weakness. Diagnosis can be made using nerve conduction studies, where characteristically there is delay in conduction velocities over the region of compressed nerve. The results of decompressive surgery are not as good as in non-diabetic patients (Dawson 1993). Non-surgical treatments include symptomatic relief with non-steroidal anti-inflammatory agents and immobilisation using splints.

Table 1.2: Varieties of focal and multifocal neuropathies.

Cranial nerve palsies

III nerve palsy

VI nerve palsy

Compression neuropathies

Median nerve

Ulnar nerve

Peroneal nerve

Lateral cutaneous nerves of the thigh

Diabetic amyotrophy

Diabetic truncal radiculoneuropathy

1.2 Central Nervous System Involvement in Diabetes Mellitus

Most research on diabetic complications has focused on the kidney, retina, PNS, small and larger blood vessels. The CNS, which is connected structurally and functionally to the PNS, has been largely ignored. In recent years, with significant improvements in neuroimaging methods, it has become evident that diabetes causes significant CNS complications. These result in important cerebrovascular and functional impairments (Mijnhout, Scheltens et al. 2006). The CNS is affected by both the metabolic and vascular consequences of diabetes.

1.2.1 Central Nervous System Involvement in Diabetic Neuropathy

1.2.1.1 Spinal Cord

Largely considered a disease of the PNS, there is increasing evidence to support concomitant involvement of the CNS in DPN. In fact, patho-anatomical studies dating back to the 1900's were the first to report the distribution and character of lesions in the CNS in diabetes. Involvement of the spinal cord reported in these postmortem studies, demonstrated axonal loss, gliosis, and demyelination within the spinal cord (Leichtentritt 1893; Pryce 1893; Williamson 1904; Reske-Nielsen and Lundbaek 1968; Reske-Nielsen, Lundbaek et al. 1970). Olsson et al. demonstrated in an autopsy study of nine patients with diabetes that the predominant spinal cord lesion was degeneration of long tracts (Olsson, Save-Soderbergh et al. 1968). When compared with age matched non-diabetic patients, there was significant demyelination of the dorsal, ventral and spinocerebellar long tracts in diabetic patients. In contrast, there were only slight changes in cord grey matter and minimal degeneration of the ventral motor neurons. Degeneration of the dorsal columns was also frequently reported in autopsy studies from this era (Woltman H.W 1929). Dolman et al demonstrated that the maximum brunt is borne by the lower segments of the cord, where the entire posterior column showed significant degeneration. In the upper segments, the lesions involved only the medial portions, indicating an ascending degeneration from the lower regions (Dolman 1963). Within degenerated areas myelin sheaths were few in number and

many of the remaining ones were thin. The posterior roots of cords from diabetic patients were atrophic, corresponding with that in the posterior columns. The dorsal root ganglia and anterior roots were, by contrast, well preserved. Another consistent finding from these early autopsy studies was changes to the blood vessels within the CNS. Sclerotic changes in the arteries and intraneural arterioles of the brain and spinal cord were frequently reported (Fagerberg 1959; Dolman 1963).

The early spinal cord autopsy data, originating mainly from the 1920-30's (Woltman H.W 1929)(ref) are difficult to interpret for a number of reasons: Firstly, the knowledge of neuroanatomy and in particular, functional neuroanatomy was limited. Secondly, most patients in the pre-insulin or early insulin use era suffered catastrophic systemic disease which relates poorly with the vast majority of patients we see now; furthermore, the diagnostic aids, and thus the ability to exclude other disease processes such as neurosyphilis, were poor. Thirdly, subsequent modern advances in the management of diabetes which have resulted in better glycaemic, blood pressure and lipid control have resulted in alterations in the current clinicopathological presentation of DPN; and, finally, the classifications and syndromes described bear little relation to those we presently use. Indeed, they predate Joslin and Root's original characterisation of DPN (Joslin E.P 1939'). Despite these limitations, variable pathological changes were observed within the spinal cord. What is fascinating, certainly in the context of this thesis, is that the changes within the peripheral nerve were always much more consistent, which in 1929 prompted Woltman and Wilder to state that 'the degenerations noted in the spinal cord are unimportant' (Woltman H.W 1929).

The 1980's saw a proliferation of studies conducted to evaluate CNS function in diabetes using somatosensory evoked potentials (SEP). SEP provides an indirect measure of spinal cord as well as supraspinal (central) conduction velocity. A peripheral nerve is stimulated, usually with an electrical current, and the latency measured at various levels including the spinal cord, brainstem and cortex. Studies using SEP have had variable

results, especially in the presence of peripheral nerve dysfunction (Gupta and Dorfman 1981), although most do suggest some element of central conduction delay in diabetes (Collier, Reid et al. 1988; Pozzessere, Rizzo et al. 1988; Pozzessere, Rizzo et al. 1989; Nakamura, Noritake et al. 1992). Ziegler et al. conducted the most significant SEP study in DPN (Ziegler, Muhlen et al. 1993). This study determined proximal, spinal and central nerve conduction in different stages of DPN using somatosensory potentials evoked by tibial nerve stimulation. One hundred patients with type 1 diabetes (37 No DPN, 37 subclinical DPN and 36 symptomatic DPN) and 51 healthy volunteers were recruited. Patients with symptomatic neuropathy demonstrated the greatest degree of cortical and peripheral sensory conduction deficit compared to other study groups. There were also significant correlations between SEP components with indices of peripheral and autonomic function tests. Hence, dysfunction along the somatosensory afferent pathways in type 1 diabetes is characterized by peripheral conduction deficits, absent spinal components and reduced or absent amplitudes of the cortical complex. These results indicate a degree of dysfunction within both central and peripheral nerves in DPN.

Further evidence for CNS involvement in DPN arose from observations by Tesfaye et al in a study assessing electrical spinal cord stimulation in painful DPN. This invasive technique is used, albeit infrequently, to alleviate neuropathic pain that is unresponsive to conventional drug treatment. However, this technique was ineffective in patients with severe loss of vibration and joint position sense. This is possibly due to the inability of electrical stimulation of the dorsal columns because of degeneration/atrophy that accompanies DPN. This observation led to further studies looking at the extent of involvement of the spinal cord in DPN.

In a pilot study, using non-invasive in-vivo MR imaging, Eaton et al measured the cord cross-sectional area of 19 patients with DPN, ten diabetic patients with No DPN and ten Healthy Volunteers. All patients underwent MR imaging of the spinal cord with measurements of cord cross sectional area at three anatomical levels (lower cervical [C4/5],

upper [T3/4] and lower [T8/9] thoracic regions). They demonstrated a significantly lower cord area in the cervical and upper thoracic regions in patients with DPN compared to Healthy Volunteers, indicating extensive disease in these areas (Eaton, Harris et al. 2001). Subgroup analysis of DPN patients showed no significant difference in cord area to explain the presence or absence of painful symptoms. These results suggested that the abnormalities previously reported in post mortem studies reflected a neuropathic process affecting the spinal cord. One theory postulates that damage to the peripheral nerve causes secondary spinal cord “shrinkage “ due to degeneration or atrophy, so called “dying back” phenomenon. And another postulates that the primary insult may be to the CNS, with the well-documented peripheral changes occurring as secondary phenomenon. It is also conceivable that both PNS and CNS involvement occur concomitantly resulting in the findings described.

1.2.1.2 Brain

Findings from the spinal cord studies described above, suggest that the metabolic insult of diabetes has a generalised effect on the whole nervous system, and made us question whether the brain too may be involved. Anatomical studies have demonstrated that ascending sensory pathways of the spinal cord terminate within the thalamus before higher order sensory projections are sent to the somatosensory cortex (Wilson, Kitchener et al. 1999). Recent studies have shown that the thalamus does not merely act as a sensory relay station but also modulates/processes the information that is presented to the cortex (McCormick and Bal 1994).

In vivo proton MR spectroscopy (H-MRS) is a non-invasive technique that can provide metabolic information from different body tissues, including the brain. It has been used to study the classification and pathophysiology of various neurological conditions including neoplasms, viral and retroviral infections, ischaemia, demyelination and some forms of epilepsy and dementias (Wilkinson, Lunn et al. 1997; Wilkinson, Griffiths et al. 2001; Wilkinson, Hadjivassiliou et al. 2005). Conventional MRI and H-MRS rely on the same

physical principles to collect MR signal, but differ in the way the data is processed, displayed and interpreted. H-MRS produces data presented in spectrum form that contains several resonances or peaks. Recent MR spectroscopy studies in diabetes have focused on its metabolic impact on the developing brain (Sarac, Akinici et al. 2005) and the cerebral consequences of hypoglycaemia (Rankins, Wellard et al. 2005) or diabetic ketoacidosis (Wootton-Gorges, Buonocore et al. 2005). N-acetyl aspartate (NAA) is a prominent peak that is used as a marker of neuronal integrity and function. Observations of cerebral NAA in diabetes that have been reported are contradictory and most studies did not quantify the presence of DPN in their subgroups with diabetes (Kreis and Ross 1992). Thus, it remains unclear if the changes observed are the result of diabetes or the consequences of neuropathy.

1.2.1.3 Painful DPN and neuroimaging

Painful DPN is a severe disabling chronic condition that affects a significant number of patients with diabetes. The exact pathophysiological mechanisms of neuropathic pain remain unknown although based on experiments in animal models both peripheral and central mechanisms have been postulated (Cervero and Laird 1996). However, there are no consistent differences in peripheral nerve morphological parameters between painful DPN and painless DPN (Malik, Tesfaye et al. 1994). It is likely that the pathophysiological changes resulting in pain may in part, lie elsewhere within the nervous system. Recent advances in modern, non-invasive, neuroimaging methods have led to better understanding and refinement of pain processing in the brain. Many studies based on positron emission tomography (PET) and functional MRI (fMRI) have investigated changes in brain activity in response to various experimental stimuli inducing pain. This has led to the characterisation of a network of brain areas that consistently activate in response to acute pain, forming the "pain matrix" involved in the different dimensions of pain perception (Melzack 1999). These areas include: primary and secondary somatosensory cortex, insular, anterior cingulate and prefrontal cortices and the thalamus. Other regions such as basal ganglia, cerebellum,

amygdala, hippocampus and areas within the parietal and temporal cortices can also be active dependent upon the particular set of circumstances for that individual (Apkarian, Bushnell et al. 2005). Many of these studies, however, have been performed in healthy volunteers following acute pain stimulation, and changes in the brain associated with chronic pain have been less thoroughly investigated.

Several positron emission tomography studies have investigated changes in basal regional cerebral blood flow (CBF) related to spontaneous continuous non-diabetic neuropathic pain. These studies mainly report a significant decrease in regional CBF in the hemithalamus contralateral to the pain (Laterre, De Volder et al. 1988). In many instances, this decrease in thalamic activity was reversed by therapeutic interventions providing significant symptomatic relief (Di Piero, Jones et al. 1991). These results, however, appear contrary to electrophysiological findings in animal and human studies of chronic pain, which have demonstrated abnormal hyperactivity of thalamic neurons (Hua, Garonzik et al. 2000). There is no satisfactory explanation for the observed relative decrease in thalamic relative CBF in neuropathy pain patients (Moisset and Bouhassira 2007). Some investigators have postulated that this decrease may be a compensatory mechanism for inhibiting excessive nociceptive inputs or to the uncoupling between blood flow and neuronal activity in patients experiencing neuropathic pain (Iadarola, Max et al. 1995). The observed decrease in thalamic activity appears to be reversible following various types of analgesic procedures suggests that this decrease results from functional impairment rather than degenerative process.

Increases in relative CBF have been reported in the anterior insula, posterior parietal cortex, inferior and lateral prefrontal cortex and the anterior cingulate cortex in several other non-diabetic neuropathic pain studies (Hsieh, Belfrage et al. 1995). These data suggest that changes in basal brain activity in patients with chronic neuropathic pain involve only a small portion of the "pain matrix" (described below) associated with acute pain. Further studies with better characterisation of neuropathy are required to confirm the pattern of brain activity

associated with spontaneous neuropathic pain in diabetes. Given the complex, multidimensional clinical entity that is painful DPN, it is likely to be mediated by a number of diverse pathophysiological brain mechanisms and possess a unique “pain matrix” of its own. A greater understanding of the specific features of painful DPN will help advance our understanding of the mechanisms of this troublesome complication of diabetes and may result in the development of more rationale (mechanism-based) treatment approaches.

The pathogenesis of diabetic CNS complications is not limited to the effects of neuropathy, but is likely that a multifactorial process involving adverse effects of chronic hyperglycaemia, recurrent hypoglycaemia and cerebrovascular disease maybe collectively responsible. A comprehensive review of the impact of these factors on the CNS is found in Appendix 1.

1.3 Summary

Chronic complications are significant problems in patients with diabetes mellitus and are major causes of morbidity and mortality. It has become increasingly clear that the brain and spinal cord are also susceptible to diabetic end organ damage. Recognition that diabetes results in important complications of the CNS should prompt further research into understanding the pathological mechanisms that underpin these effects. Advances in neuroimaging will enable us to define at what stage during the course of diabetes pathologic changes develop in the brain and will hopefully result in new strategies to prevent or reverse the damage caused by diabetes mellitus.

1.4 Aims of Thesis

- To review and appropriately modify the MR acquisition protocol and spinal cord area measurement technique to improve overall precision and accuracy. To patient these modifications to stringent quality control studies.
- To assess and confirm involvement of the spinal cord occurs in DPN using non invasive MR imaging techniques and investigate if this occurs early in patients with subclinical DPN.
- To establish the involvement of the thalamus is DPN using H-MRS.
- To investigate the aetiology of thalamic neuronal dysfunction in DPN by testing a metabolic hypothesis using H-MRS.
- To investigate the aetiology of thalamic neuronal dysfunction in DPN by testing a vascular hypothesis using MR perfusion imaging.
- To assess the therapeutic efficacy and side effect profile of CBME (sativex) in severe intractable painful DPN in a randomised double blind placebo controlled trial.

2 QUALITY ASSESSMENTS OF METHODOLOGICAL TECHNIQUES

2.1 Introduction

Central nervous system involvement in DPN is increasingly being recognised. As highlighted in the previous chapter, early post mortem studies have demonstrated axonal loss, gliosis and demyelination of the spinal cord in patients with of long standing duration of diabetes (Olsson, Save-Soderbergh et al. 1968). A pilot study was conducted to explore the possible involvement of the spinal cord in DPN by using MR imaging and performing spinal cord cross sectional area (CSA) measurements. Subsequently a larger study (Study 2) was designed to confirm the findings of the pilot study but also to investigate if spinal cord involvement occurs in patients with early, sub-clinical DPN. The pilot study used a manual outlining method with pixel counting to estimate cord CSA. The advantage of this technique is that it is quick and simple to perform, but, it is however operator dependent.

The accuracy and reproducibility of the measurement technique are important factors in obtaining high quality spinal cord CSA results in Study 2. The various methods available to estimate cord CSA can be broadly classified into two categories: computer assisted semi automated and manual outlining techniques. Although these techniques are widely used in multiple sclerosis studies, there have been few formal studies of the accuracy and reproducibility of these measurement techniques (Losseff, Webb et al. 1996). Hence, before commencing Study 2 we performed a quality control (QC) study to compare the performance of a computerised, semi-automated cord CSA measurement technique with the manual outlining technique used in the pilot study. There were two aims in this QC study. The first was to compare the accuracy and longitudinal reproducibility of the manual outlining and semi-automated computer assisted techniques in measuring CSA utilising test tube phantoms (TTP). The more accurate and reproducible of the two techniques would be chosen as the method of choice for measuring cord CSA in human patients. The second aim

was to assess the inter- and intra-observer (novice vs expert) variability of cord CSA in human patients.

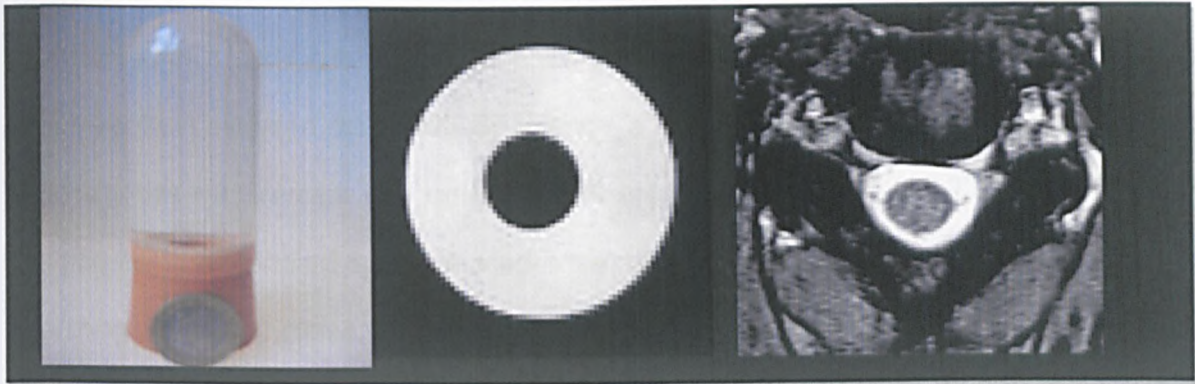
2.2 Phantom Studies

2.2.1 Materials and Methods

This study is a replicated, fully crossed multi-factor, single-centre methodology study. Experiments were divided into TTP and patient studies.

2.2.2 Test tube phantoms

Test tube phantoms containing doped water of varying contrasts, with similar cross-sectional dimensions to human spinal cords were employed (Figure 2.1a). Phantoms were made using test tubes specifically commissioned from Scientific Glass Laboratories Ltd. (Stoke-on-Trent, UK) to exact measurements. To mimic the spinal cord within the spinal canal, a smaller test tube was placed within a larger one and each filled with water. Three inner test tubes with different CSA (63.63mm^2 , 78.55mm^2 and 95.04mm^2) were used. Each outer test tube had the same CSA. Five drops of gadolinium were added to the outer test tube to represent bright white cerebrospinal fluid (CSF) as seen on T2 weighted MR image of the cervical spine (Figure 2.1b and c). Test tube phantoms (Figure 2.2) were orientated in the transverse scan plane and aligned centre of the test object at magnetic isocentre (red line) with the light beam, laser or zero point of the bed scale.



(a)

(b)

(c)

Figure 2.1: (a) Test tube phantom, (b) cross sectional images of test tube phantoms acquired following from magnetic resonance imaging, (c) magnetic resonance image of the cervical spine. CSF, cerebrospinal fluid.



Figure 2.2: Test tube phantoms

2.2.3 MR imaging protocol

Introduction of a semi-automated spinal cord CSA measurement technique relies on good image contrast between cord and CSF. Hence, a new MR acquisition protocol was designed to satisfy this requirement and optimise cord area measurements. All TTP underwent MR imaging using a standard spinal phased-array, receive only radiofrequency coil on a system operating at 1.5T (Eclipse, Philips Medical Systems, Cleveland, Oh, USA). T2* weighted imaging was performed axially from C1-T2 using a gradient echo technique [echo time (TE) = 17.9 ms, repetition time (TR) = 800ms; slice thickness = 4mm, in plane resolution = 0.78mm X 0.96mm].

2.2.4 Cross sectional area measurement

Inner test tube CSA measurements begin by first defining the boundary between the inner test tube and the doped water surrounding it. To delineate this boundary we used both the manual outlining method and the new computerised semi-automated method. The MR propriety software (TwinStar, Philips Medical Systems, Cleveland, Oh, USA) then estimates the CSA of the inner test tube by pixel counting within the boundary margins. The accuracy of each measurement technique was tested against the known CSA of the inner test tubes (Figure 2.3).

2.2.4.1 Manual outlining CSA measurement

Online scanner software (TwinStar, Philips Medical Systems, Cleveland, Oh, USA) with a 'free hand' drawing utility was used to manually outline the boundary between the inner test tube and surrounding doped water (Figure 2.4).

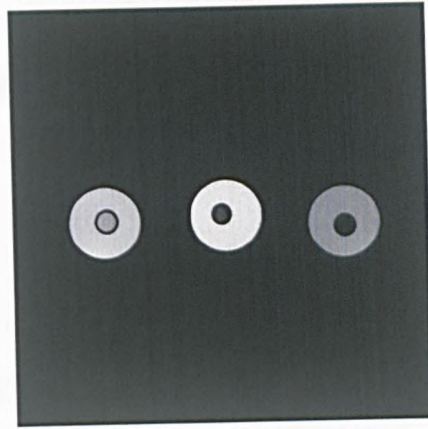


Figure 2.3: Magnetic resonance image of test tube phantoms with three different inner test tube cross sectional areas.

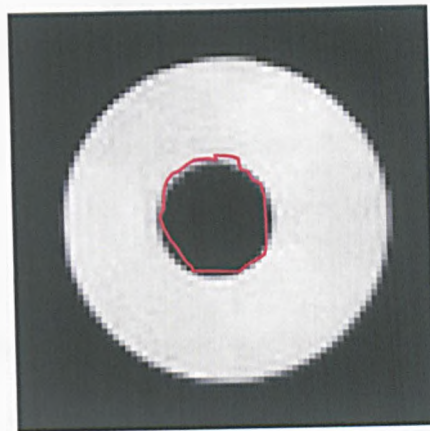


Figure 2.4: Cross sectional magnetic resonance images of the test tube phantom demonstrating measurement of inner test tube cross sectional area using the manual outlining technique.

2.2.4.2 Semi-automated CSA measurement

The computer automatically delineates the boundary between inner test tube and doped water using a thresholding method (Figure 2.5) (Losseff, Webb et al. 1996). A simple formula, shown below, is used to calculate the signal intensity of pixels that occupy the boundary between the two.

If the signal intensity (SI) of the inner test tube and doped water were uniform in their composition then a contour drawn in the image at a SI halfway between the SI of the inner test tube and doped water would be the true position of the boundary. To calculate the boundary SI, a region of interest (ROI) is drawn within the doped water outside the inner test tube [Area Outer (AO), Figure 2.5a] and another within the inner test tube [Area Inner (AI), Figure 2.5b]. The computer then calculates the signal intensity (SI) and area (mm²) of both inner and outer regions. From these two regions the mean CSF SI is calculated as follows:

$$\text{Mean doped water}_{(SI)} = \frac{SI_{(AO)} \times (AO) - SI_{(AI)} \times (AI)}{(AO - AI)}$$

The boundary signal intensity is taken as the mean of inner test tube SI and doped water SI:

$$\text{Boundary}_{(SI)} = \frac{CSF_{(SI)} + \text{Cord}_{(SI)}}{2}$$

Using the semi-automated region growing technique in DISPIImage on a Sun Microsystems workstation (Plummer 1992) the investigator chooses a point ('seed) at, or near, the boundary of the doped water and inner test tube (Figure 2.4c). The program then constructs a border around the inner test tube at the boundary SI. The algorithm starts contouring following from the "strongest edge point" (a border representing the greatest change in intensity from one pixel to another) in the neighbourhood of a user-selected point. The strongest edge point is found through a search over a 5 X 5 pixels squared area with the selected point in the centre. From the "starting point" (the first strongest edge point) found by the algorithm, the program finds the direction of the next contour point by searching north,

east, south and west from the starting point for the direction with the strongest gradient. The next contour point also has to be above the threshold at the starting point. The program then traces a contour from the most recent point following the same principle described above. The contour is complete when it traces back to the starting point (Figure 2.4d). The algorithm uses an interpolated path through the images, for instance, the contour traces its path, based on the gradient calculated with relevant fractions of contributing neighbouring image pixels.

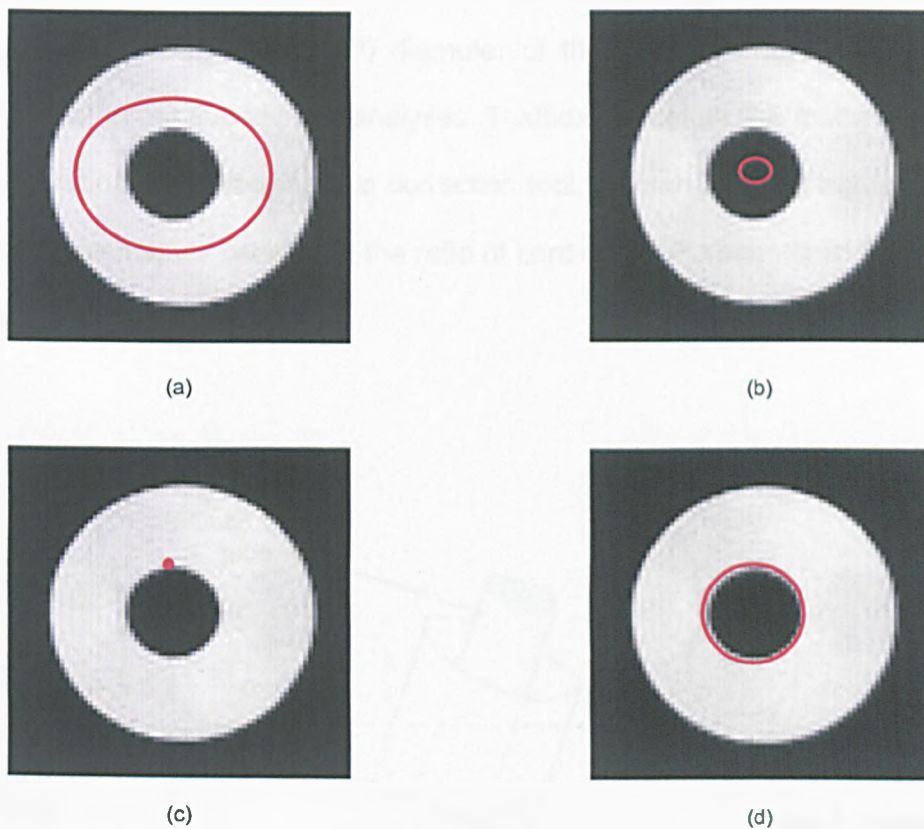


Figure 2.5: Cross sectional magnetic resonance images of the test tube phantoms demonstrating measurement of inner test tube cross sectional area using the semi automated computer assisted technique. (a) A ROI is drawn within the doped water outside the inner test tube and another within the inner test tube (b). Using the semi-automated region growing technique (in Displmage) the investigator chooses a point ('seed) at, or near, the boundary of the doped water and inner test tube (c). The program then traces a contour back to the starting point (d).

2.2.5 Errors of cross-sectional area measurement

Figure 2.6 below is a schematic representation of differences in inner test tube cross-sectional area measurements caused by variation in TTP positioning. Inner test tube CSA measurement is also dependent on the angle at which the transverse sections of the inner test tube image are acquired (Figure 2.7). As a result of such variations actual inner test tube cross-sectional area in slice B is greater than slice A. As all structures in a cross-sectional image will experience the same amount of artificial scaling from such effects, we also measured the antero-posterior (AP) diameter of the outer test tube and used this as a "correction tool" in our subsequent analyses. Textbox 2.1 details the mathematical proof and hence justification for employing this correction tool. Instead of using actual inner test tube CSA measurements, we calculated the ratio of cord CSA:AP diameter of the outer test tube and used this in the final analysis.

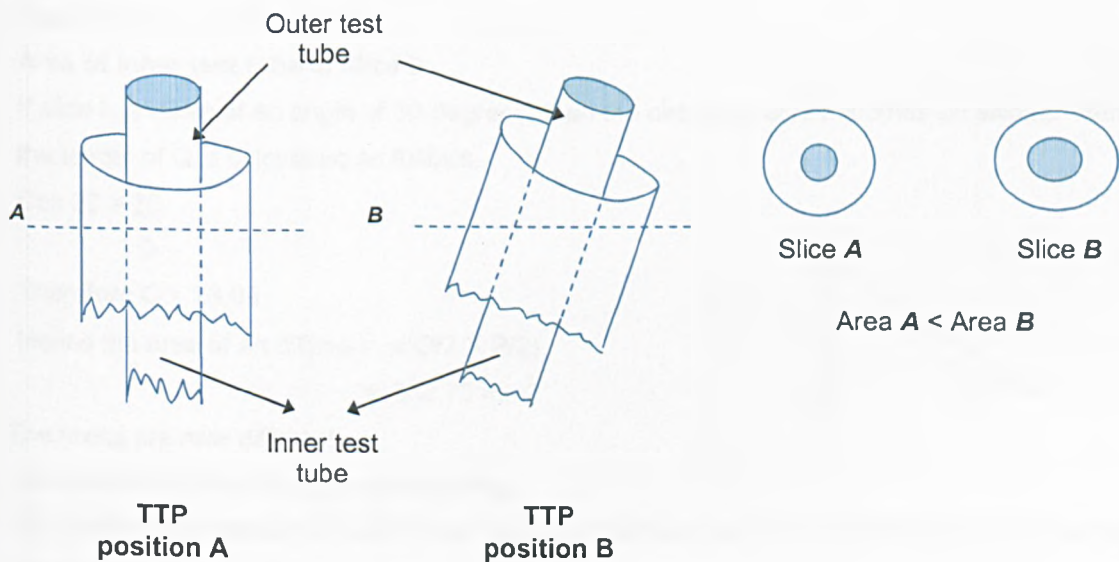


Figure 2.6: Differences in inner test tube cross-sectional area measurement caused by variation in patient positioning. TTP, test tube phantom.

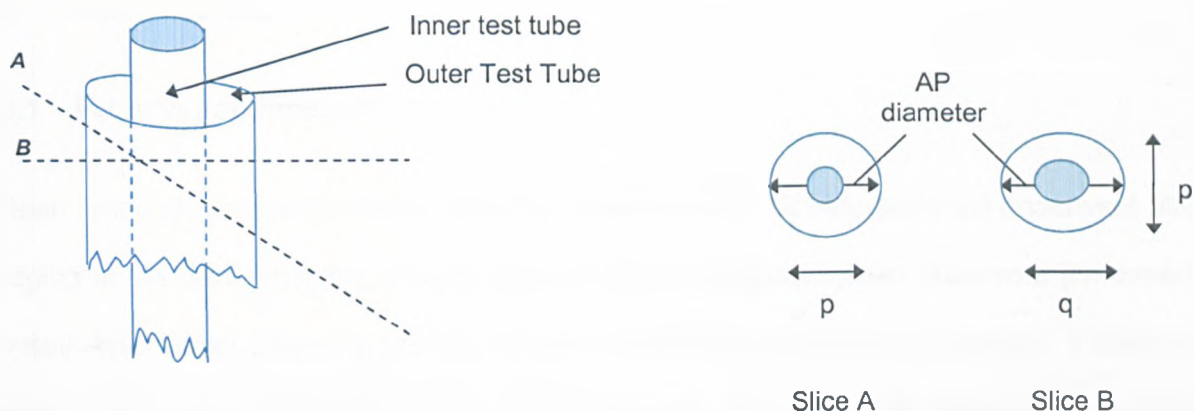


Figure 2.7: Errors in inner test tube cross sectional area that can result from altering the slice angle.

Textbox 2.1

Assumptions

- Inner test tube cross sectional area is a circle
- error in slice positioning is only in the anterior posterior direction

Area of inner test tube in slice a:

$$\pi (P/2)^2$$

Giving arbitrary values

$$P = 20$$

$$\text{Then the area} = 314.16 \text{ mm}^2$$

Area of inner test tube in slice b:

If slice b is taken at an angle of 30 degrees, then the circular shape becomes an ellipse. Hence the length of Q is calculated as follows.

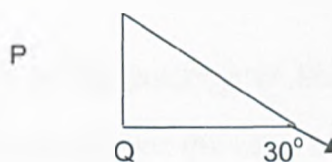
$$\cos 30 = \frac{20}{Q}$$

$$Q$$

$$\text{Therefore } Q = 23.09$$

$$\text{Hence the area of an ellipse} = \pi(Q/2 \times P/2)$$

$$= 362.70 \text{ mm}^2$$



The areas are now different.

Correction of error in slice positioning.

By dividing both areas by the AP outer test tube diameter, we correct for this error and the results are then the same as shown below.

$$APa = \text{AP diameter of outer test tube as measured in slice a,} = 50$$

$$APb = \text{AP diameter of outer test tube as measured in slice b,} = 50/\cos 30, = 57.73$$

$$\text{Therefore the corrected area of inner test tube for slice a is: } 314.16/50 = 6.28 \text{ mm}$$

$$\text{and the corrected inner test tube area in slice b is: } 362.70/57.73 = 6.28 \text{ mm}$$

2.3 Human Studies

2.3.1 Patients and methods

Fifteen randomly selected patients (Healthy Volunteers and diabetic patients) underwent MR imaging of the cervical spine. Images acquired were analysed by two observers [an expert (myself- involved in analysing all data acquired) and a novice (CJE- an assessor trained to perform the analysis specifically for the QC studies)]. The same MR acquisition protocol used in the TTP study was used here. Sagittal imaging of the entire cervical spine was performed. Pilot images were viewed to ensure the cross sectional images were taken perpendicularly to the cord and that the anatomical level of disc space C2/C3 was correct. A single cross sectional image was chosen in the middle of disc space C2/C3 for cord CSA measurements. The method chosen to analyse the cervical cord CSA was the most accurate and reproducible of the two techniques assessed in the TTP study. Inter- and intra-observer variability was assessed by averaging ten measurements of cord CSA at the level C2/C3 for each patient. Observers were blinded to the name and group of patients. Patients were subsequently rescanned after being repositioned on the scanner to determine the scan-rescan variation.

2.3.2 Inter-patient variation in cord CSA

Another potential confounding factor that could impact on the outcome of Study 2 is the substantial inter-patient variation in spinal cord CSA. This would limit the value of actual cord area measurements in a cross-sectional study. Figure 2.8 below is a schematic representation of the consequences of inter-patient variability in spinal cord CSA on a cross-sectional study. Patient A is a larger diabetic patient who develops neuropathy and subsequent cord atrophy. Patient B is a smaller diabetic patient who does not develop neuropathy. Comparing actual cord area sampled at time X will show no difference in cord CSA between patients A and B. However, the spinal cord has shrunk from a larger size in patient A and remains unchanged in patient B. To account for this inter-patient variability we

used the AP diameter of the bony canal as a correction tool. We used the spinal cord CSA to AP canal diameter ratio as an index to account for inter-patient variations in Study 2. This index also served a secondary purpose of correcting for errors in transverse slice positioning of the spinal cord (as detailed above).

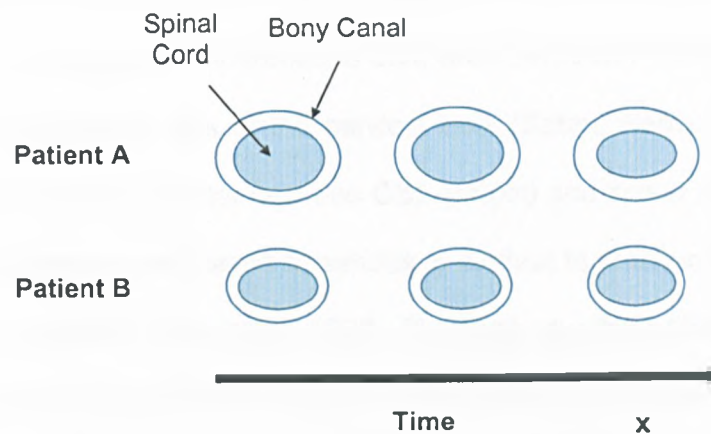


Figure 2.8: Variations in cord cross-sectional area in two patients over time.

2.4 Data Analysis

2.4.1 Study end points

Parameters that were calculated and served as end points:

Test tube phantom studies:

1. Inner test tube CSA

CSA area estimated over 24 slices using both manual outlining and semi-automated region growing technique.

2. Anterior posterior (AP) diameter

Using a scaled ruler provided by the scanner image workstation (TwinStar, Philips Medical Systems, Cleveland, Oh, USA) the outer test tube AP diameter was measured.

Patient studies:

1. Cervical cord CSA to AP canal diameter ratio

The CSA measurement method that proved most accurate and reproducible in the TTP studies was subsequently used to calculate cervical cord CSA at the level C2/C3. This level was chosen as: 1) the greatest difference in cord area between Painless DPN and Healthy Volunteers in the pilot study was at the cervical level (Eaton, Harris et al. 2001); 2) CSF encircles the cord and the contrast between CSF (bright) and spinal cord (dark) allows the use of a semi-automated voxel based thresholding method to measure cord cross-sectional area (Figure 3.1, (Losseff, Webb et al. 1996); 3) positioning the patient to ensure that the cord was in the middle of the spinal canal (i.e. not touching bone) was facilitated as the neck is kept in the more comfortable, neutral position and 4) there are fewer disc prolapses at this level (Thorpe, Kidd et al. 1993).

Using a scaled ruler provided by the Twinstar (Philips Medical Systems, Cleveland, Oh, USA) software, the AP diameter of the spinal canal was measured and the ratio of cord CSA and AP canal diameter was calculated. The inter- and intra-observer reproducibility of this technique was assessed by both an expert (myself) and a novice (CJE).

2.4.2 Statistical Analysis

Accuracy

Accuracy was assessed as the difference between the observed value and the actual target value for each end point of TTP studies.

Absolute accuracy was calculated as follows:

(Observed – Target)

The percentage accuracy was calculated as follows:

$$\frac{[(\text{Observed} - \text{Target}) \times 100]}{\text{Target}}$$

The number of significant measurement errors was determined for each TTP measurement end point. Measurement error for each end point was defined as greater than $\pm 2\%$ of target.

The accuracy of both measurement techniques were assessed by measuring the constant and proportional bias compared to known reference measurements of each TTP. The ideal measurement technique will produce equivalent results with reference measurements and therefore have a constant bias of 0 and proportional bias of 1. The hypothesis test compares constant and proportional bias of each measurement technique against the ideal values. If the p-value is statistically significant then the bias differs from this ideal value.

Precision

Overall reproducibility was assessed by determining the coefficient of variation (CV = standard deviation/mean) of each end point across all measurements compared to the reference standard.

Finally, we compared the agreement between the two measurement techniques using Bland Altman plots (Bland and Altman 1986). In the human studies, reproducibility was expressed as both the mean centred coefficient of variation and the standard deviation of the amount of variation in the observed values.

2.5 Results

2.5.1 Test Tube Phantom Studies

Actual CSA of the three TTP used for QC analysis were 63.63mm², 78.55 mm² and 95.04 mm². The absolute and percentage accuracy of both measurement techniques for each TTP is displayed in Table 2.1. Percentage and absolute accuracy of the semi-automated inner

test tube CSA measurement technique was significantly lower compared to the manual outlining method at each level. Of the 24 measurements made for each TTP, the number of measurement errors for the semi automated technique ranged from 6 to 11 compared with 14 to 20 for the manual outlining method. Accuracy of CSA measurements using semi-automated technique was far superior with constant bias closer to zero and proportional bias closer to 1 across all TTP sizes (Table 2.1). These biases were not significantly different from the hypothetical ideal value. Whereas, the proportional bias for measurements made using the manual outlining technique was significantly different from the ideal value (proportional bias = 1.07, $p = 0.03$; 95% CI 1.03:1.11). The scatter plot (Figure 2.9) shows the observations of reference (X) plotted against the test method (Y).

The coefficient of variation for the semi-automated technique for repeated measures of different size TTPs ranged from 1.3% to 2.5%. The CV range for the manual outlining technique was much higher between 5.2% to 6.6%. Figure 2.10 contains precision plots (see below) showing the standardised observations from the mean for each measurement technique applied to each TTP. Most observations derived from the semi-automated technique were within two standard deviations of the mean and hence this proved to be the most reproducible technique. The Bland Altman plots show little correlation between the two measurement techniques. The average difference (bias) between measurement techniques ranged between 2.57 to 4.38 (Table 2.2).

Reference value of outer test tube AP diameter was 42mm. Mean AP diameter of measured using the analysis software was 40.7(0.17); 95% CI 0.12:0.36. The coefficient of variation for the AP diameter of repeated measures was 0.4%.

2.5.2 Human Studies

The intra-observer standard deviation and coefficient of variation for the experienced observer were 0.01 and 1.0% respectively. The novice observer had a standard deviation of 0.02 and a coefficient of variation of 2.0% for repeated measurements of the same image.

The inter-observer reproducibility coefficient of variation was 1.4% and standard deviation of 0.014. The coefficient of variation and standard deviation of the measurements made by the experienced operator on the scan-rescan series of images were 2.0% and 0.02 respectively.

Table 2.1: Accuracy assessment of the semi-automated and manual outlining methods.

	CB	p	PB	p
Manual outlining	-0.87(-4.29:2.54)	0.19	1.07(1.03:1.11)	0.03
Semi-automated	0.33(-5.89:6.56)	0.62	1.01(0.93:1.09)	0.37

CB, Constant Bias; PB, Proportional Bias; Results expressed as Bias,95% Confidence Interval.

Table 2.2: Assessment of the semi-automated and manual outlining cross sectional area measurements.

	CSA (mm ²)	AA*	AA (%)*	ME*	CV (%)	Bias*
TTP A	78.55 [#]					
Manual outlining	83.0(5.1); 3.9:7.2	4.17(0.3-14.7)	6.89(0.5-18.8)	20	6.6	-3.8(-6.3:-1.2)
Semi-automated	79.5(1.9); 1.5:2.8	1.24(0.3-5.4)	1.58(0.3-6.9)	12	2.5	
TTP B	63.63 [#]					
Manual outlining	67.2(3.4);2.7:4.8	3.37(-3.9-10.0)	5.30(-6.3-15.7)	20	5.4	-2.6(-4.1:-1.0)
Semi-automated	64.6(0.8); 0.6:1.2	0.99(-1.2-2.4)	1.55(-1.8-3.8)	10	1.3	
TTP C	95.04 [#]					
Manual outlining	100.8(4.9);3.8:6.9	6.07(0.4-15.5)	6.4(0.4-16.3)	18	5.2	-4.4(-6.6:-2.1)
Semi-automated	96.3(1.5);1.2:2.2	1.74(0.2-4.0)	1.83(0.2-4.2)	7	1.6	

CSA, Cross Sectional Area; **AA**, Absolute Accuracy; **AA%**, Percentage Absolute Accuracy; **ME**, Measurement Errors; **CV**, Coefficient Variation and **TTP**, Test Tube Phantom. Results expressed as mean(SD),95%CI, median(range) and bias(95%CI). * p<0.05. # Reference Value.

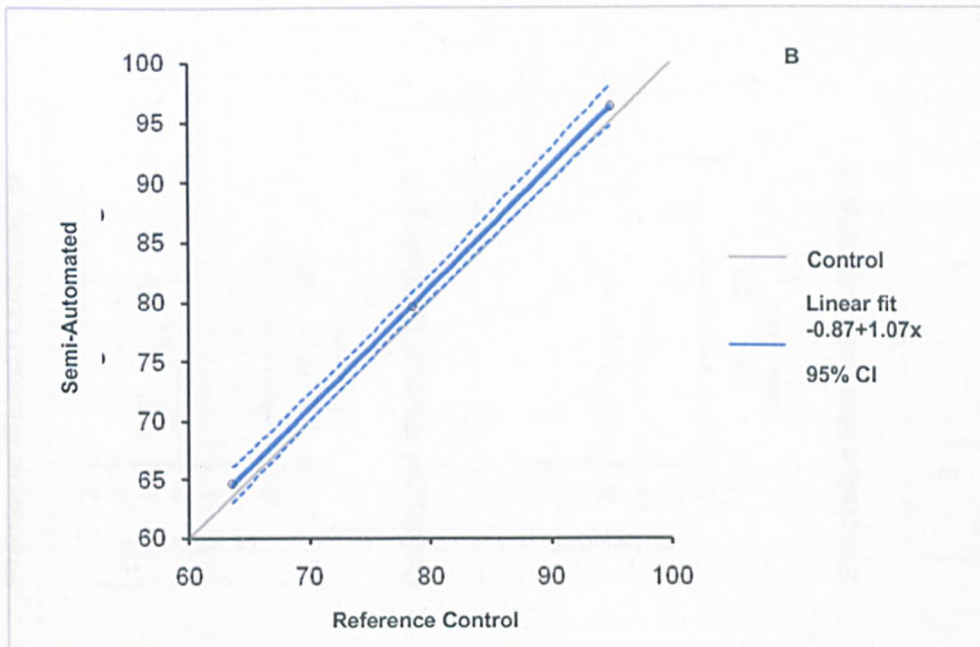
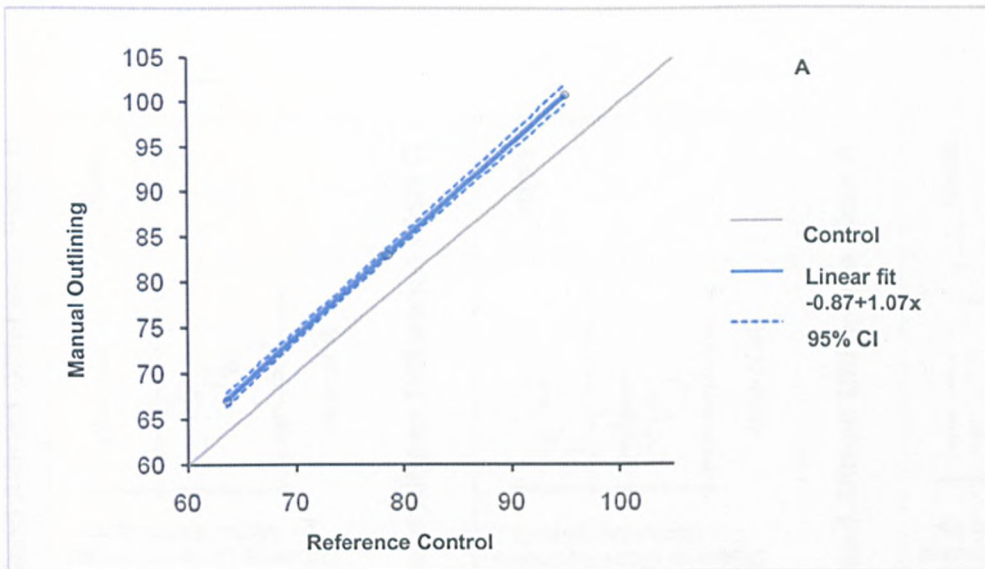
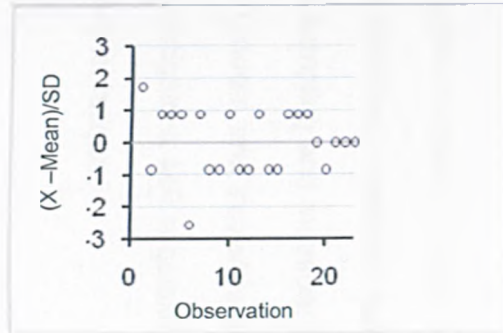


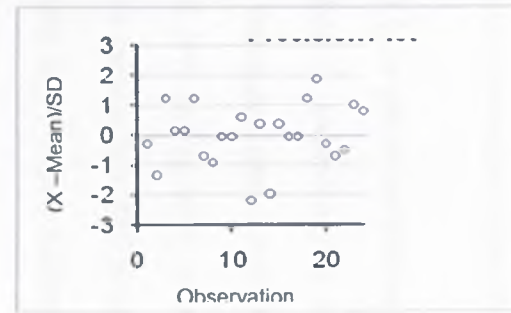
Figure 2.9: Linear Fit of Cross Sectional Area Measurements vs Reference Control A Semi-Automated, B Region Growing. 95% CI, 95% Confidence Intervals

Figure 2.10: Precision and Bland-Altman Plots of Cross Sectional Area measurements

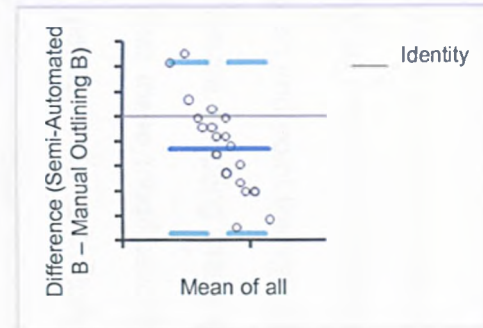
Precision Semi-Automated B



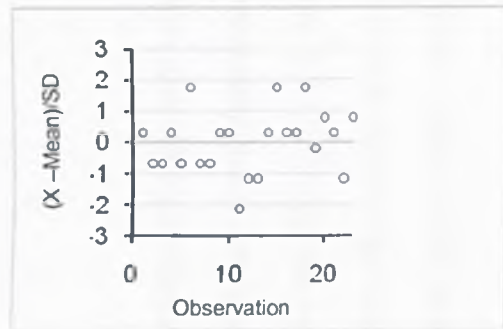
Precision Manual Outlining B



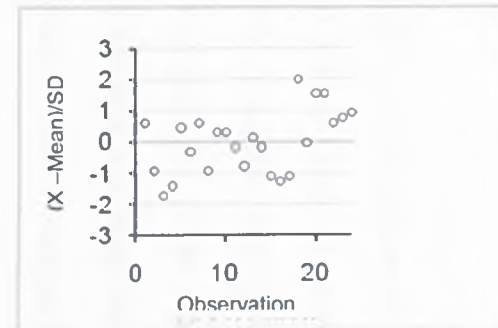
Bland Altman Difference Plots B



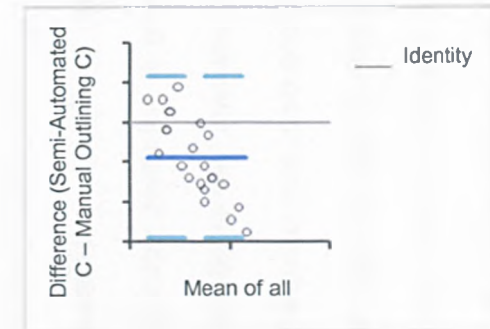
Precision Semi-Automated C



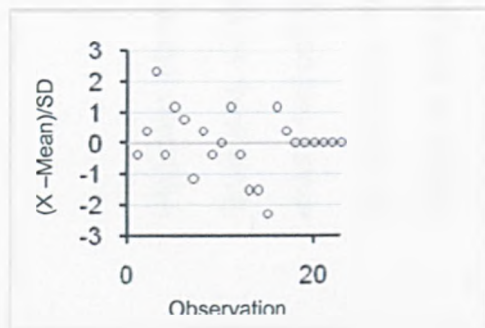
Precision Manual Outlining C



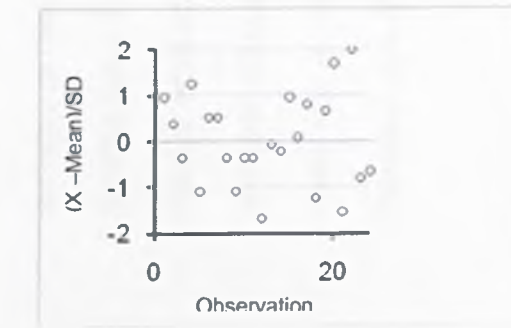
Bland Altman Difference Plots C



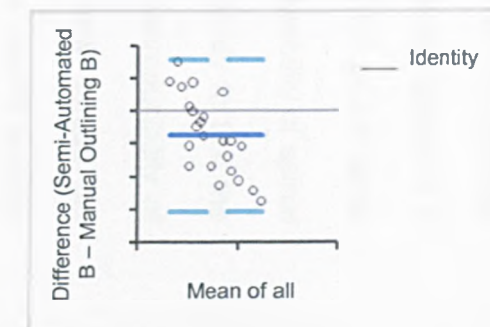
Precision Semi-Automated A



Precision Manual Outlining A



Bland Altman Difference Plots A



2.6 Discussion

Having analysed the original pilot study by Eaton et al. (Eaton, Harris et al. 2001), we identified certain aspects of its methodology and analysis plan, that if modified may improve the accuracy and precision of cord CSA. The adoption of a new image acquisition protocol, a semi-automated cord CSA measurement technique and the ratio of cord CSA to AP canal diameter as an end point theoretically minimised error and improved reproducibility of spinal cord CSA. Before adopting these changes, however, we designed a QC study to test the hypothesis that these modifications will result in improved accuracy and reproducibility of study end points. This QC study enabled a uniform assessment of new versus old procedures using in-house manufactured TTP and human patients. These results provide insight into the accuracy and reproducibility associated with the measurement of spinal cord CSA due to the procedural changes proposed.

Test tube phantom studies demonstrated a high degree of accuracy with relatively lower number of measurement errors and high degree of reproducibility of the semi-automated computer assisted technique. The cumulative change in percentage accuracy was overall low using both measurement techniques. With lower percentage scores, however, the semi-automated technique had the least accuracy drift over the three different inner test tube size ranges. Hence, the semi-automated technique was chosen to measure spinal cord CSA in the QC human studies. These latter inter- and intra-observer variability studies demonstrated acceptable coefficient of variation.

Potential sources for error in cord CSA measurements are summarised in Figure 2.11. Once identified, measures can be put in place to minimise these errors. Errors can be divided into sampling and measurement errors. Each of these can be divided into two further categories; random and systemic errors. Individually these errors can originate from study patients, investigators, MR acquisition protocols and MR equipment and the spinal cord CSA measurement technique.

Systemic sampling and measurement errors of cord CSA estimation were assessed by the TTP accuracy protocols. Accuracy is a function of systemic error (i.e. the greater the error the less accurate the variable) and defined as how closely the measurement is to the actual or true value. The reference standard used was a specifically commissioned TTP, built to set dimensions to mimic a range human spinal cord CSA. Two main additional sources of errors, which can affect the accuracy, were identified as being investigator bias and instrument bias. Investigator bias is defined as the consistent distortion on the reporting of a measurement whether conscious or subconscious. Standardising the MR acquisition technique and introducing the semi-automated computer assisted cord CSA measurement method reduced the impact of this bias. In addition, investigators will be blinded to the spinal cord images of patients during analysis.

Instrument bias is the result of a faulty function of a mechanical instrument (MR scanner) and/or measurement technique. The MR scanner is calibrated on a regular basis and will proceed as per departmental and manufacturer specified guidelines. The introduction of a new acquisition protocol has improved the contrast between spinal cord and CSF, thereby improving the efficiency of the semi-automated cord CSA measurement technique. Furthermore, statistical tests comparing constant and proportional bias against ideal values demonstrated that the semi-automated measurement technique was not significantly different from the ideal value and hence has greater overall accuracy. Percentage absolute accuracy calculations showed that the manual outlining methods was particularly flawed at the extreme ends of inner test tube CSA sizes. The semi-automated measurement technique, on the other hand, displayed less drift in accuracy over the size ranges.

The second set of errors assessed in this study is random sampling and measurement errors, which were assessed by precision TTP and human QC studies. Precision is defined as the ability to provide the same results from the same patient on 2 or more occasions and is susceptible to random errors. The precision protocol utilised TTP and

human patients to test the reproducibility and inter/intra-observer variability of both the sampling and the chosen CSA measurement technique. With smaller coefficient of variations in both TTP and human studies, the semi-automated CSA measurement technique appeared to be less susceptible to random errors.

In this study, precision was dependent on three factors, first the equipment used (MR scanner and cord CSA measurement technique), second the investigator and finally the patients studied. Inter- and intra-operator repeatability using the semi-automated computer assisted technique confirmed it was also more reproducible. Additional strategies that will be employed to improve reproducibility include standardising measurement methods (operating manuals detailing protocols in use for the acquisition of images, recording procedure, positioning patients and step by step guide to using the semi-automated cord CSA measurement technique will be employed), training (investigators will be trained in methods for image post processing for measuring spinal cord CSA), automating (introduction of a semi-automated spinal cord measurement technique will improve precision by reducing human error) and repetition (repeating measurements [spinal cord CSA and AP canal diameter] and taking the mean will reduce the impact of random error).

Another strategy that will be employed to minimise errors of spinal cord CSA measurement is supplementation. By measuring several different variables to represent the characteristics of interest (spinal cord CSA) will add both accuracy and precision to the study. An index (spinal cord area:AP canal diameter ratio) which, combines several measurements can also enhance precision. This index has the added benefit of accounting for both inter-patient variations of cord CSA and errors caused by slice positioning (described above).

This QC study has aspects that may limit its interpretation. We did not test accuracy of measurement techniques on human patients. The accuracy would be expected to fall because of the introduction of MR susceptibility artefacts. In summary, this study provides a

quantitative assessment of the accuracy and reproducibility of two cord cross-sectional area measurement techniques using simulated testing of TTP and human patient. Overall, because of the better accuracy and precision of the semi-automated computer assisted technique, we chose to use this in the subsequent larger spinal cord DPN study.

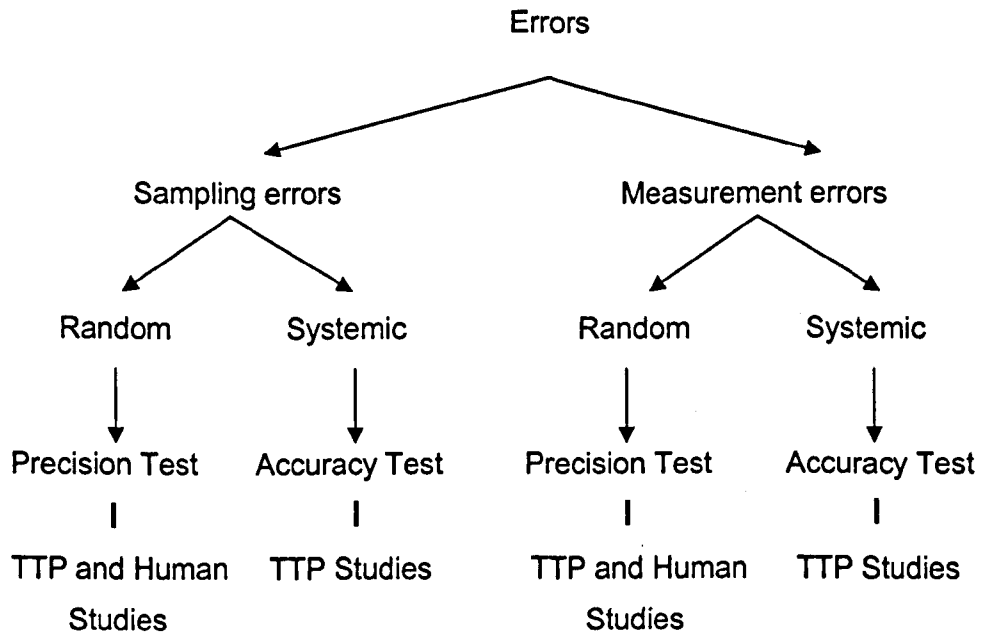


Figure 2.11: Measurement and sampling errors.

3 EARLY INVOLVEMENT OF THE SPINAL CORD IN DIABETIC DISTAL SYMMETRICAL POLYNEUROPATHY

3.1 Introduction

Distal symmetrical diabetic sensorymotor polyneuropathy is the commonest form of neuropathy in the western world with important health and financial implications (Boulton 1997; Vinik, Park et al. 2000; Boulton, Kirsner et al. 2004). Although various vascular and metabolic factors have been implicated, a complete understanding of the pathogenesis of DPN remains elusive (Dyck and Giannini 1996; Cameron, Eaton et al. 2001). Consequently, apart from glycaemic control, we have no proven rational treatments. DPN has hitherto been considered a disease of the PNS only with CNS involvement largely overlooked. However, knowledge of the full extent of nervous system involvement is crucial for a greater understanding of the pathogenesis of DPN and may have an important role in the development of effective therapies.

Involvement of the spinal cord was first suggested by post mortem studies in the 1960's and 1970's. Pryce, Leichtentritt and Williamson described loss of myelinated fibres and gliosis of the dorsal columns of the spinal cord in diabetic patients (Leichtentritt 1893; Pryce 1893; Williamson 1904). Further histopathological changes reported within the spinal cord include long tract, nerve root and dorsal root ganglion degeneration with demyelination and axonal loss (Reske-Nielsen and Lundbaek 1968; Reske-Nielsen, Lundbaek et al. 1970). However, many of these studies did not examine patients with DPN specifically and it is therefore impossible to conclude whether these changes were due to neuropathy or diabetes *per se*. More recently, the observation that electrical spinal cord stimulation failed in relieving neuropathic pain in patients with severe loss of vibration and joint position sense, suggests that the spinal cord may be involved in the disease process (Tesfaye, Watt et al. 1996).

In a recent pilot study, Eaton et al have demonstrated a significant reduction in cross-sectional area of the cervical spine using MR imaging in patients with advanced DPN

compared to Healthy Volunteers (Eaton, Harris et al. 2001). However, as the relevance of these findings to the pathogenesis of DPN is dependent on whether spinal cord shrinkage occurs early, a larger, adequately powered study was conducted.

3.2 Methods

3.2.1 Patients

Two hundred and twenty type 1, male diabetic patients from the Royal Hallamshire Hospital Diabetes Register were screened for the study (October 2001-January 2004). Selection criteria included, type 1 diabetes diagnosed for more than five years and age between 18 - 65 years. Exclusion criteria included significant back problems (defined as either known degenerative back disease or symptoms which have occurred on a regular basis, or have required consultation for investigation and treatment), history of spinal trauma, non-diabetic neuropathies, history of alcohol consumption of more than 20 units a week, painful neuropathy, diabetic neuropathies other than DPN (e.g. mononeuropathies, proximal motor neuropathies etc), claustrophobia or other factors which precluded MR imaging. A total of 122 diabetic patients fulfilled the inclusion and exclusion criteria and were willing to undergo neurophysiological assessments (detailed below). Of these, 98 subsequently underwent MR imaging. Seventeen withdrew from the study before imaging and seven had contraindications to imaging. We also recruited 24 age and sex matched Healthy Volunteers and eight patients with hereditary sensory motor neuropathy. Patients with hereditary sensory motor neuropathy were diagnosed following nerve conduction studies and genetic testing, and all had the disease phenotype. Members from the same family were excluded to avoid bias. All patients gave written, informed consent to enter the study, which had prior approval by the South Sheffield Regional Ethics Committee.

3.2.2 Assessment of neuropathy

All patients underwent: 1) assessment of neuropathic symptoms [Neuropathy Symptom Score (NSS); (Dyck and Thomas 1999)] and 2) neurological examination from which the Neuropathic Impairment Score of the Lower Limbs [NIS (LL); (Dyck and Thomas 1999)] was derived and 3) 7 tests of nerve function, in order to determine the “NIS LL+7 tests” Neuropathy Composite Score (NCS) (Dyck, Davies et al. 1997; Dyck, Litchy et al. 2003). Further details of the neurophysiological assessments and a summary how the NCS, was calculated (Dyck and Thomas 1999) please refer to Appendix 2. Vibration perception threshold was assessed using the Computer Assisted Sensory Evaluation IV (CASE IV, Minnesota, USA) system employing standard techniques (Dyck, O'Brien et al. 1993; Dyck, Zimmerman et al. 1993). The electrophysiological measurements (peroneal motor nerve conduction velocity, distal latency and compound muscle action potential; sural sensory nerve action potential; and tibial motor nerve distal latency) were assessed with surface electrodes at a stable skin temperature (31°C; Medelec, Synergy Oxford Instruments, Oxford, UK). Autonomic function assessment of heart rate variation with deep breathing was performed with continuous electrocardiographic monitoring (Dyck and Thomas 1999). The same, trained physician (DS) performed all the above assessments on each patient.

According to Dyck et al., the minimum criterion for the presence of DPN is a NCS \geq 4.5 (Painless DPN, as patients with painful DPN were excluded from this study) (Dyck, Litchy et al. 2003). Patients with a NCS of 0 were classified as having No DPN (Dyck and Thomas 1999). Diabetic patients with NCS between 1 and 4.5, and hence objective evidence of functional nerve impairment, were classified as having Subclinical DPN. In all patients, assessments to stage the severity of neuropathy were made within 7 days of MR imaging.

3.2.3 Magnetic resonance imaging protocol

All patients underwent MR imaging of the cervical spine using a standard spinal phased-array receive only radiofrequency coil on a system operating at 1.5 Tesla (Eclipse, Philips Medical Systems, Cleveland, Oh, USA). T2* weighted imaging was performed axially from C1-T2 using a gradient echo technique [echo time (TE) = 17.9 ms, repetition time (TR) = 800ms; slice thickness = 4mm, in plane resolution = 0.78mm X 0.96mm]. Cord cross-sectional area was measured at the level of disc space C2/C3 in all the patients. Total imaging time was 15 minutes.

Before the analysis was performed, an experienced neuroradiologist reviewed the standard images acquired (axial T2, sagittal T1 and T2) to exclude any anatomical abnormalities. A simple four point scoring system was employed to quantify the degree of degenerative vertebral disease affecting patients in this study (normal = 0, thecal indentation only = 1, thecal indentation touching the cord = 2, cord compression = 3). There were no significant differences in the scores for degenerative disc disease between the five groups studied. None of the patients recruited had cord compression.

3.3 Data analysis

3.3.1 Spinal cord area measurements

Figure 3.1 shows an example of the images obtained. Average cord area measurements from three slices through disc space C2/C3 were calculated. An assessor, who was blinded to patient identity, performed the analysis.

Absolute cord area measurements can be of limited value in cross-sectional studies because of substantial inter-patient variation in cord area. In addition the cord cross-sectional area is also dependent on the angle at which the cord image is acquired. Although axial slices of the spinal cord were aligned parallel to cervical disc C2/C3, the spinal cord may not have been sectioned perpendicularly due to variations in patient positioning. The

resultant errors and further details of the correction methods employed are discussed in Chapter 2. In summary, as all structures in an image will experience the same amount of artificial scaling from such effects (Pelletier, Garrison et al. 2004), we also measured the AP diameter of the spinal canal and used it as a “correction tool” in all our subsequent analyses. The AP diameter of the spinal canal was measured in the slice below the C2/C3 intervertebral disc using the tools on the proprietary MR system’s software (Eclipse, Philips Medical Systems, Cleveland, Oh, USA).

Hence, in order to account for both inter-patient variations and errors caused by patient positioning in the scanner, we subsequently analysed the actual cord area measurements in two ways:

- **Actual cord area adjusted for patient demographics and spinal canal AP diameter**
 - a) To account for errors caused by patient positioning in the scanner, we adjusted actual cord area means for AP diameter of the spinal canal and
 - b) To account for errors caused by inter-patient variability we included age, weight and height as additional covariates in the analysis of covariance (see below).
- **Normalised cord area**
 - a) To account for errors caused by patient positioning, we calculated a “normalised” cord area by dividing each individual’s actual cord area with their AP diameter of the spinal canal (cord canal ratio) and
 - b) Subsequently adjusted normalised cord area for age, weight and height to account for inter-patient variability.

3.3.2 Statistical analysis

All analyses were performed using the statistical package SPSS 11.1. Baseline characteristics were described as means and standard deviation for normally distributed variables and as medians and 5th and 95th percentiles for variables with a skewed distribution. Nerve conduction velocities were below the detection threshold in 18 patients for the sural nerve and 4 patients for the peroneal nerve. In all analyses, these extremely low values were assumed to equal 0.

We used analysis of covariance (ANCOVA) to compare differences in actual cord areas between groups (Healthy Volunteers, those with hereditary sensory motor neuropathy, No DPN, Subclinical DPN and Painless DPN) using age, height, weight and AP spinal canal diameter as covariates. The relation between actual cord area and individual attributes of nerve function (e.g. nerve conduction velocities, vibration perception threshold etc.) and NCS was analysed in more detail among patients with diabetes (n=98), using linear regression. For the final analysis we used ANCOVA to analyse differences in normalised cord area between groups and on this occasion used age, height and weight as covariates.

3.3.3 Reproducibility

The reproducibility of both MR acquisition and cord area measurement techniques was assessed (Chapter 2). A series of 18 patients, representing the range of spinal cord size, were selected. Each patient was scanned, removed from the scanner, repositioned and then rescanned (scan-rescan series). One set of images was then analysed twice by two investigators using the same cord area measurement technique as described above. One investigator was experienced with the technique having analysed all patients, and the other with no previous experience of the technique except a brief instruction beforehand. Images from both acquisitions (scan-rescan series) were then analysed by the experienced investigator to assess the variation in cord area caused by the MR acquisition technique.

Reproducibility was expressed as both the mean centered coefficient of variation and the standard deviation of the amount of variation in the observed values.

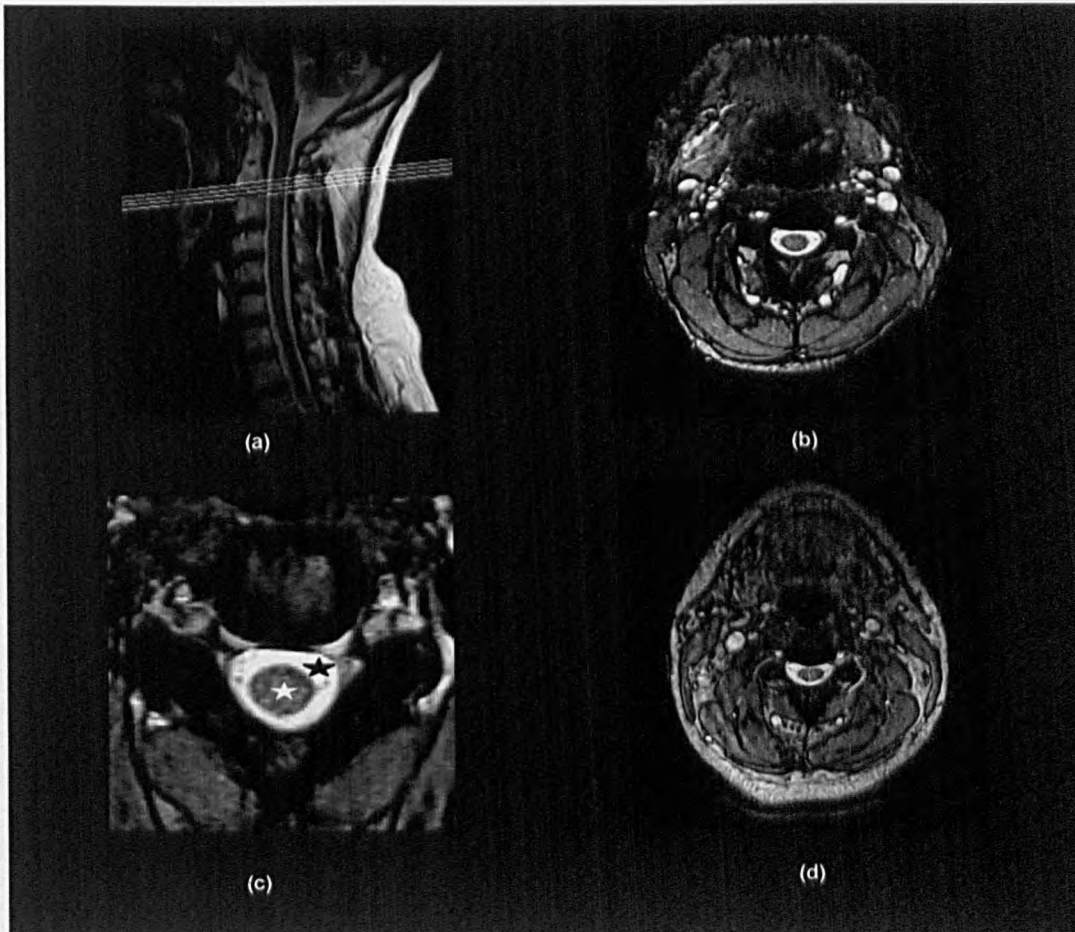


Figure 3.1: T_2 -weighted magnetic resonance imaging of the cervical spine.

(a) Spin echo magnetic resonance mid-sagittal image of a Healthy Volunteer depicting the prescription of axial slices acquired at the level of C2/C3 parallel to disc space C2/C3. (b) Axial images at the level of disc space C2/C3 showing a section of the cervical cord in a non-diabetic control. Magnified gradient echo MR axial images of the cervical spinal cord of (c) a Healthy Volunteers and (d) an age matched patient with Painless DPN illustrating differences in cord size and shape. The high contrast between cerebrospinal fluid (★) and spinal cord (☆) used to define the edge of the cord is evident.

3.4 Results

3.4.1 Demographics

Table 3.1 shows demographic details of the three diabetic groups (No DPN, Subclinical DPN and Painless DPN), Healthy Volunteers and patients with hereditary sensory motor neuropathy. Table 3.2 and 3.3 shows the results of the neurophysiological and MR assessments for each of the five groups.

	No DPN	Subclinical DPN	Painless DPN	HV	HSMN
Group Size	22	26	50	24	8
Age (years)	38.9(8.8)	42.6(10.5)	49.2(9.0)	47.6(13.1)	52.6(15.4)
Height (cm)	177.4(5.6)	176.7(6.2)	174.8(7.0)	175.6(5.4)	176.3(5.6)
Weight (Kg)	85.2(11.2)	81.8(11.5)	82.7(13.3)	81.4(14.0)	80.9(14.2)
DD (years)	18.0(8.8)	17.2(9.7)	26.2 (10.7)	N/A	N/A

No DPN, diabetic patients with no neuropathy (NCS = 0); **Subclinical DPN**, diabetic patients with early neuropathy ($1 < \text{NCS} < 4.5$); **Painless DPN**, diabetic patients with established neuropathy ($\text{NCS} > 4.5$); **HV**, Healthy Volunteers; **HSMN**, patients with hereditary sensory motor neuropathy; **DD**, Duration of diabetes.

Table 3.2: Subgroup Neurophysiological Assessments.

	No DPN	Subclinical DPN	Painless DPN	HV	HSMN
SV	43.6 (37.7-48.4)	38.7 (34.4-49.3)	31.0 (0.0-40.9)	44.6 (32.1-50.8)	ND
SA	10.6 (2.3-24.0)	8.3 (3.4-27.9)	2.1 (0.0-13.4)	11.1 (6.4-28.1)	ND
PV	43.3 (7.0)	41.7 (3.8)	34.7 (6.9)	44.6 (3.1)	ND
PA	5.8 (2.9)	5.5 (1.7)	2.5 (2.0)	7.0 (3.1)	ND
HRDB	1.5 (0.2)	1.4 (0.2)	1.3 (0.2)	1.4 (0.2)	1.3(1.2)

Actual cord cross-sectional area measurements, normalised cord cross-sectional area, sural sensory nerve conduction velocity (**SV, m/s**), sural sensory nerve action potential (**SA, mA**), common peroneal nerve conduction velocity (**PV, m/s**), common peroneal compound muscle action potential (**PA, mA**), R-R variability with heart rate deep breathing (**HRDB**), and of the patients (**No DPN**, diabetic patients with no neuropathy; **Subclinical DPN**, diabetic patients with early neuropathy; **Painless DPN**, diabetic patients with established neuropathy; **HV**, Healthy Volunteers and **HSMN**, patients with hereditary sensory motor neuropathy). Results are given as mean (\pm SD), and median (5th; 95th percentile) for variables with a skewed distribution. **ND**, not detectable. **N/A**, not applicable.

Table 3.3: Sugroup Neurophysiological Assessments and Cervical Cord Cross-sectional Area Measurement.

	No DPN	Subclinical DPN	Painless DPN	HV	HSMN
Vib JND	15.4 (2.4)	18.3 (3.0)	21.2 (2.6)	17.5 (3.0)	ND
NCS	0.0 (0.0-0.0)	2.0 (1.0-4.0)	10.5 (5.0-29.5)	0.0 (0.0-0.0)	N/A
NSS	0.0 (0.0-0.0)	0.0 (0.0-2.8)	2.0 (0.0-10.5)	0.0 (0.0-0.0)	N/A
NSS + NIS LL	0.0 (0.0-0.0)	0.0 (0.0-4.8)	9.5 (0.0-42.5)	0.0 (0.0-0.0)	N/A
Actual CA	93.9 (7.6)	92.5 (8.8)	87.1 (9.1)	91.2 (9.0)	93.7 (11.2)
AP	14.0 (1.8)	15.2 (1.4)	14.9 (1.9)	13.4 (1.3)	13.8 (2.1)
Normalised CA	67.9 (7.0)	61.2 (6.6)	59.0 (7.2)	69.1 (7.5)	68.7 (7.4)

Vibration “just noticeable difference” (**Vib JND**), Neuropathy Composite Scores (**NCS**), Neuropathy Symptom Score (**NSS**), Neuropathy Impairment Score of the Lower limbs (**NIS LL**), actual cord area (**Actual CA**) and AP diameter of bony canal (**AP**) of the patients (**No DPN**, diabetic patients with no neuropathy; **Subclinical DPN**, diabetic patients with early neuropathy; **Painless DPN**, diabetic patients with established neuropathy; **HV**, Healthy Volunteers and **HSMN**, patients with hereditary sensory motor neuropathy).

3.4.2 Reproducibility

The intra-observer standard deviation and coefficient of variation for the experienced observer were 0.01 and 1.0% respectively. The novice observer had a standard deviation of 0.02 and a coefficient of variation of 2.0% for repeated measurements of the same image. The inter-observer reproducibility coefficient of variation was 1.4% and standard deviation of 0.014. The coefficient of variation and standard deviation of the measurements made by the experienced operator on the scan-rescan series of images were 2.0% and 0.02 respectively.

3.4.3 Spinal cord area assessments

Figure 3.2 shows the mean actual cord area per group, after adjustment for age, height, weight and AP diameter of the spinal canal. Diabetic patients with no neuropathy (No DPN group) had a similar, adjusted mean actual cord area as the Healthy Volunteers and the patients with hereditary sensory motor neuropathy. We found that adjusted actual cord area was progressively smaller in Subclinical DPN and Painless DPN groups. Compared to diabetic patients with No DNP, those with Subclinical DNP had an actual cord area that was 3.8 mm² smaller (95 percent confidence interval: -0.8–8.4) and those with Painless DPN had a cord area that was 9.1 mm² smaller (95 percent confidence interval: 4.9–13.4). The test for an inverse linear trend across the three diabetic groups showed a p-value of 0.001.

20.5 percent and 10.3 percent of Painless DPN and Subclinical DPN patients respectively had spinal cord atrophy as defined by an adjusted actual cord area less than two standard deviations below that of normal controls. Table 3.4 and 3.5 shows the relations between the actual cord area and the measures of nerve conduction velocity, NSS and NISLL among the patients with diabetes. Adjusting for age, height, weight and AP diameter of the spinal canal, we found that higher sural and peroneal nerve conduction velocities were associated with larger spinal cord areas. The amplitudes in both nerves were not related to the actual cord area at a statistically significant level. Adjusted actual cord area was inversely related to NCS with each point associated with a cord area reduction of 0.36 mm²

(95 percent confidence intervals 0.16–0.56). Heart rate variability and vibration perception threshold (“just noticeable difference”) were not related to actual cord area.

Normalised cord area, obtained by dividing calculated cord area with the AP canal diameter (as described above), was subsequently analysed. Figure 3.2B shows the mean normalised cord area per group, after adjustment for age, height and weight. We confirmed all the relations described above between normalised cord areas and nerve conduction velocities, and NCS (Table 3.5), except the relation with the peroneal motor nerve conduction velocity. Comparison of normalised cord area after adjustment for age, height and weight, revealed that both Painless DPN (mean 58.8mm, 95 percent confidence interval 56.7–60.7) and Subclinical DPN (61.5mm, 58.8–64.2) groups had a significantly lower normalised cord area compared to No DPN patients (67.8mm, 64.7–70.9) [$p < 0.0001$ and $p = 0.02$ respectively]. The difference in normalised cord area measurements between Painless DPN and Subclinical DPN did not reach statistical significance [$p = 0.11$]. Furthermore, diabetic patients with No DPN had normalised cord area measurements, which were not statistically different from Healthy Volunteers (69.2mm, 66.4–72.0) and hereditary sensory motor neuropathy patients (69.1mm, 64.2–74.1) [$p = 0.51$ and $p = 0.98$ respectively]. When compared with hereditary sensory motor neuropathy, diabetic patients with Subclinical DPN and Painless DPN had a lower normalised cord area [$p < 0.0001$ and $p = 0.01$ respectively].

Table 3.4: Relation Between Actual Spinal Cord Area and Clinical and Neurophysiological Assessments Diabetic Patients.

	Actual Cord Area, adjusted for age, height, weight and A-P diameter			
	β	95% CI	Partial r	p-value
SV	0.17	0.06:0.27	0.32	0.002
SA	0.20	-0.10:0.49	0.14	0.18
PV	0.32	0.07:0.56	0.27	0.01
PA	0.29	-0.42:1.00	0.09	0.42
HRDB	3.62	-5.93 - 13.18	0.09	0.45
Vib JND	-0.47	-1.07 - 0.12	-0.17	0.12
NCS	-0.36	-0.56 - -0.16	-0.35	0.001
NSS	-1.00	-1.70:0.28	-0.28	0.01
NSS+ NIS LL	-0.26	-0.41:0.11	-0.34	0.001

Sural sensory nerve conduction velocity (**SV, m/s**), sural sensory nerve action potential (**SA, mA**), common peroneal nerve conduction velocity (**PV, m/s**), common peroneal compound muscle action potential (**PA, mA**), Neuropathy Symptom Score (**NSS**), Neuropathy Impairment Score of the Lower Limbs (**NIS LL**), β is the linear regression coefficient, indicating the change in cord area (in mm²) per unit change in each of the independent variables. Partial r is the partial correlation coefficient obtained from the linear regression model. 95 percent confidence interval (**95%CI**).

Table 3.5: Relation Between Normalised Spinal Cord Area and Clinical and Neurophysiological Assessments Diabetic Patients.

	Normalised Cord Area, adjusted for age, height, weight	
	Partial r	P-value
SV	0.28	0.01
SA	0.14	0.20
PV	0.18	0.10
PA	-0.02	0.82
NSS	-0.25	0.01
HRDB	0.11	0.31
Vib JND	-0.17	0.10
NCS	-0.27	0.01
NSS+NIS LL	-0.28	0.01

Sural sensory nerve conduction velocity (**SV, m/s**), sural sensory nerve action potential (**SA, mA**), common peroneal nerve conduction velocity (**PV, m/s**), common peroneal compound muscle action potential (**PA, mA**), Neuropathy Symptom Score (**NSS**), R-R variability with heart rate deep breathing (**HRDB**), vibration “just noticeable difference” (**Vib JND**) Neuropathy Impairment Score of the Lower Limbs (**NIS LL**). Partial r is the partial correlation coefficient obtained from the linear regression model. The normalised cord area is the cord area divided by the antero-posterior diameter of the spinal canal.

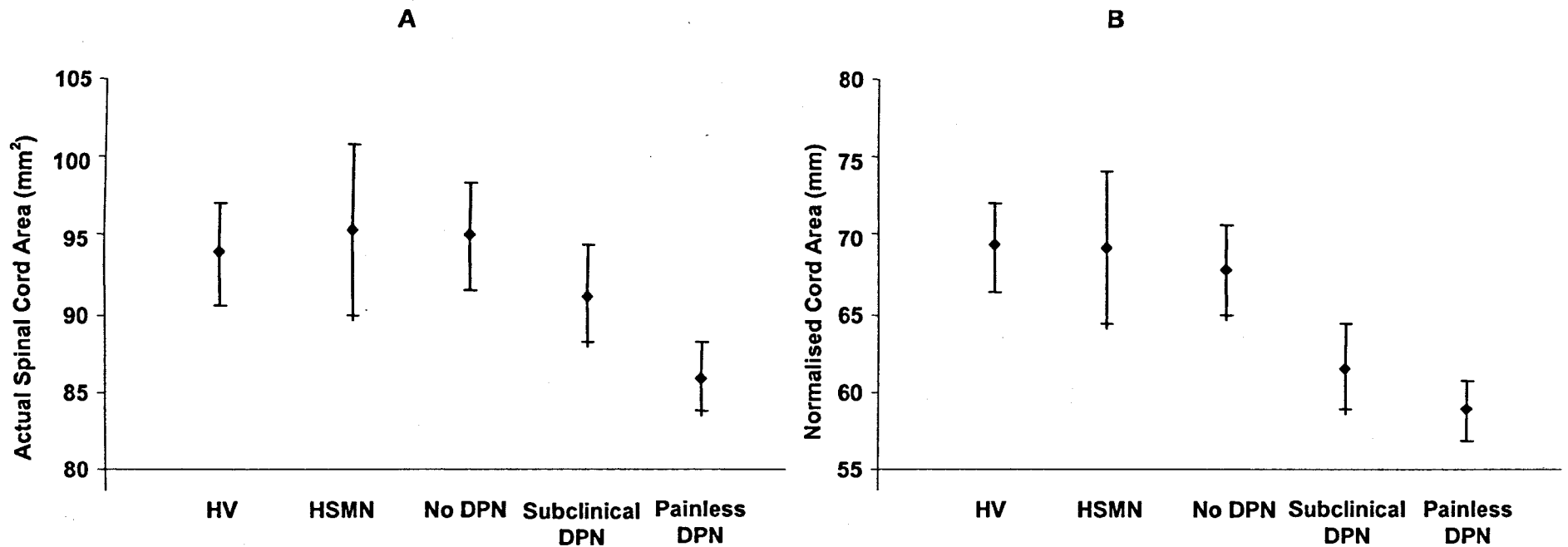


Figure 3.2: Group spinal cord area measurements

A Actual spinal cord area (mm², mean \pm 95 percent confidence interval) per group, adjusted for age, height, weight and antero-posterior diameter of the spinal canal. P-value for trend inverse linear relation of actual cord area in diabetic groups: 0.001.

B Normalised spinal cord area (mm, mean \pm 95 percent confidence interval) per group, adjusted for age, height and weight.

3.5 Discussion

Distal symmetrical diabetic polyneuropathy has traditionally been considered a disease of the peripheral nerve only, with potentially important areas such as the spinal cord being largely overlooked. In a pilot study, using an MR imaging technique, Eaton et al demonstrated that cervical spinal cord area was significantly reduced in advanced DPN compared to normal control patients (Eaton, Harris et al. 2001). In this larger study we confirmed the results of the pilot study and, more importantly, also clearly demonstrate that spinal cord atrophy is an early process being present not only in Painless DPN but also even in patients with relatively modest impairments of nerve function (Subclinical DPN). The significant trend across diabetic groups and the continuous relations found with the NCS indicate a continuing loss of cord area as the disease progresses. Significant correlations were found between cord area and neurophysiological parameters. Cord area was not significantly different between age and sex matched Healthy Volunteers and diabetic patients without DPN. In contrast, unlike DPN, patients with hereditary sensory motor neuropathy (neuropathy control group) had normal cord areas suggesting that the pathological process in this disease is confined to the peripheral nerve.

Actual spinal cord area measurements are well recognized to be affected by patient demographics and positioning in the scanner (Sanfilippo, Benedict et al. 2004). We therefore, carefully accounted for these variations using two methods, both of which indicated early involvement of the spinal cord in DPN. The first employed a statistical method, which adjusted group means of actual cord area for age, weight, height and AP canal diameter. The second technique involved calculating a normalised cord area before adjusting group means for age weight and height. This "normalisation", as used in studies of brain atrophy, is not only an individualised index of cord atrophy but also minimises the effects of patient positioning in the scanner. In addition, systematic errors were minimised by employing a semi-automated computerised technique for measuring actual cord cross-sectional areas

(Losseff, Webb et al. 1996). These steps taken improved both the precision and accuracy and hence reliability of the technique.

The San Antonio Consensus Conference on Diabetic Neuropathy recommended that, for full classification of DPN, at least one measure from each of the following categories needs to be assessed: DPN symptoms, clinical examination, electrodiagnostic tests, quantitative sensory tests and autonomic function tests (ADA 1988). All these clinical and neurophysiological assessments were carried out in each patient to accurately characterise DPN. We then used the NCS, derived from the sum of the NIS LL+7 tests, to quantify the severity of DPN. The NCS has been shown to be more sensitive and reproducible than single attributes of nerve function for the detection and staging of DPN (Dyck, Litchy et al. 2003). In the present study, a minimum score of 4.5 was used to diagnose Painless DPN. However, we regard DPN as a disease continuum, and therefore studied patients with NCS between 1 and 4.5. This, "Subclinical DPN" group, although not fulfilling minimal criteria for the diagnosis of DPN, nonetheless has evidence of impaired nerve function. Thus, the detection and accurate quantification of DPN, crucial for the study, was conducted using well validated methods by a trained physician (Dyck and Thomas 1999).

The findings of this study clearly demonstrate that the neuropathic process in man is not confined to the peripheral nerve and does involve the spinal cord. Worryingly, this occurs early in the neuropathic process. Even at the Subclinical DPN stage, extensive and perhaps even irreversible damage may have occurred. Indeed, with these results in mind, it is not surprising that the variety of therapeutic options so far attempted in DPN have not been successful (Pfeifer and Schumer 1995).

A group of patients with hereditary sensory motor neuropathy, an autosomal dominant inherited form of neuropathy, was studied to represent a disease control group as 1) it is well recognized to only affect the PNS and 2) vascular factors have not been implicated in its pathogenesis (Harding and Thomas 1980; Llewelyn, Thomas et al. 1988).

The absence of spinal cord involvement in hereditary sensory motor neuropathy argues against a “dying back” mechanism, i.e. peripheral nerve damage causing secondary spinal cord “shrinkage” in a progressive “dying back” fashion, in DPN. Hence, it is likely that the insult of diabetes is generalised, concomitantly affecting the PNS and CNS. The significant correlation between neurophysiological parameters and cord area in DPN supports this.

There is increasing evidence for the involvement of microvascular factors in the pathogenesis of axonal loss in the peripheral nerve (Malik 1997). Nerve biopsy and in-vivo studies in man have revealed the presence of endoneurial microangiopathy (Malik, Tesfaye et al. 1994) impaired nerve blood flow (Teskaye, Harris et al. 1993; Eaton, Harris et al. 2003) and nerve hypoxia (Newrick, Wilson et al. 1986). Additionally, the severity of peripheral nerve fiber loss correlates with the degree of endoneurial microangiopathy. Post-mortem findings of microvascular disease within the spinal cord (Olsson, Save-Soderbergh et al. 1968), similar to that seen in the peripheral nerve (Teskaye, Malik et al. 1994), suggest that the same pathogenic mechanisms may be involved in both areas. It is, therefore, likely that the metabolic insult of diabetes (hyperglycemia, insulin resistance, dyslipidemia, hypertension etc.) has a generalised effect on the nervous system with similar vascular processes and axonopathy (neuronal loss) resulting in the observed cord atrophy in DPN (DeFronzo, Hendler et al. 1982; Teskaye, Stevens et al. 1996).

Our secondary objective was to evaluate the effectiveness of this technique as an early marker for DPN, as we currently have no “microalbuminuria equivalent” for DPN. By the time DPN is clinically detectable, severe peripheral nerve fiber loss and endoneurial microangiopathy is present (Malik, Veves et al. 2001). Furthermore, unlike other microvascular complications of diabetes, the early diagnosis of DPN currently relies on a combination of detailed clinical and neurophysiological assessments, that not only are time consuming and costly, but also have major limitations including high inter-observer variability, insensitivity to changes over time and non-linearity of the measured parameters (Perkins and Bril 2003). Our findings suggest that spinal cord cross-sectional area

measurement using this quick, non-invasive and operator independent MR technique, may serve as an additional tool in the early detection and accurate quantification of DPN.

Finally, this demonstration of early spinal cord involvement builds on a history of hints throughout the literature that DPN is more than just a disease of the peripheral nerve, and calls into question the concept of pure “peripheral” diabetic neuropathy. Apart from glycaemic control, there are no treatments that are able to halt the neuropathic process in diabetes. A complete understanding of the full extent of CNS involvement is crucial to elucidating the pathogenesis of DPN and facilitating the development of rational treatments. Recognition that DPN is, in part, a disease which affects the whole nervous system, should trigger a critical rethinking of this disorder, opening a new direction for further research. Prospective studies are now required to determine the natural history of cord involvement In DPN.

4 THALAMIC NEURONAL DYSFUNCTION IN PATIENTS WITH TYPE I DIABETES MELLITUS AND CHRONIC SENSORIMOTOR DISTAL SYMMETRICAL POLYNEUROPATHY

4.1 Introduction

Diabetes is a leading cause of neuropathy (Boulton, Malik et al. 2004) with important associated health (Jeffcoate and van Houtum 2004) and economic implications (Boulton 1997). Relatively little is known about the pathophysiology underlying DPN (Dyck and Giannini 1996; Cameron, Eaton et al. 2001; Malik, Veves et al. 2001) although it has recently been shown to be associated with cardiovascular risk factors (Tesfaye, Chaturvedi et al. 2005).

Research into DPN has focused mainly on the PNS with CNS involvement being overlooked. However, we have demonstrated a significantly lower cross-sectional area of the cervical spine in DPN on MR imaging (Eaton, Harris et al. 2001). In Chapter 3, we reported that spinal cord 'atrophy' is present not only in patients with Painless DPN but also in those with early (Subclinical) DPN (Selvarajah, Wilkinson et al. 2006). This suggests that the metabolic insult of diabetes has a generalised effect on the whole nervous system, and has made us question whether the brain too may be involved.

Anatomical studies have demonstrated that ascending sensory pathways of the spinal cord terminate within the thalamus before higher order sensory projections are sent to the cortex (Wilson, Kitchener et al. 1999). The representation of body surface in the form of somatotopic maps is a fundamental feature of somatosensory organisation. Such maps have been demonstrated at all levels of the somatosensory system including the thalamus in many mammalian species. Within the central core of the ventroposterior thalamus, the tactile receptors of the body surface are represented as a single complete map of the contralateral body. In apparently all mammals, the central region of the ventroposterior complex is composed of a lateral division (VPI), which represents the body and a medial division (VPM),

which represents the face (Figure 4.1). The thalamus is not merely the principal sensory relay station but is thought to process/modulates the information that is presented to the cortex. It is exposed to rich modulatory influences that come from the cortex, the thalamic reticular nucleus, and the brain stem (Sherman and Guillery 2002). This modulation represents an important function of the thalamic relay, allowing it to modulate transmission to the cortex in accord with current attentional needs. The modulation can be global or it can be highly localised and specific; it can allow for changing interactions between one circuit (or part of one circuit) and another in a complex pattern of interactions. Hence, in the presence of sensory nerve dysfunction that accompanies painless DPN, the specific aim of this study was to investigate whether thalamic function is also impaired.

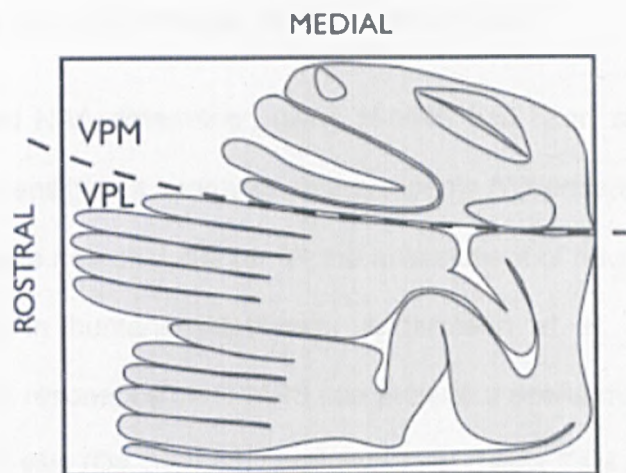


Figure 4.1: Somatotopic representation of tactile receptors of the body surface in the thalamus. VPL, ventroposterior lateral, VPM, ventroposterior medial.

The investigative modality used to test this hypothesis was H-MRS. In-vivo H-MRS is a non-invasive technique that can provide metabolic information from different body tissues, including the brain. In the latter context, it has been used to study the classification and pathophysiology of various neurological conditions including neoplasms, viral and retroviral infections, ischaemia, demyelination and some forms of epilepsy and dementias. Spectroscopy and imaging can be performed during the same patient examination, the information yielded by H-MRS often being considered an adjunct to that provided by imaging

(Wilkinson, Griffiths et al. 2001). Indeed, in some pathologies, neurochemical abnormalities may be present on H-MRS prior to abnormalities being detected on imaging (Wilkinson, Hadjivassiliou et al. 2005). Conventional MRI and H-MRS rely on the same physical principles to collect the MR signal, but differ in the way the data is processed, displayed, and interpreted. H-MRS, as used in the present study, produces spectra that contain several resonances or peaks. In brain parenchyma, the three major peaks detected are due to N-acetyl groups, total creatine (Cr) and choline (Cho) containing compounds. Immunohistochemical studies have suggested that N-Acetyl Aspartate (NAA), the major constituent of the N-acetyl group resonance at long echo time (TE), is localised exclusively in neurons and their processes throughout the CNS (Moffett, Namboodiri et al. 1991; Simmons, Frondoza et al. 1991; Urenjak, Williams et al. 1993).

In-vivo cerebral NAA determined using H-MRS has been shown to correlate with histological neuronal density in a variety of animal models (Wilkinson, Lunn et al. 1997). It is also used as a surrogate neuronal marker for the assessment of neuroprotective therapeutic compounds/strategies in humans (Matthews, Andermann et al. 1990). It is generally accepted that the NAA resonance on H-MRS can provide a useful marker for brain neuronal and axonal integrity in-vivo (De Stefano, Matthews et al. 1995; Tsai and Coyle 1995; Hugg, Kuzniecky et al. 1996; Nakano, Ueda et al. 1998).

An H-MRS study was conducted utilising NAA resonance as a surrogate marker for neuronal function to test the hypothesis that damage to the peripheral sensory nerves caused by diabetes mellitus may be accompanied by thalamic neuronal dysfunction. This study also aimed to characterise the relationship between thalamic neuronal biochemistry and traditional neurophysiological assessments of the peripheral nerves reflecting severity of painless DPN.

4.2 Methods

4.2.1 Patients

Twenty eight right handed male patients with diabetes from the Royal Hallamshire Hospital Diabetes Register were screened for the study. To be eligible, participants had to fulfil the following inclusion criteria: type 1 diabetes diagnosed for more than five years and age between 18 - 65 years. Patients were excluded for the following reasons: cerebrovascular disease, significant back problems (either known degenerative back disease or symptoms which have occurred on a regular basis, or have required consultation for investigation and treatment), history of spinal trauma, non diabetic neuropathies, history of alcohol consumption of more than 20 units a week (one measure of alcohol e.g. glass of wine equals one unit), diabetic neuropathies other than DPN (e.g. mononeuropathies, proximal motor neuropathies etc), painful DPN, internal diseases potentially affecting cerebral metabolism (hepatic disease, Cushing's disease etc), diabetic ketoacidosis in the preceeding six months, hypoglycaemia in the previous 24 hours, claustrophobia or factors which precluded MR imaging (e.g. cardiac pacemaker). Six age- and sex-matched non-diabetic Healthy Volunteers were also recruited. All patients gave written, informed consent before participation in the study, which had prior approval by the South Sheffield Regional Ethics Committee.

4.2.2 Assessment of neuropathy

Detailed neurological assessment was undertaken to identify the presence and quantify the severity of neuropathy in each patient. Neuropathic symptoms were documented by completion of the NSS questionnaire and then the outcome of a detailed neurological examination was graded by defined criteria according to the standard NIS questionnaire (Dyck and Thomas 1999). All patients underwent: 1) perception cooling detection thresholds acquired from the dorsal aspect of the right foot using the Computer Assisted Sensory Evaluation IV (CASE IV, W.R. Electronics, Stillwater, MN, USA) system employing standard

techniques (Dyck, O'Brien et al. 1993; Dyck, Zimmerman et al. 1993); 2) cardiac autonomic function tests performed with a computer assisted technique (Dyck and Thomas 1999) and 3) nerve conduction studies performed, at a stable skin temperature of 31°C and a room temperature of 24°C, using a Medelec electrophysiological system (Synergy Oxford Instruments, Oxford, UK). The following nerve attributes were measured: 1) sural sensory nerve action potentials and conduction velocities and 2) common peroneal and tibial motor nerve distal latency, compound muscle action potential and conduction velocity. Further details on the neurophysiological procedures employed can be found in Appendix 2.

Based on these clinical and neurophysiological assessments diabetic patients were divided into two groups: 1) **No DPN** consisting of asymptomatic patients with normal clinical and neurophysiological assessments; 2) **Painless DPN**, comprising of pain free patients with both clinical and neurophysiological abnormalities (at least two abnormalities of neurophysiologic assessment)(ADA 1988). In addition, a NCS derived from the assessments described above (NISLL+7) was calculated (Dyck, Davies et al. 1997; Dyck, Litchy et al. 2003). A full description of the method of calculation has been described in Appendix 2. This scoring system takes into account the findings of neurological examination and neurophysiological assessments with a higher score indicating a more severe neuropathy.

4.2.3 Magnetic resonance spectroscopy protocol

All patients underwent H-MRS examination on a 1.5T MR system (Eclipse, Philips Medical Systems, Cleveland, Ohio) using a standard quadrature receive-only head coil. Prior to spectroscopy, a set of transaxial T2-weighted images (TE=90ms; TR=10500ms, ETL=16; 30 contiguous slices of thickness 5mm; acquisition matrix=256x256 over a 240mm field of view) was acquired using a fast-spin-echo technique to guide the placement of the spectroscopic region-of-interest. Single-voxel spectra were obtained from an 8ml cubic volume of interest placed within the right thalamus to encompass the ventral posterior lateral sub-nucleus

(Figure 4.2). Care was taken to avoid inclusion of ventricular CSF within the spectroscopic voxel. Two spectra were acquired from each patient:

- 1) Long echo time (long TE) (TE=135ms, TR=1600ms) using a point-resolved (PRESS) technique (Figure 4.3).
- 2) Short echo time (short TE) (TE=20ms, TR=5000ms) using a stimulated-echo acquisition mode (STEAM) technique with a mixing time of 12ms (Figure 4.4).

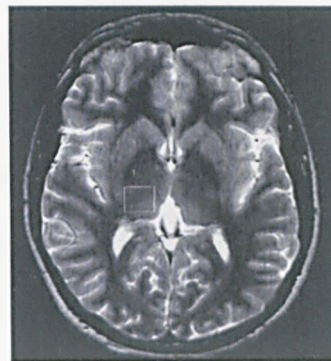


Figure 4.2: Axial section of the brain with voxel positioned to encompass the ventroposterior thalamic subnucleus.

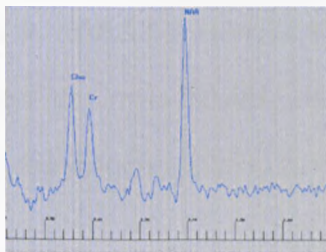


Figure 4.3

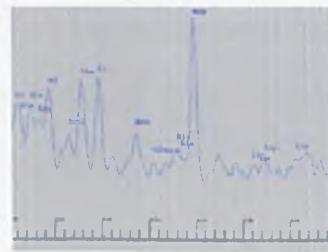


Figure 4.4

Figure 4.3: Example spectra obtained at long echo time using a point-resolved acquisition technique.

Figure 4.4: Example spectra obtained at short echo time using a stimulated-echo acquisition technique.

For each acquisition, an automated shimming algorithm was used prior to water suppression to maximise the homogeneity of the magnetic field within the spectroscopic volume of interest. Following shimming, the optimum amplitude of the water suppression pulse was determined manually in an iterative fashion. A total of 1024 data points were sampled and averaged 192 times on both occasions. A reference water spectrum (suppression pulse set at zero) was obtained automatically following the acquisition of the water suppressed data.

4.3 Data analysis

MR spectra were analysed and reviewed independently by an MR physicist with extensive experience using H-MRS in neurological diseases and clinical research studies. Throughout the analyses the assessor was unaware of the group classification of individual patients. All post acquisition processing was performed using fully integrated proprietary software from the manufacturer of the MR system. By convention long TE results are expressed as ratios under the three prominent resonances assigned to Cho (3.22ppm), Cr (3.02ppm) and NAA (2.02ppm) ie. NAA:Cho; NAA:Cr and Cho:Cr ratios. Short TE results were calculated as the areas under the *myo*-Inositol (ml at 3.56ppm), Cho (3.22ppm), Cr (3.02ppm) and NAA (2.02ppm) resonances relative to that of unsuppressed water.

4.3.1 Statistical method

All analyses were performed using the statistical package SPSS 11.1. Subgroup demographics were described as means and standard deviation for normally distributed variables and as medians and 5th and 95th percentiles for variables with a skewed distribution.

The appropriate tests for normality were conducted to guide subsequent statistical analysis. The appropriate tests for normality were conducted to guide subsequent analysis. Subgroup H-MRS metabolite endpoints were compared using non-parametric tests

(Kruskal–Wallis). Any relationships between these endpoints and neurophysiological assessments were analysed in more detail among participants with diabetes, using Spearman's rank correlation coefficients.

4.4 Results

4.4.1 Subgroup Demographics

Demographics of the study groups are summarised in Table 4.1. The mean ages, height and weight of the Painless DPN, No DPN and Healthy Volunteer groups were not significantly different (ANOVA, $p=0.63$, 0.62 , 0.12 respectively). As expected, patients in the DPN group had a longer duration of diabetes ($p=0.02$) and higher HbA1c levels ($p=0.02$).

Table 4.1: Subgroup Demographics [mean(SD)].			
	HV	No DPN	Painless DPN
Group Size (n)	6	8	10
Age (years)	42.5(15.4)	42.9(8.7)	48.3(11.5)
DD (years)*	N/A	11.6(7.7)	22.1(8.8)
HbA1c (%)*	N/A	7.8(0.9)	9.1(1.2)
Weight (Kg)	82.7(15.1)	91.6(15.6)	80.0(6.9)
Height (m)	176.2(13.6)	171.6(6.4)	178.6(5.3)
NCS	N/A	0.57(0.8)	7.83(3.3)

HV, Healthy Volunteers; **No DPN**, Diabetic patients with no neuropathy; **Painless DPN**, Diabetic patients with painless neuropathy. **DD**, Diabetes Duration, **NCS**, Neuropathy Composite Score, **N/A**, not applicable. * denotes statistically significant difference between No DPN and Painless DPN ($p=0.02$).

4.4.2 MR spectroscopy assessments

The Painless DPN group had lower mean NAA:Cr ratios at long TE (median [range] 1.55 [0.81]; $p=0.04$) than No DPN participants (1.79 [0.98]) and healthy volunteers (1.90 [0.42]) (Table 4.2, Figure 4.5a). NAA:Cho ratio was also lowest in participants with Painless DPN (1.55 [0.56]; $p=0.02$) compared with No DPN (1.80 [0.46]) and healthy volunteers (0.76 [0.28], Figure 4.5b). The Cho:Cr ratio obtained at long TE did not vary significantly between the groups (Painless DPN 1.04 [0.6], No DPN 0.95 [0.82], Healthy Volunteers 1.06 [0.76]; $p=0.87$).

Analysis of mean NAA metabolite resonances obtained at short-TE revealed no statistically significant difference between these groups ($p=0.07$). No significant differences were observed with any of the other metabolites at short TE between any of the groups.

4.4.3 Spectroscopy and neurophysiological correlations

A significant positive association between both sural amplitude ($r =0.61$, $p=0.004$) and sural nerve conduction velocity ($r =0.58$, $p=0.006$) and long TE NAA:Cr ratio signal was observed among participants with diabetes. In addition, the NAA:Cr ratio signal was significantly associated with peroneal latency ($r =-0.53$, $p=0.01$), amplitude ($r =0.67$, $p=0.001$) and velocity ($r =0.37$, $p=0.06$) in this group. Similar correlations among participants with diabetes were noted with tibial nerve latency ($r =-0.51$, $r=0.02$), but not with velocity ($r =0.23$, $p=0.18$). In this groups, vibration perception threshold was related to NAA:Cho ratio at a significant level ($r =-0.70$, $p=0.004$), and we also observed a significant correlation between heart rate variability with deep breathing and NAA:Cr ratio ($r =-0.46$, $p<0.05$). In addition, the NAA:Cr ratio was also found to relate to overall NCS ($r =-0.53$, $p=0.03$) among diabetic participants.

Table 4.2: H-MRS subgroup metabolite ratios (acquired at long echo time) and metabolite areas relative to that of unsuppressed water (acquired at short echo time).

	Healthy Volunteers	No DPN	Painless DPN
Long TE			
NAA:Cho	1.74(0.11)*	1.80(0.15)*	1.58(0.16)*
NAA:Cr	1.87(0.15)	1.82(0.35)	1.55(0.24)
Cho:Cr	1.08(0.14)	0.95(0.28)	1.01(0.13)
Short TE			
NAA	0.78(0.08)	0.88(0.10)	0.77(0.11)
Cho	0.52(0.16)	0.50(0.11)	0.49(0.16)
Cr	0.60(0.18)	0.58(0.19)	0.59(0.06)

N-Acetyl Aspartate to Choline ratio (**NAA:Cho**), N-Acetyl Aspartate to Creatine ratio (**NAA:Cr**), Choline to Creatine ratio (**Cho:Cr**), N-Acetyl Aspartate (**NAA**), Choline (**Cho**), Creatinine (**Cr**). **No DPN**, diabetic patients with no neuropathy, **Painless DPN**, diabetic patients with painless DPN. Results are given as mean (SD) for variables with a normal distribution. * ANOVA p=0.01, Painless DPN vs No DPN p=0.005, Painless DPN vs Healthy Volunteers p=0.004.

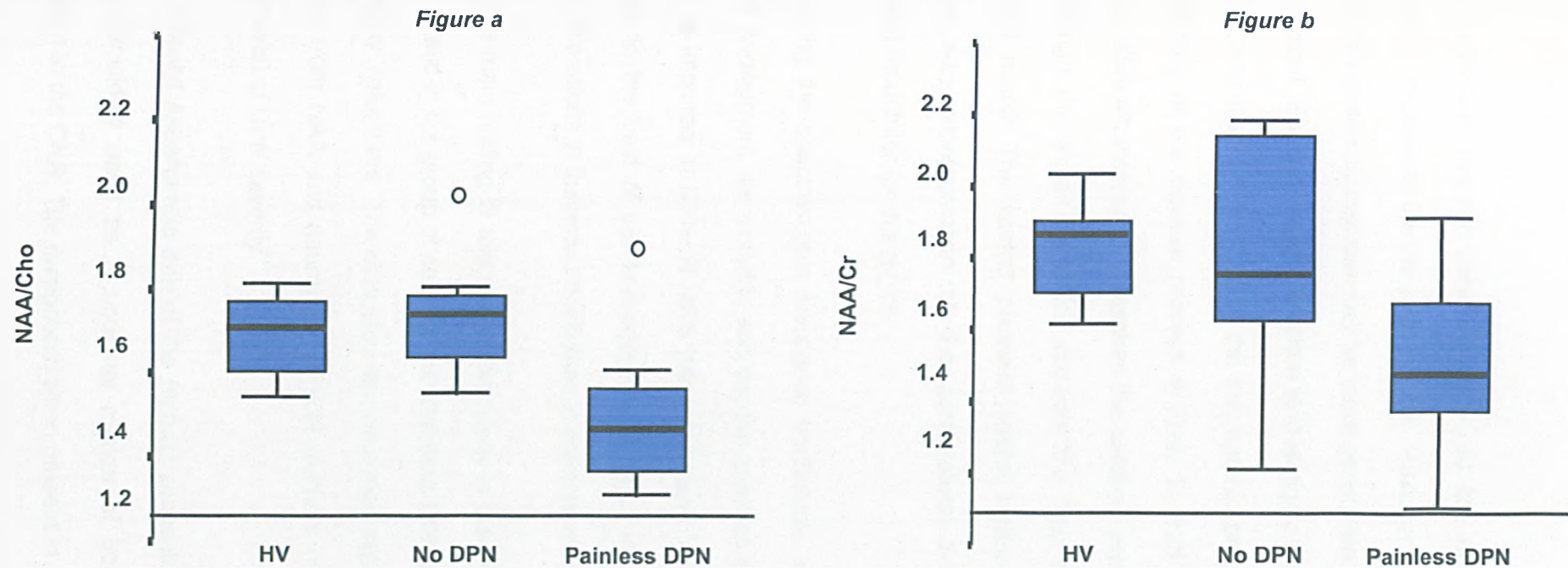


Figure 4.5 a and b: Mean N-Acetyl Aspartate/Choline [NAA/Cho (A)] and N-Acetyl Aspartate/Creatine [NAA/Cr (B)] ratios at long echo time in Healthy Volunteers (HV), diabetic patients with neuropathy (Painless DPN) and no neuropathy (No DPN).

The inferior and superior margins of each box represent the first and third quartiles, the central line is the mean, and the bars at either end represent the standard deviations. \circ represents outliers. * ANOVA $p=0.01$, Painless DPN vs No DPN $p=0.005$, Painless DPN vs Healthy Volunteers $p=0.004$.

4.5 Discussion

Distal symmetrical diabetic polyneuropathy, as the name indicates, has hitherto been considered a disease of the peripheral nerve. However, evidence is now emerging that the impact of this metabolic disorder may be more generalised than previously thought, involving the spinal cord. It seems logical therefore to investigate if key areas of the brain, such as the thalamus, may also be involved in the neuropathic process, as a fuller appreciation and understanding of the disease process is likely to lead to more effective treatments. All somatosensory information that reaches the cerebral cortex from the spinal cord must first pass through the thalamus, which controls the flow of information to the cortex and subcortical nuclei. The ventral posterior lateral nucleus of the thalamus contains the somatosensory representation of the contralateral body surface and serves as an information modulator for this region.

Using the spectroscopic resonance attributable to NAA as a surrogate marker of neuronal involvement, we aimed to address the question whether thalamic sensory neuronal function is impaired in DPN. H-MRS has been used to investigate a wide range of CNS diseases; to the best of our knowledge no H-MRS study has been previously reported showing alterations in thalamic metabolites in patients with DPN.

The main finding of this preliminary study is the significantly lower long TE thalamic NAA/Cho ratio in the group of patients with Painless DPN compared to patients with No DPN and Healthy Volunteers. The data also demonstrates significant correlations between short TE signal from NAA and neurophysiological markers (overall NCS and individual nerve function tests) of DPN severity.

N-acetyl Aspartate is one of the highest concentrations of free amino acids in the brain. It is widely used as a neuronal marker of both brain pathology and disease progression in the CNS. The compound, when present in the brain appears to reflect a high

degree of cellular integration, and therefore may be a unique metabolic construct of the intact vertebrate brain. It is believed that a reduction in the NAA resonance or NAA/Cho ratio is associated with neuronal and/or axonal loss, loss of neuron viability and dysfunction. By showing metabolite signal changes, our results may reflect the presence of thalamic neuronal dysfunction in patients with Painless DPN. As NAA is measured in a voxel i.e. a unit of volume, reduction in NAA could be due to either a decreased density of neurons or a change in neurochemistry accompanying neuronal injury/dysfunction. We used two H-MRS acquisition techniques at different echo times (short TE and long TE) to interrogate NAA signals within the ventral posterior lateral thalamic subnucleus. Each technique can provide unique information on the structure and function of neuronal cell bodies and axons. Reduced NAA signal at short TE is thought to represent irreversible neuronal loss/shrinkage whereas possible reversible neuronal injury/dysfunction can be implicated when the NAA/Cho signal ratio is reduced at long TE [17]. We demonstrated a significant difference in NAA/Cho ratio at long TE but no significant difference in the NAA resonance at short TE between the patient groups. In such a scenario our findings may reflect thalamic neuronal dysfunction in DPN rather than overt neuronal death.

Previous H-MRS studies in diabetes have mainly focused on its metabolic impact on the developing brain (Cameron, Kean et al. 2005; Sarac, Akinci et al. 2005) and the cerebral consequences of hypoglycaemia (Criego, Tkac et al. 2005; Rankins, Wellard et al. 2005) or diabetic ketoacidosis (Wootton-Gorges, Buonocore et al. 2005). Observations of cerebral NAA-moieties in diabetes mellitus that have previously been reported are contradictory. Kreis et al. found several metabolic abnormalities in the brain of patients with diabetes (Kreis and Ross 1992). In particular they found a significantly lower NAA/Cr ratio in the parietal region of patients with diabetes compared with that in age-matched controls. This contrasts with a study by Geissler et al. that found no differences in NAA signal between patients with diabetes and Healthy Volunteers in either the parietal or the occipital lobes (Geissler, Frund

et al. 2003). Neither of these studies, however, performed detailed neurophysiological assessments to quantify DPN nor focused H-MRS examination on the thalamus.

The mechanism of thalamic involvement is unclear. One possible explanation for thalamic neuronal dysfunction in DPN may be that loss of afferent input, as a result of peripheral nerve damage, subsequently causes changes to occur at progressively higher levels in the CNS ("dying back" mechanism). The correlations observed between NAA acquired at short TE, duration of diabetes and severity of neuropathy would seem to support this. Another possible explanation is that the observed changes in the thalamus may be occurring concomitantly to the changes seen in the PNS. Nonetheless, thalamic neuronal involvement whether early, late or concomitant, is likely to result in disturbed sensory gating in DPN. This may have consequences on sensory perception and pain modulation. Further studies utilising these techniques on a subgroup of patients with painful DPN are necessary to elucidate this.

Taken together with the previously described early spinal cord involvement in DPN, the possibility of thalamic sensory neuronal dysfunction suggests that nervous system involvement is not merely confined to the peripheral nerves but involves the spinal cord and brain. Prospective studies are required to determine at what stage during the course of the disease these abnormalities occur. It is noteworthy that a variety of therapeutic interventions specifically targeted at peripheral nerve damage in DPN have thus far been ineffective, and it is possible that this may in part be due to inadequate appreciation of the full extent of CNS involvement in DPN.

Major advances in non invasive MR imaging including H-MRS, tractography and functional MR imaging offer non-invasive techniques capable of elucidating various aspects of brain pathophysiology and function in DPN. Recognition that DPN is, in part, a disease which affects the whole nervous system, should trigger a critical rethinking of this disorder, opening a new direction for further research.

5 RAISED LEVELS OF GLUTAMATE/GLUTAMINE IN DIABETES, A CLUE TO THE PATHOGENESIS OF THALAMIC NEURONAL DYSFUNCTION IN DISTAL SYMMETRICAL DIABETIC POLYNEUROPATHY

5.1 Introduction

Distal symmetrical diabetic sensory motor neuropathy is one of the commonest diseases to affect the nervous system and a leading cause of amputations in the western world (Hollingshead 1991). Involvement of the CNS is increasingly being recognised. Using H-MRS, we have reported the presence of thalamic neuronal dysfunction in patients with Painless DPN (Selvarajah, Wilkinson et al. 2008). The thalamus plays a central role in somatosensory perception (Wilson, Kitchener et al. 1999) and understanding the pathological changes that lead to thalamic impairment is vitally important. Although these changes are currently unknown, it is likely that metabolic and vascular processes that have been implicated in the pathogenesis of diabetic microvascular complications are involved.

In this chapter the possible role of metabolic factors in the pathogenesis of thalamic neuronal dysfunction in DPN were examined. Glutamate is the main excitatory neurotransmitter in the brain (Shepherd and Haganir 2007) and has been implicated in the induction of neuronal injury and apoptosis in several neurodegenerative disorders (e.g. Alzheimer's disease) (Mattson and Magnus 2006). Astrocytes are essential for maintaining cerebral glutamate homeostasis, responsible for recycling released glutamate back to neurons in the form of glutamine (Takahashi, Billups et al. 1997). Thalamic astrocyte dysfunction may contribute to elevated extracellular glutamate, and hence excitotoxicity to thalamic neurons and glia in diabetic patients with DPN. Little is known regarding changes in glutamate levels in the thalamus in diabetics patients with DPN.

This study used H-MRS to assess thalamic metabolite abnormalities in diabetic patients with DPN. We hypothesized that diabetic patients would show elevated glutamate/glutamine (Glx) resonance due to a hyperglutamertergic state, decreased

myoinositol (ml), an astrocyte marker, due to possible astrocyte dysfunction (Brand, Richter-Landsberg et al. 1993).

5.2 Methods

5.2.1 Patients

Twenty four right handed male patients with diabetes from the Royal Hallamshire Hospital Diabetes Register were screened for the study. To be eligible, participants had to fulfil the following inclusion criteria: type 1 diabetes diagnosed for more than five years and age between 18 - 65 years. Patients were excluded for the following reasons: cerebrovascular disease, significant back problems (either known degenerative back disease or symptoms which have occurred on a regular basis, or have required consultation for investigation and treatment), history of spinal trauma, non diabetic neuropathies, history of alcohol consumption of more than 20 units a week (one measure of alcohol e.g. glass of wine equals one unit), diabetic neuropathies other than DPN (e.g. mononeuropathies, proximal motor neuropathies etc), painful DPN, internal diseases potentially affecting cerebral metabolism (hepatic disease, Cushing's disease etc), diabetic ketoacidosis in the preceding six months, hypoglycaemia in the preceding 24 hours, claustrophobia or factors which precluded MR imaging (e.g. cardiac pacemaker). Six age- and sex-matched non-diabetic Healthy Volunteers were also recruited. All patients gave written, informed consent before participation in the study, which had prior approval by the South Sheffield Regional Ethics Committee.

5.2.2 Assessment of Neuropathy

Detailed neurological assessment was undertaken to identify the presence and quantify the severity of neuropathy in each patient. Neuropathic symptoms were documented by completion of the NSS questionnaire and then the outcome of a detailed neurological examination was graded by defined criteria according to the standard NIS questionnaire

(Dyck and Thomas 1999). All patients underwent: 1) vibration and cooling perception thresholds acquired from the dorsal aspect of the right foot using the Computer Assisted Sensory Evaluation IV (CASE IV, W.R. Electronics, Stillwater, MN, USA) system employing standard techniques (Dyck, O'Brien et al. 1993; Dyck, Zimmerman et al. 1993); 2) cardiac autonomic function tests performed with a computer assisted technique (Dyck and Thomas 1999) and 3) nerve conduction studies performed, at a stable skin temperature of 31°C and a room temperature of 24°C, using a Medelec electrophysiological system (Synergy Oxford Instruments, Oxford, UK). The following nerve attributes were measured: 1) sural sensory nerve action potentials and conduction velocities and 2) common peroneal and tibial motor nerve distal latency, compound muscle action potential and conduction velocity. Please refer to Appendix 2 for further details on the neurophysiological procedures employed.

Based on these clinical and neurophysiological assessments diabetic patients were divided into two groups: 1) **No DPN** consisting of asymptomatic patients with normal clinical and neurophysiological assessments; 2) **Painless DPN**, comprising of pain free patients with both clinical and neurophysiological abnormalities (at least two abnormalities of neurophysiologic assessment) (ADA 1988). In addition, a NCS derived from the assessments described above (NISLL+7) was calculated (Dyck, Davies et al. 1997; Dyck, Litchy et al. 2003). A full description of the method of calculation is described in Appendix 2. This scoring system takes into account the findings of neurological examination and neurophysiological assessments with a higher score indicating a more severe neuropathy.

5.2.3 Magnetic Resonance Spectroscopy Protocol

All patients underwent H-MRS examination on a 1.5T MR system (Eclipse, Philips Medical Systems, Cleveland, Ohio) using a standard quadrature receive-only head coil. Prior to spectroscopy, a set of transaxial T2-weighted images (TE=90ms; TR=10500ms, ETL=16; 30 contiguous slices of thickness 5mm; acquisition matrix=256x256 over a 240mm field of view) were acquired using a fast-spin-echo technique to guide the placement of the spectroscopic

region-of-interest. Single-voxel spectra were obtained from an 8ml cubic volume of interest placed within the right thalamus to encompass the ventral posterior lateral sub-nucleus (Figure 4.2). Care was taken to avoid inclusion of ventricular CSF within the spectroscopic voxel. Short echo time (short TE, TE=20ms, TR=5000ms) using a stimulated-echo acquisition mode (STEAM) technique with a mixing time of 12ms.

For each acquisition, an automated shimming algorithm was used prior to water suppression to maximise the homogeneity of the magnetic field within the spectroscopic volume of interest. Following shimming, the optimum amplitude of the water suppression pulse was determined manually in an iterative fashion. A total of 1024 data points were sampled and averaged 192 times on both occasions. A reference water spectrum (suppression pulse set at zero) was obtained automatically following the acquisition of the water suppressed data.

5.3 Data Analysis

MR spectra were analysed and reviewed independently by an MR physicist with extensive experience using H-MRS in neurological diseases and clinical research studies. Throughout the analyses the assessor was unaware of the group classification of individual patients. All post acquisition processing was performed using fully integrated proprietary software from the manufacturer of the MR system. By convention short TE results were calculated as the areas under the Glx (2.1-2.5ppm), *myo*-Inositol (ml 3.56ppm) and NAA (NAA 2.02ppm) resonances relative to that of unsuppressed water.

5.3.1 Statistical Method

All analyses were performed using the statistical package SPSS 11.1. Subgroup demographics were described as means and standard deviation for normally distributed variables and as medians and 5th and 95th percentiles for variables with a skewed distribution. Analysis of variance (ANOVA) was used to compare subgroup demographics.

The appropriate tests for normality were conducted to guide subsequent statistical analysis. Group (Healthy Volunteers, No DPN and Painless DPN) mean Glx and NAA metabolite areas at short TE relative to unsuppressed water were compared using ANOVA. Mean differences in cerebral metabolites were also analysed using analysis of covariance (ANCOVA) with age as a covariate.

5.4 Results

5.4.1 Subgroup Demographics

Demographic details of the study groups are summarised in Table 5.1. The mean ages, height and weight of the Painless DPN, No DPN and Healthy Volunteers groups were not significantly different (ANOVA, $p=0.63$, 0.62 , 0.12 respectively). As expected, patients in the No DPN group had a longer duration of diabetes and higher HbA1c levels ($p=0.02$).

Table 5.1: Subgroup Demographics.			
	HV	No DPN	Painless DPN
Group Size (n)	6	8	10
Age (years)	42.5(15.4)	42.9(8.7)	48.3(11.5)
DD (years)*	N/A	11.6(7.7)	22.1(8.8)
Weight (Kg)	82.7(15.1)	91.6(15.6)	80.0(6.9)
HbA1c (%)*	N/A	7.8(0.9)	9.1(1.2)
NCS	N/A	0.57(0.8)	7.83(3.3)

HV, Healthy Volunteers; No DPN, Diabetic patients with no neuropathy; Painless DPN, Diabetic patients with neuropathy. DD, Duration of Diabetes, N/A, not applicable. NCS, Neuropathy Composite Score. Mean(SD), * denotes statistically significant difference between No DPN and Painless DPN ($p=0.02$).

5.4.2 MR Spectroscopy Assessments

5.4.2.1 Glutamine and glutamate

Two patients (one patient from each diabetic subgroup) were excluded from the final analysis because of poor spectra quality. Spectroscopic data obtained at short TE are summarised in Table 5.2. In the full sample of patients with diabetes thalamic Glx levels were significantly higher [mean(SD) 0.47(0.12)] compared with Healthy Volunteers [0.27(0.13), $p=0.001$]. A one-way ANOVA revealed a main effect of group [Healthy Volunteers, 0.27(0.14); No DPN, 0.53(0.13); Painless DPN, 0.45(0.12)] on thalamic Glx levels ($p=0.002$). Post hoc pairwise comparisons showed that Healthy Volunteers had significantly lower Glx in comparison with all diabetic subgroups [No-DN ($p=0.001$; 95% confidence interval (95%CI) -0.41:-0.12), Painless DPN ($p=0.006$; 95%CI -0.31:-0.06)]. There was no significant difference in Glx between diabetic subgroups ($p=0.15$; 95%CI -0.03:0.20).

5.4.2.2 myoInositol

Mean ml signal of patients with diabetes [0.38(0.16)] was lower compared with Healthy Volunteers [0.53(0.15); $p=0.67$, 95% CI -0.29:0.01]. Diabetic patients with Painless DPN had lowest ml levels [0.36(0.16)] compared with patients with No DPN [0.42(0.17)] and Healthy Volunteers [0.52(0.15), ANOVA $p=0.14$]. Post hoc pairwise comparisons showed that Painless DPN patients were significantly lower compared with Healthy Volunteers ($p=0.05$; 95% CI -0.32:<0.001).

Table 5.2: H-MRS patient group metabolite areas relative to that of unsuppressed water (acquired at short echo time).

	Healthy Volunteers (n=6)	Diabetes (n=24)	
Short TE			
Glx*	0.27(0.14)	0.48(0.12)	
ml	0.52(0.15)	0.38(0.16)	
	Healthy Volunteers	No DPN (n=16)	Painless DPN (n=8)
Short TE			
Glx*	0.27(0.14)	0.53(0.13)	0.45(0.11)
ml	0.52(0.15)	0.42(0.17)	0.36(0.16)

Glutamate-glutamine (**Glx**), myoinositol (**ml**), N-Acetyl Aspartate (**NAA**). No DPN, diabetic patients with no neuropathy, Painless DPN, diabetic patients with painless DPN. Results are given as mean (SD) for variables with a normal distribution. * ANOVA $p < 0.05$.

5.5 Discussion

Apart from good glycaemic control, there is an overall lack of effective treatments for DPN. This may be because most therapeutic agents have been developed to target pathological changes in the peripheral nerves (Ziegler, Pritchett et al. 2007), with changes occurring within the CNS being overlooked. We have previously demonstrated that the pathological process of DPN may also affect the thalamus, which plays an important role in somatosensory perception. A better understanding of the mechanisms that lead to thalamic neuronal dysfunction may aid the development of new therapeutic agents.

The main finding of this chapter is significantly elevated Glx levels in all patients with diabetes compared with Healthy Volunteers. There was no significant difference in Glx levels between diabetic patients with DPN or without DPN. There was a trend for lower ml signal in patients with Painless DPN compared with No DPN and Healthy Volunteers. Elevated Glx may reflect a hyperglutamatergic state, while decreased ml suggests decreased glial content or function in the thalamus of diabetic patients.

The Glx peak on H-MRS is made up of two compounds, glutamate and glutamine. Glutamate serves as an important excitatory neurotransmitter and has functional role in synaptic plasticity crucial to learning and memory (Lessmann 1998). It is also a potentially damaging agent, involved in neurodegenerative diseases. The pathological effect of glutamate occurs when present in excessive levels as shown in Alzheimers's disease (Lipton 2005). Glutamate in the CNS comes mainly from either glucose, via the Krebs's cycle, or glutamine which is synthesised by glial cells and taken up by neurons. Therefore elevated thalamic Glx levels in our diabetic subgroup could represent either a generalised 'diabetes' effect or a susceptibility to develop thalamic neuronal dysfunction or both.

Unlike previous papers that have used H-MRS to probe changes in brain metabolites related to diabetes (Kreis and Ross 1992; Geissler, Frund et al. 2003), which have shown

increases in ml, in this study there were no significant differences in thalamic ml across study groups. Conversely, however, in this study ml was non-significantly lower in diabetic subgroups. Most of the previous studies, however, did not include the thalamus or quantify the presence of DPN. Myo-inositol is a glial marker and is typically elevated during glial activation or hypertrophy associated with active inflammation or demyelination, such as in the white matter of patients with HIV (Wilkinson, Lunn et al. 1997) or multiple sclerosis (Fernando, McLean et al. 2004). The absence of ml elevation in our diabetic subgroup, especially in patients with DPN, is also different from degenerative brain disorders, such as Alzheimer's and frontotemporal dementia, which show increased ml along with decreased NAA (Kantarci, Knopman et al. 2008; Small, Bookheimer et al. 2008). In these degenerative dementias, elevated ml is thought to reflect a glial response to the neuronal injury. Conversely, our findings suggest decreased or dysfunctional glial response despite abnormal neuronal function (decreased NA:Cho- Chapter 4). Reduced ml has also been demonstrated in the peripheral nerves of diabetic rats and implicated in the pathogenesis of DPN.

Data from animal literature suggests that diabetes can significantly impair glial function (Lechuga-Sancho, Arroba et al. 2006). An important function of glia, especially astroglia, is reuptake of glutamate from the extracellular space after neurotransmission, astroglial dysfunction might prevent this reuptake and lead to excess extracellular glutamate concentration. Glutamate accumulation can lead to overstimulation of postsynaptic glutamate receptors resulting in intracellular calcium overload and neuronal injury/cell death (Lipton and Rosenberg 1994). Hence, elevated thalamic Glx levels amongst patients with diabetes may provide the substrate for subsequent neuronal injury. Glutamate reuptake is also important for shaping of excitatory post-synaptic currents; dysfunctional or decreased glutamate reuptake by glia may also interfere with neuronal function (Lisman 2003).

One limitation of this study is that with a standard short TE H-MRS sequence (TE = 30ms) on a 1.5T MR scanner, the spectral peaks of glutamate and glutamine were

overlapping and difficult to distinguish. Our H-MRS acquisition protocol did not allow sufficiently accurate expression of the glutamate component of the total Glx signal intensity as an absolute concentration of glutamate. Future studies performed on a 3T scanner with spectral editing analyses will aim to address this limitation. A second potential limitation is the inability of H-MRS to distinguish between synaptic and extrasynaptic Glx. We have demonstrated elevated Glx in our diabetic subgroups suggesting these patients have an overall higher precursor pool of glutamate. In a model proposed by Sanacora et al, they postulate that impaired glial function results in elevated extrasynaptic glutamate and glutamine levels (Kugaya and Sanacora 2005). In their model, they distinguished synaptic glutamate levels from extrasynaptic concentrations and suggest that reduced glial function could lead to decreased synaptic release and increases in extrasynaptic glutamate secondary to spillover. The accumulation of glutamate in the extrasynaptic space may trigger a cascade of events that results in neuronal dysfunction/death.

Finally, the thalamus may be inherently susceptible to the complications of diabetes as it is more metabolically active given its central role in somatosensory perception. Thalamic astrocyte dysfunction secondary to diabetes and resultant glutamatergic dysfunction maybe the pathogenetic mechanism leading to thalamic neuronal dysfunction. Given the pathophysiological importance of glutamate further evaluation of the relationship between thalamic H-MRS abnormalities and DPN is needed.

6 CEREBROVASCULAR PERFUSION ABNORMALITIES IN DISTAL SYMMETRICAL DIABETIC POLYNEUROPATHY AND TYPE 1 DIABETES MELLITUS

6.1 Introduction

The presence of thalamic sensory neuronal dysfunction in DPN suggests that CNS involvement is not limited to the spinal cord but also other important areas in the brain, responsible for somatosensory perception, may be affected (Selvarajah, Wilkinson et al. 2008). Although the pathogenesis of thalamic involvement remains unknown, it is likely that both vascular and metabolic aetiological factors that have been postulated in the pathogenesis of DPN and other microvascular complications of diabetes (retinopathy and nephropathy) are involved. A better understanding of the pathogenesis of thalamic dysfunction in DPN could provide new therapeutic targets to treat or prevent this condition.

Neurophysiological and neuropsychological studies of the pathophysiological disturbances caused by diabetes on the human brain reveal both functional and structural abnormalities (Mijnhout, Scheltens et al. 2006). These structural abnormalities appear to mimic those attributable to normal aging (atrophy and leukoariosis), but seem to develop prematurely. Diabetes alters endothelial function and permeability of the blood brain barrier, thus affecting microcirculation and regional metabolism (Horani and Mooradian 2003). Single proton emission computer tomography (SPECT) studies suggest that chronic hyperglycaemia alters CBF in the frontal, temporal, parietal, occipital and cerebellar ROI. These studies analysed regional cerebral perfusion by subdividing the brain into different anatomical ROI and not by regions defined by major vascular territories (anterior cerebral artery [ACA], middle cerebral artery [MCA] and posterior cerebral artery [PCA]). A more accurate assessment of cerebral perfusion abnormalities in diabetes is obtained by investigating regional perfusion of the major vascular territories.

Advances in modern in-vivo MR technology have enabled the acquisition of high quality multi-slice echo planar imaging of the brain with sub-second temporal resolution.

Chelates of gadolinium, a rare earth metal, are commonly used as exogenous contrast agents in MR imaging because of its modulatory effect on both T1 and T2* of tissue within their sphere of influence. This is coupled with fast T2*-weighted MR imaging, which provides the ability to detect the passage of a bolus of gadolinium as it passes through the capillary bed. The result is a spatial map of the brain parenchymal perfusion characteristics (Patel, Siewert et al. 1995).

This study sought to utilise dynamic contrast enhanced MR perfusion to test our hypothesis that thalamic neuronal dysfunction that accompanies DPN is associated with unique perfusion abnormalities and diabetes results in global perfusion abnormalities of the major vascular territories. Thus the aims of this study were firstly, to investigate regional cerebrovascular perfusion in type 1 diabetes and secondly to assess the characteristics of thalamic microcirculation in DPN.

6.2 Methods

6.2.1 Patients

Eighteen right handed male patients with type 1 diabetes from the Royal Hallamshire Hospital Diabetes Register were recruited consecutively for the study. To be eligible, participants had to fulfil the following inclusion criteria: type 1 diabetes diagnosed for more than five years and age between 18 - 65 years. Patients were excluded for the following reasons: stroke, myocardial infarction, congestive cardiac failure, arrhythmia, other clinically important cardiac disease, non diabetic neuropathies, history of alcohol consumption of more than 20 units a week (one measure of alcohol e.g. glass of wine equals one unit), diabetic neuropathies other than DPN (e.g. mononeuropathies, proximal motor neuropathies etc), kidney or liver transplant, renal failure, carotid stenosis and neurological or other systemic disorders, diabetic ketoacidosis in the preceding six months and hypoglycaemia in the preceding 24 hours. Patients with MRI incompatible, metal implants, pacemakers, arterial stents, claustrophobia, history of allergy to iodine or radiological contrast compounds

also were excluded. Diabetic patients were treated for hypertension when clinically diagnosed and all patients were on statins. Medications that could potentially affect cerebrovascular perfusion characteristics were omitted on the morning of the test. These included antihypertensives and antiarrhythmics medications. Six age- and sex-matched non-diabetic Healthy Volunteers who were normotensive and not being treated for any systemic disease were also recruited. All patients gave written, informed consent before participation in the study, which had prior approval by the South Sheffield Regional Ethics Committee.

6.2.2 Assessment of Neuropathy

Detailed neurological assessment was undertaken to identify the presence and quantify the severity of neuropathy in each patient. Neuropathic symptoms were documented by completion of the NSS questionnaire and then the outcome of a detailed neurological examination was graded by defined criteria according to the standard NIS questionnaire. All patients underwent: 1) vibration and cooling perception thresholds acquired from the dorsal aspect of the right foot using the Computer Assisted Sensory Evaluation IV (CASE IV, W.R. Electronics, Stillwater, MN, USA) system employing standard techniques; 2) cardiac autonomic function tests performed with a computer assisted technique and 3) nerve conduction studies performed, at a stable skin temperature of 31°C and a room temperature of 24°C, using a Medelec electrophysiological system (Synergy Oxford Instruments, Oxford, UK). The following nerve attributes were measured: 1) sural sensory nerve action potentials and conduction velocities and 2) common peroneal and tibial motor nerve distal latency, compound muscle action potential and conduction velocity.

Based on these clinical and neurophysiological assessments diabetic patients were divided into two groups: 1) **No DPN** consisting of asymptomatic patients with normal clinical and neurophysiological assessments; 2) **Painless DPN**, comprising of pain free patients with both clinical and neurophysiological abnormalities (at least two abnormalities of

neurophysiologic assessment) and 3) **Painful DPN**, patients with painful symptoms together with clinical and neurophysiological abnormalities.

6.2.3 Magnetic Resonance Perfusion Protocol

MR examinations were performed on a 1.5T system (Eclipse, Philips Medical Systems, Cleveland, Ohio, USA). The MR protocol included the acquisition of transaxial, cranial images using dual-echo fast spin-echo (TE = 20, 90ms; TR = 2000ms) and fluid-attenuated inversion recovery (FLAIR) (TE = 95.9ms; TR = 6000ms; TI = 1800ms) techniques, the latter being acquired both before and after the perfusion assessment.

Parenchymal perfusion was assessed using a multi time point, single shot T2* weighted EPI sequence (TE_{eff} = 60ms; TR = 1.4s; acquisition matrix = 192 x 188, zero filled prior to Fourier transformation to 256 x 256; FOV = 25cm). Twelve 5mm thick contiguous axial slices were acquired over the cerebrum every 1.4s for a total imaging time of 98s, yielding 70 time points. Exogenous perfusion contrast was provided by a 20ml bolus of gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA, Magnevist, Schering AG, Germany), which was followed by a 20ml saline flush, administered intravenously using a power injector (Spectris, Medrad, Netherlands) at a rate of 5ml/sec starting at the 10th imaging time point.

Post acquisition processing was performed using software integrated with the imaging system, in a manner similar to that described previously (Doerfler, Eckstein et al. 2001). As the bolus of gadolinium chelate passes through a ROI, a loss in signal occurs as the transverse relaxation rate increases due to localised dephasing. For each scan episode, the timing of the signal change within user defined ROI's were obtained with respect to those defined by the signal time [S(t)] course of a circular ROI placed within the proximal intracranial internal carotid artery. Haemodynamic anatomical ROI's were applied to outline eight regions as illustrated on Figure 6.1 and 6.2. These regions outline major intracranial

vascular territories (ACA, MCA and PCA) and deep brain nuclei (thalamus and caudate nucleus). The caudate nucleus was chosen as an internal control for subgroup comparisons.

6.3 Data analysis

Two levels of analyses were performed. Firstly, we compared perfusion of the major vascular territories (ACA, MCA and PCA; Figure 6.2) between Healthy Volunteers with all diabetic patients. Variables in these analyses were derived using Model A (described below). Next, we subdivided diabetic patients into three neuropathy subgroups (No DPN, Painful DPN and Painless DPN) and compared deep brain nuclei perfusion characteristics with Healthy Volunteers. Variables for this latter analysis were derived using Model A and B (described below).

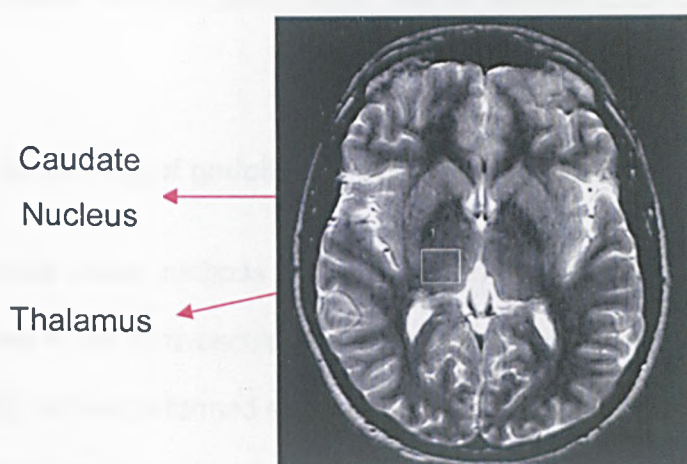


Figure 6.1: Cross sectional image of the cerebral cortex at the level of the thalamus. Red circles denote regions of interests within the thalamus and caudate nucleus.

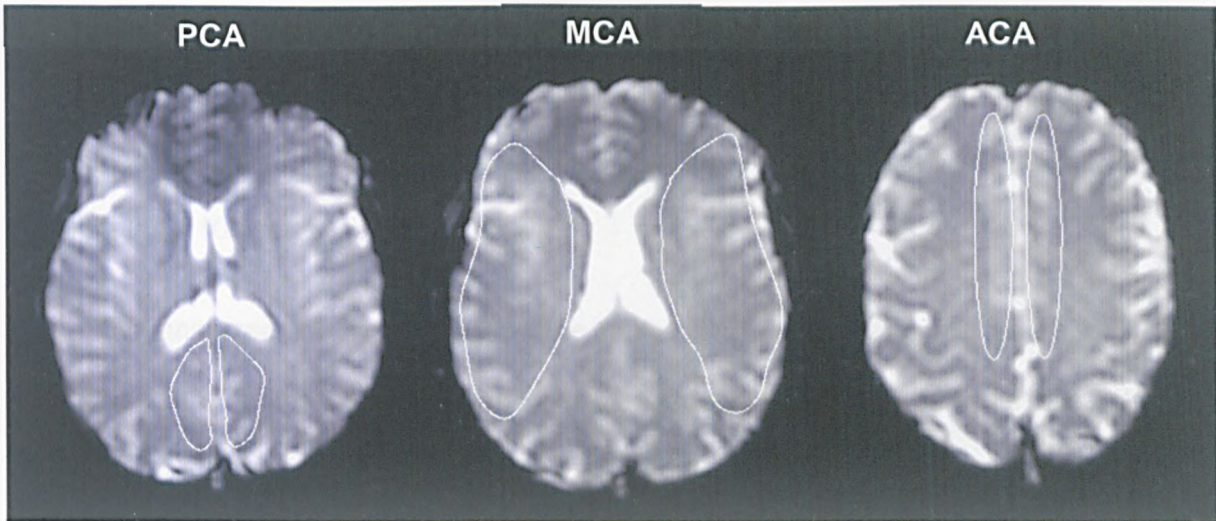


Figure 6.2: White circles are regions of interests within the three major vascular territories; ACA, anterior cerebral artery, MCA, middle cerebral artery and PCA, posterior cerebral artery

6.3.1 Modelling of gadolinium concentration-time curves

Exogenous tracer methods in MR perfusion imaging use a model that assumes the tracer is restricted to the intravascular compartment and does not diffuse into the extracellular space. Imaging can be performed either dynamically (rapid imaging over time during bolus injection) or in the steady state (imaging after a constant infusion has reached an equilibrium concentration in the blood). In this study we chose the former imaging technique. The signal time course of the average pixel value within each ROI was inverted to produce a concentration time profile.

Figure 6.3 shown as typical concentration-time profile of the passage of gadolinium through the thalamus in a healthy volunteer. From this profile, several peaks are found, which suggests that gadolinium has not diffused or mixed well in the body within the short observation period (100 sec). The mixing is assumed to be a function of the distance from the heart to the ROI, and vascularity in relation to cardiac output. Thus, the exposure of the

ROI to gadolinium takes place as episodes of high concentrations followed by lower values. Several peaks are seen until the mixing has been complete in the body. The height of the peaks is expected to go down as gadolinium is distributed and eliminated in each cycle. Gadolinium signal rerun does not return to the baseline (zero), but shows an apparent constant level 100s after administration.

We analysed this concentration time curve using two models. The first model (Model A) analysed the first pass of gadolinium bolus through the ROIs, while the second model (Model B) analysed in detail the whole concentration-time profile of the contrast material over 100sec as it recycles through the thalamus.

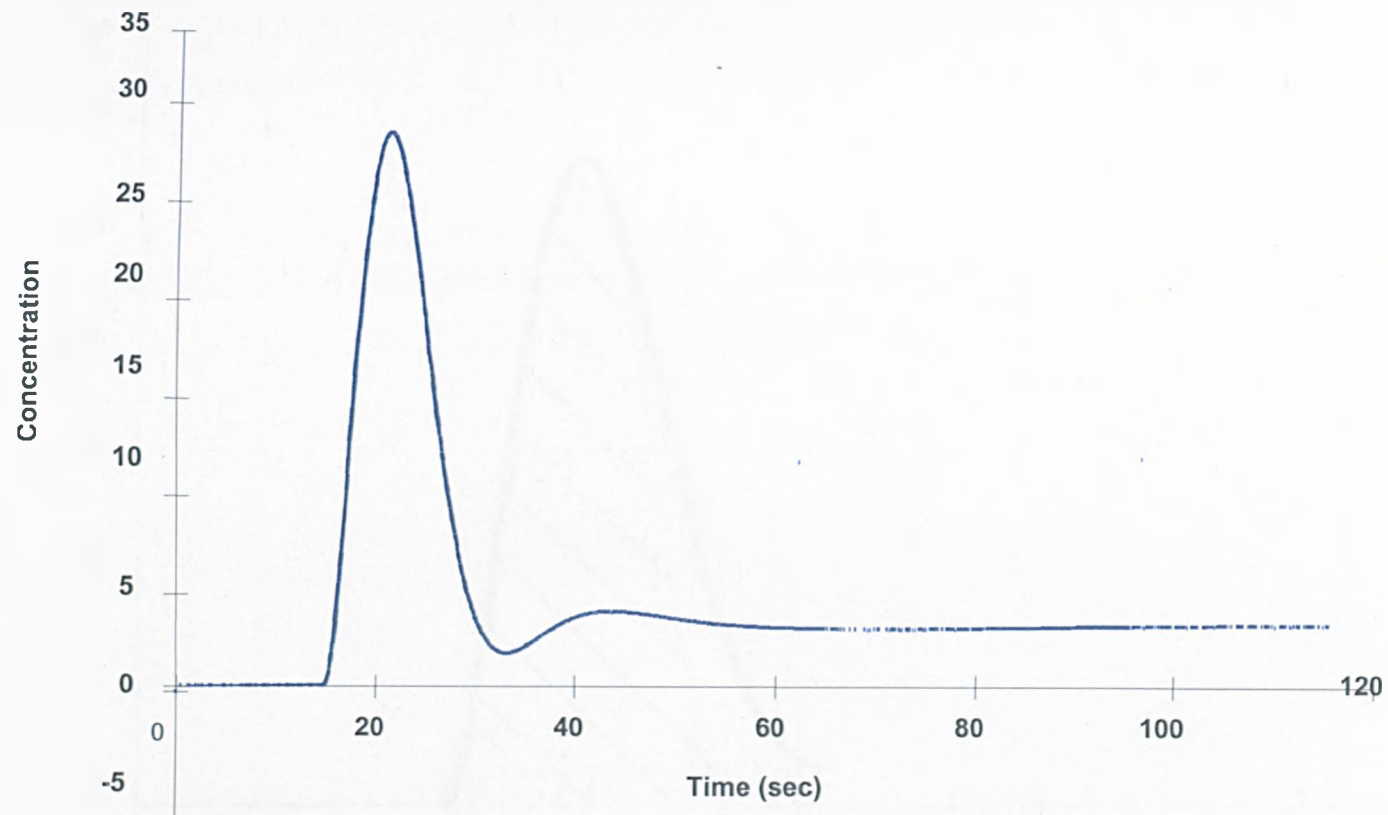


Figure 6.3: Relative concentration-time profile of gadolinium passage through the thalamus in a healthy volunteer.

Model A

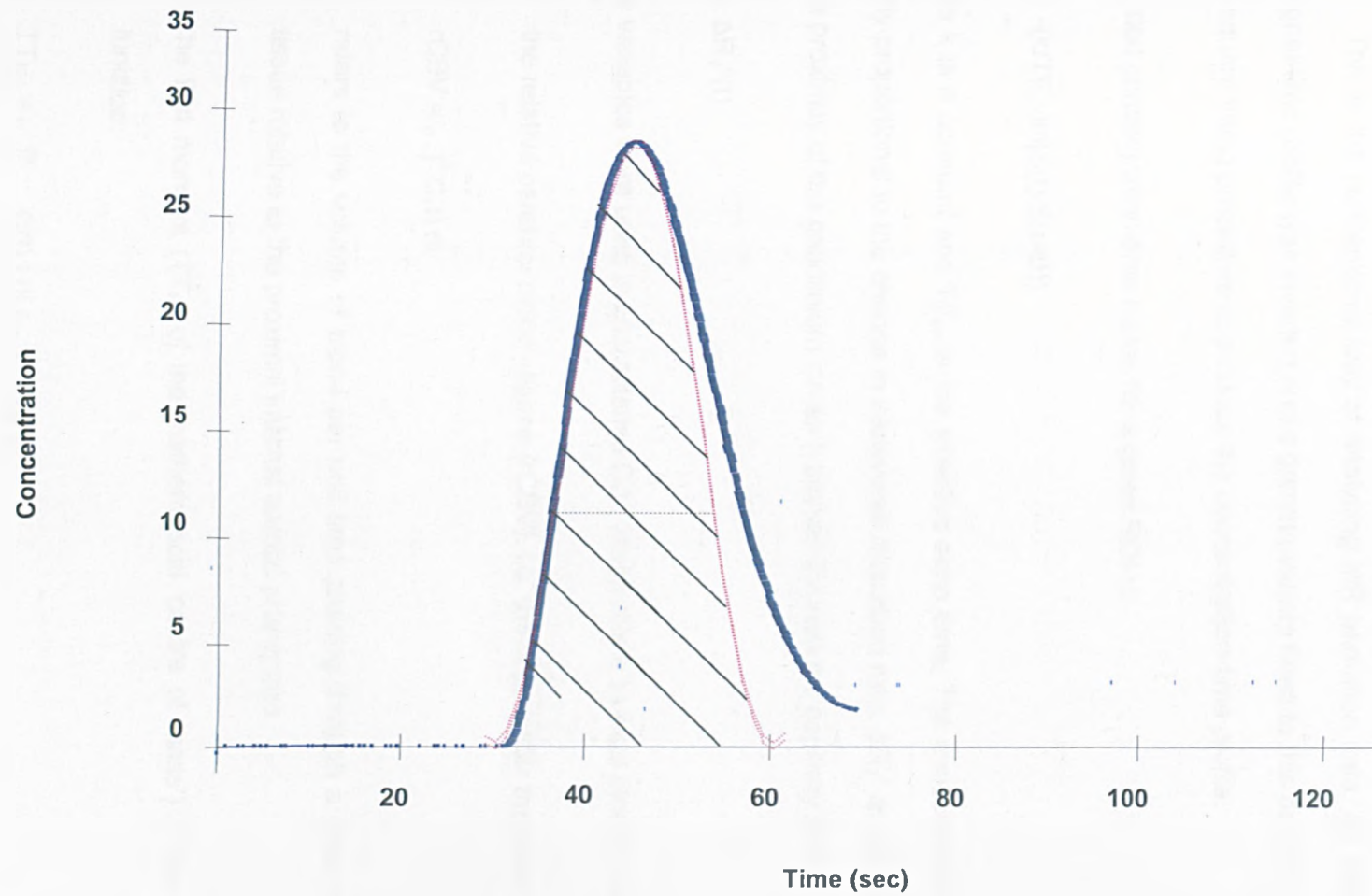


Figure 6.4: First pass relative concentration time profile of gadolinium through the thalamus in a healthy volunteer.

Blue line in Figure 6.4 represents the observed profile; red dotted line represents the gamma-variate fit. The area under the red dotted line (shaded) represents the cerebral blood volume (CBV) and the black dotted line denotes the first moment transit time.

This is the conventional way of analysing MR perfusion data. As mentioned above, the signal-time profile was inverted and a gamma-variate fitted to this data using a non-linear least square fitting procedure to produce the concentration-time profile.

The fitted concentration-time curve, for a given ROI is:

$$C(t) = -(k/TE_{\text{eff}})\ln[S(t)/S(t=0)]$$

Where k is a constant and TE_{eff} is the effective echo time. The concentration-time curve is directly proportional to the change in transverse relaxation rate, ΔR_2^* at time t brought about by the proximity of the gadolinium ion as it passes through the capillary bed:

$$C(t) \propto \Delta R_2^*(t)$$

Three variables were used to characterise $C(t)$, which relate to local blood volume and flow:

- the relative cerebral blood volume (rCBV), i.e. the area under the fitted curve:

$$rCBV = \int_0^{\infty} C(t) dt$$

refers to the volume of blood per unit time passing through a given region of brain tissue relative to the proximal internal carotid artery; and

- the first moment (TT_{FM} of the mathematical 'centre of mass') of the fitted gamma-function:

$$TT_{\text{FM}} = \int_0^{\infty} C(t).t dt / \int_0^{\infty} C(t) dt$$

refers to the average time it takes blood to pass through a given region of brain tissue, measured in seconds.

- finally we calculated the relative cerebral blood flow (rCBF) through each ROI by:

$$rCBF = \frac{rCBV}{TT_{FM}}$$

refers to the average volume of blood passing through the ROI per unit time.

We calculated the average rCBV, TT_{FM} and rCBF for all ROIs in the right and left cerebral hemispheres. Study end points were mean average rCBV, TT_{FM} and rCBF. These were compared between Healthy Volunteers and all diabetic patients.

Model B

With the assistance of ARH and colleagues, we constructed a mathematical model to describe the concentration-time profile of the passage of gadolinium through the thalamic ROI, using pharmacokinetic drug modelling principles.

The following empirical model was derived:

$$\text{Concentration of gadolinium} = A(1 - e^{-k_{el}(t-t_{lag})}) + C.(e^{-k \cos((B(t-t_{lag})+B_2)(t-t_{lag}))})$$

This model consists of three components.

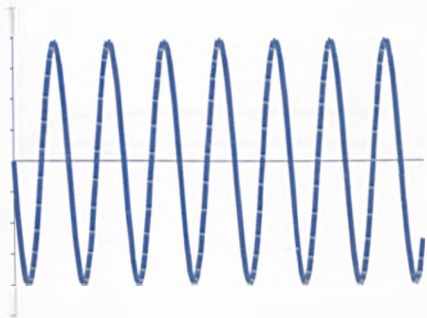
Component A: to represents the episodic delivery profile, which are seen as waves of descending heights until it becomes a smooth line.

Component B: to account for accumulation of the gadolinium in the organ that reaches a constant level after several peaks.

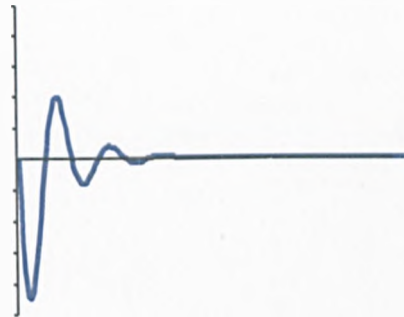
Component C: to account for the delay in bolus arrival time (t_{LAG})

Component A

A. Waves



B. Concentration = $C \cdot \sin(B \cdot t)$



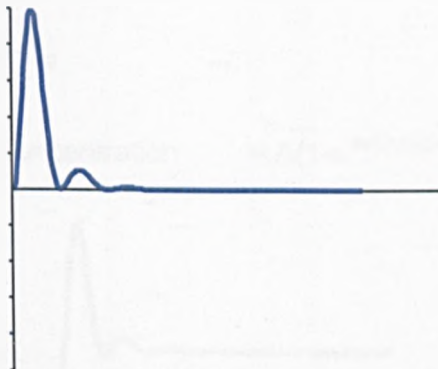
C. Concentration = $C \cdot e^{-k \sin t} \cdot \sin(B \cdot t)$



D. Concentration = $C \cdot e^{-k \sin t}$



E. Concentration = $C \cdot e^{-k \sin t} + C \cdot e^{-k \sin t} \cdot \sin(B \cdot t)$



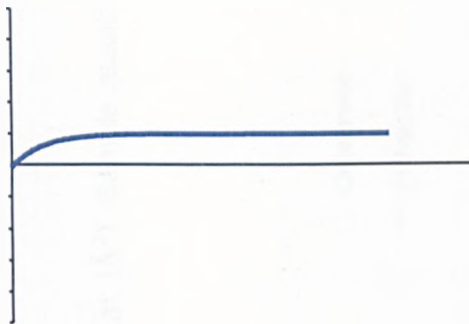
F. Concentration = $C \cdot e^{-k \cos t} - C \cdot e^{-k \cos t} \cdot \cos(B \cdot t)$

$$= C \cdot e^{-k \cos t} \times [1 - \cos(B \cdot t)]$$

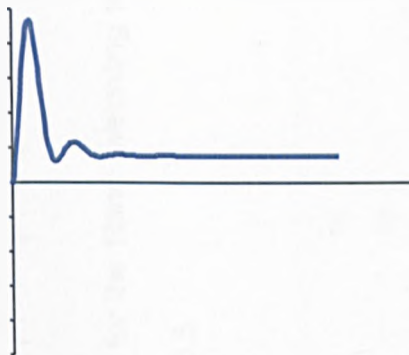
G. Concentration = $C \cdot e^{-k \cos t} \times [1 - \cos(B \cdot t + B_2 \cdot t)]$

Component B

A. Concentration = $A(1 - e^{-k_{el}t})$



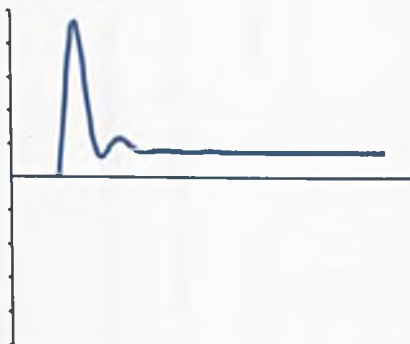
B. Concentration = $A(1 - e^{-k_{el}t}) + C.e^{-k_{cos}t} \times [1 - \cos(B.t + B_2).t]$



Component C

t to $t - t_{lag}$

A. Concentration = $A(1 - e^{-k_{el}(t-t_{lag})}) + C.e^{-k_{cos}(t-t_{lag})} \times [1 - \cos((B.(t-t_{lag}) + B_2).(t-t_{lag}))]$



Final Fitted Result

WinNolin Professional (Pharsight Co. Ver. 4.0.1) was used for the fitting (weighting scheme was set at $1/y^2$). Sample results for a healthy volunteer and diabetic patient with DPN are shown in Figure 6.5.

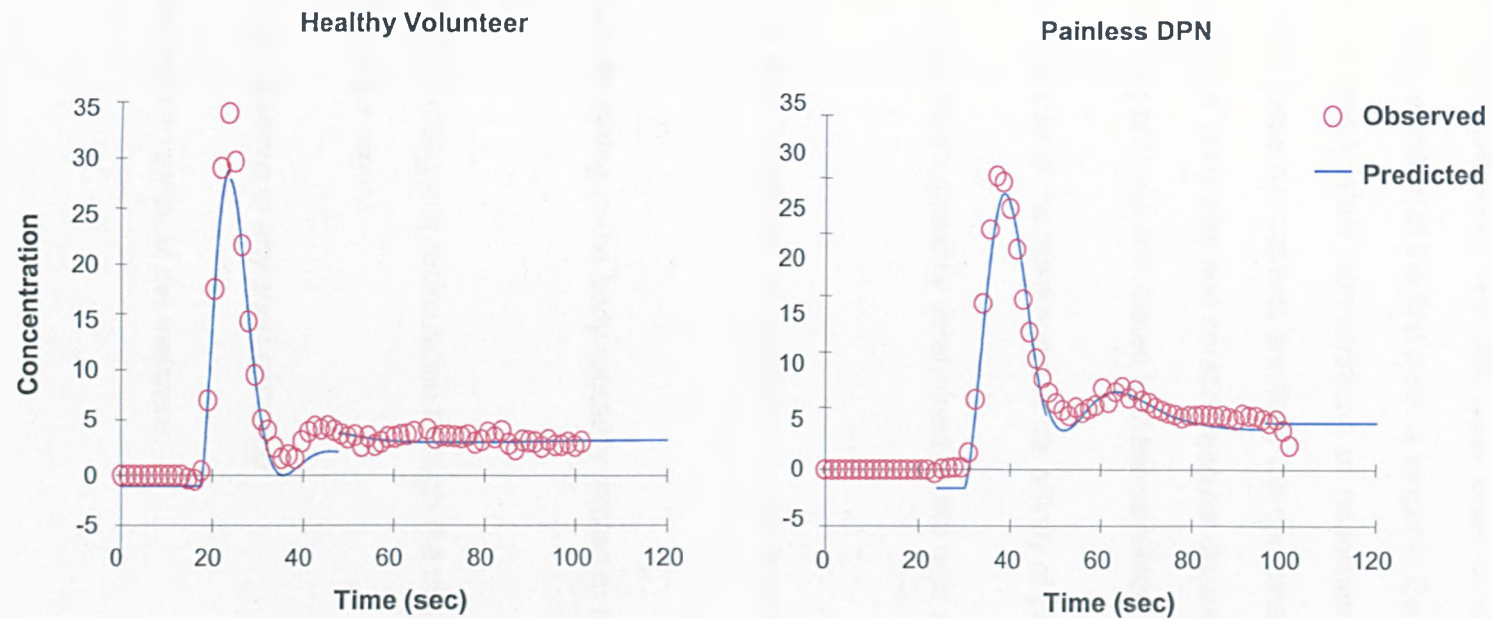


Figure 6.5: Relative concentration time profile of gadolinium DTPA tracer bolus as it passes through the left thalamus in a healthy volunteer and a patient with painless DPN. (○) represent data points of observed gadolinium concentration sampled from time 0min to 100min and (—) represent the predicted concentration of gadolinium calculated from the empirical model.

From Figure 6.5, there are several differences in the concentration time profile of gadolinium tracer bolus as it passes through the thalamus, between the two study patients. Firstly there is a delay in bolus arrival time (t_{lag}) in the patient with Painless DPN compared with the Healthy Volunteer. Secondly, the first pass peak concentration in the healthy volunteer is greater but the duration of the first peak is longer in Painless DPN. Finally it also takes longer to reach a steady state concentration of gadolinium in Painless DPN. The differences seen between these two patients are likely the combined effects of vascularity of the thalamus, cardiac output, body size and cerebrovascular disease. Seven biomarkers are used in this model which we propose are related to these variables.

A: Combined effects of the size of the thalamus and the affinity of gadolinium to the tissue.

K_{el} : the uptake rate of gadolinium, possibly determined by the ratio of organ size/affinity to its vascularity.

C: the size of the initial pool for dilution of gadolinium in the body (the smaller the size the taller the peak).

K_{cos} : the rate of gadolinium mixing in the body (possibly related to blood volume and cardiac output).

B: the constant for short circuiting and recirculation through the thalamus (possibly related to cardiac output and size of the organ).

B2: similar to B but in the absence of any short circuiting.

t_{lag} : time it takes for the bolus to arrive at the thalamus.

6.3.2 Statistical analysis

All analyses were performed using statistical package SPSS 14.0. Subgroup demographics were described as means and standard deviation for normally distributed variables and as medians and 5th and 95th percentiles for variables with a skewed distribution. Demographic and laboratory variables were compared between groups using one-way ANOVA.

- Healthy Volunteers vs Patients with Diabetes

We compared regional perfusion (Model A) of the major vascular territories (ACA, MCA and PCA) between Healthy Volunteers with all diabetic patients. Variables assessed were average rCBV, TT_{FM} and rCBF for each vascular territory.

- Healthy Volunteers vs Diabetic Subgroups

We subsequently divided diabetic patients into No DPN, Painful and Painless DPN. Using variables derived from Model A we compared thalamic and caudate nuclei perfusion characteristics of each diabetic subgroup with Healthy Volunteers. More in depth analysis of thalamic perfusion using variables derived from Model B was then performed. Non parametric median tests were used to compare biomarkers A, B, B2, C, K_{el} , K_{cos} and T_{lag} .

6.4 Results

6.4.1 Comparisons between Healthy Volunteers and patients with diabetes: regional perfusion of major vascular territories

6.4.1.1 Subgroup Demographics

The quality of the perfusion data obtained was graded using patientive assessments of the time-intensity curves. The method used is described in more detail elsewhere (Griffiths, Pandya et al. 2006). Perfusion results from one patient were excluded from the final analysis because of the poor quality of data obtained. Demographics of Healthy Volunteers (n=5) and

patients with diabetes (n=18) are displayed in Table 6.1. Age, height and weight differences did not reach statistical significance.

Table 6.1: Demographics of Healthy Volunteers and Patients with Diabetes.		
	Healthy Volunteers	All Diabetic Patients
Age (years)	45.8 (14.7)	51.9 (9.3)
Height (m)	178.2 (14.2)	174.3 (10.1)
Weight (kg)	88.2 (7.5)	86.5 (14.0)
HbA1c (%)	N/A	8.4 (0.8)
DD (years)	N/A	15.8 (10.7)

DD, Duration of Diabetes. Results presented as mean (SD). Not applicable (N/A).

6.4.1.2 Model A

Overall Healthy Volunteers had lower global rCBV compared to diabetic patients. Mean rCBV differences between these two groups, within the anterior, middle and posterior cerebral artery territories reached statistical significance (Table 6.2). Diabetic patients had longer transit times and greater rCBF compared to Healthy Volunteers but this did not reach statistical significance.

Table 6.2: Regional Cerebral Perfusion Characteristics of Healthy Volunteers and Patients with Diabetes.

		Average rCBV	Average TT _{FM}	Average rCBF
Anterior Cerebral Artery	HV	205.3 (57.2)*	31.8 (6.9)	6.7 (2.1)
	DM	264.4 (55.2)*	34.9 (5.2)	7.8 (2.2)
Middle Cerebral Artery	HV	189.0 (55.2) [§]	31.6 (6.8)	6.17 (1.9)
	DM	244.6 (43.3) [§]	34.7 (4.8)	7.2 (1.5)
Posterior Cerebral Artery	HV	190.8 (36.5) [#]	32.1 (7.2)	6.2 (1.7)
	DM	248.4 (43.8) [#]	35.2 (5.0)	7.2 (1.8)

Results are presented as mean(SD). Healthy Volunteer (HV), Patients with Diabetes (DM), relative Cerebral Blood Volume (rCBV), first moment Transit Time (TT_{FM}), relative Cerebral Blood Flow (rCBF). * p=0.05; 95% Confidence Interval (CI) -118.7:0.6, [§] p=0.03; 95% CI -103.8:-7.4, [#] p=0.01; 95% CI -102.3:-12.9.

6.4.2 Comparison between Healthy Volunteers and diabetic patients divided into neuropathy subgroups: thalamic perfusion

6.4.2.1 Subgroup Demographics

Table 6.3 presents the demographics of Healthy Volunteers and diabetic patients divided into No DPN, Painless DPN and Painful DPN. Diabetic patients with Painful DPN were significantly older than those with No DPN and Healthy Volunteers (ANOVA p=0.03, Painful

DPN vs No DPN $p=0.005$; 95% CI 5.7:28.5, Painful DPN vs Healthy Volunteers $p=0.01$; 95% CI 3.8:28.5). There were no statistical significant differences in the other demographics studied.

Table 6.3: Demographics of Healthy Volunteers and Diabetic Neuropathy Subgroups.				
	HV	No DPN	Painless DPN	Painful DPN
N	5	6	7	5
Age*	45.8 (14.7)	44.9 (7.1)	51.7 (9.1)	62.0 (3.9)
Height	178.2 (14.2)	170.4 (6.2)	176.3 (3.9)	177.8 (17.1)
Weight	88.2 (7.5)	88.4 (15.8)	80.0 (7.6)	90.4(16.5)
HbA1c	N/A	8.4 (0.2)	8.9 (0.9)	7.7 (0.9)
Retinopathy	N/A	2	2	3
Microalbuminuria	N/A	1	3	3

HV, Healthy Volunteers. Results presented as mean(SD). Distal Symmetrical Diabetic Polyneuropathy (**DPN**), not applicable (**N/A**). * ANOVA $p=0.03$.

6.4.2.2 Model A

Perfusion results of the four groups studied are summarised in Table 6.4. Overall group comparison showed that patients with Painful DPN have significantly higher mean thalamic rCBV compared with Healthy Volunteers and diabetic patients with No DPN and Painless DPN ($p=0.04$). Patients with Painful DPN had the longest thalamic tracer transit time (TT_{FM})

compared to the other study groups ($p=0.07$) and the lowest mean thalamic rCBF amongst diabetic patients ($p=0.8$). Perfusion parameters within the caudate nucleus (control region) were not significantly different between subgroups.

Table 6.4: Regional Cerebral Perfusion Characteristics of Healthy Volunteers and Diabetic Patients Divided into Neuropathy Subgroups.				
		Av rCBV	Av TT_{FM}	Av rCBF
Thalamus	HV	181.9 (51.7)*	33.7 (14.9)	5.77 (1.6)
	No DPN	202.3 (25.8)*	35.3 (13.2)	6.52 (1.3)
	Painless DPN	216.5 (65.5)*	35.9 (13.7)	6.51 (2.8)
	Painful DPN	228.7 (19.5)*	38.4 (3.6)	5.93 (0.5)
Caudate Nucleus	HV	186.8 (53.0)	33.0 (15.5)	6.03 (1.6)
	No DPN	207.4 (28.0)	35.3 (14.7)	6.95 (1.8)
	Painless DPN	237.9 (84.3)	34.9 (13.3)	7.15 (2.8)
	Painful DPN	238.1 (66.1)	37.0 (5.0)	6.41 (2.0)

Results presented as median (range). Healthy Volunteers (HV), Distal Symmetrical Diabetic Polyneuropathy (DPN), Relative Cerebral Blood Volume (rCBV), First Moment Transit Time (TT_{FM}), Relative Cerebral Blood Flow (rCBF). * Median test, $p=0.045$.

6.4.2.3 Model B

Biomarkers derived from pharmacokinetic modelling of tracer passage through the thalamus for the different study groups are summarised in Table 6.5 and displayed in Figure 6.6. We found that patients with Painful DPN had a significantly lower B2 compared with Healthy Volunteers ($p=0.04$). Subgroup differences in the other biomarkers were not statistically significant.

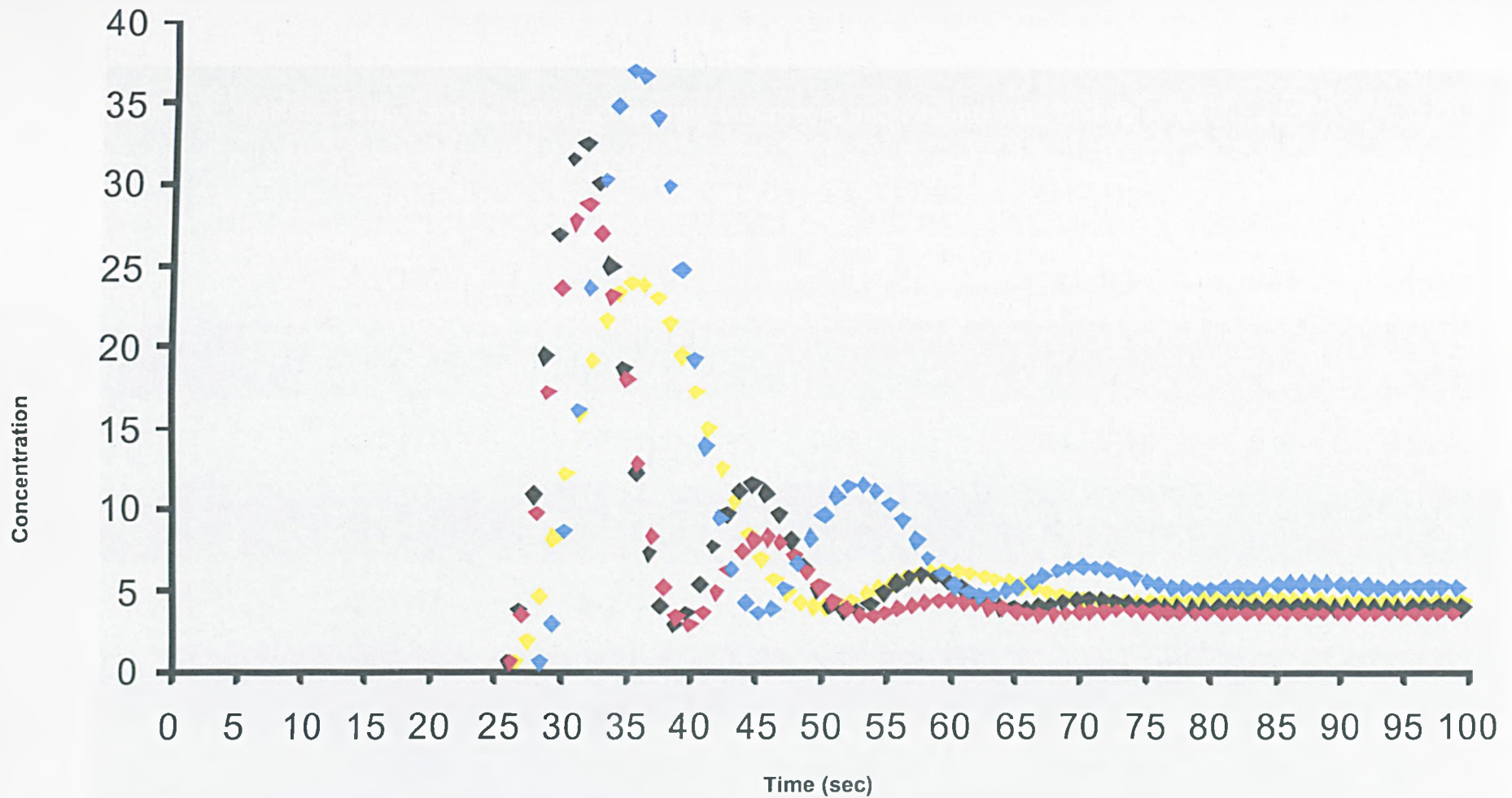


Figure 6.6: Relative concentration time profile of the passage of exogenous contrast agent (gadolinium DTPA) through the thalamus in each subgroup. [◆] Healthy Volunteers, [◆] diabetic patients with no neuropathy (No DPN), [◆] diabetic patients with painless DPN (Painless DPN) and [◆] diabetic patients with Painful DPN (Painful DPN).

Table 6.5: Thalamic Perfusion Biomarkers of Healthy Volunteers and Patients with Diabetes.

	HV	No DPN	Painless DPN	Painful DPN	P	Chi-Sq
A	3.4 (12.5)	3.0 (2.1)	3.8 (4.0)	4.7 (6.1)	0.17	5.1
B	-0.0038 (0.0)	-0.0036 (0.01)	-0.0021 (0.0)	-0.0029 (0.0)	0.17	5.1
B2	0.43 (0.1)	0.42 (0.2)	0.38 (0.1)	0.37 (0.06)	0.04	5.1
C	28.5 (8.7)	28.9 (19.7)	30.6 (21.1)	35.9 (33.3)	0.68	1.5
T_{LAG}	25.9 (11.6)	26.2 (13.8)	26.7 (11.5)	28.4 (4.6)	0.24	4.2
K_{el}	0.09 (0.4)	0.11 (1.1)	0.1 (0.7)	0.06 (0.6)	0.84	0.83
K_{cos}	0.1 (0.03)	0.12 (0.05)	0.1 (0.05)	0.09 (0.04)	0.84	0.83

Results presented as median (range). Distal Symmetrical Diabetic Polyneuropathy (DPN), Healthy Volunteers (HV).

6.5 Discussion

This preliminary study demonstrated that patients with diabetes had significantly greater rCBV in all major cerebral vascular territories (ACA, MCA and PCA) compared to age, sex and BMI matched Healthy Volunteers. This was accompanied by a non significant trend towards longer TT_{FM} and greater rCBF amongst diabetic patients. In addition, patients with Painful DPN had significantly greater thalamic rCBV compared to other Painless DPN and Healthy Volunteers. There were no differences in the perfusion characteristics of the caudate nucleus between study groups.

The finding of greater rCBV in the major vascular territories of diabetic brains suggests a persistent resting vasodilatory state. This could reflect either a compensatory mechanism, utilising cerebrovascular reserve capacity, to overcome diabetic microangiopathy or impairment of cerebral autoregulation. Previous studies have demonstrated reduced cerebrovascular reserve capacity in patients with diabetes using CO_2 inhalation and acetazolamide challenge (Paulson, Strandgaard et al. 1990; Jimenez-Bonilla, Quirce et al. 2001). In addition, similar changes in cerebral perfusion demonstrated in this study have been described in the diabetic kidney; where the early stages of diabetes is characterised by increases in renal blood flow and glomerular filtration rate. This has been shown to be secondary to impairment of renal autoregulation resulting in nitric oxide mediated renal vasodilatation.

We also found that diabetic patients with Painful DPN had significantly higher thalamic rCBV compared with other study groups. Further detailed analysis of thalamic perfusion using pharmacokinetic derived biomarkers suggests that thalamic recirculation time (B2) is longer in patients with Painful DPN compared to Painless DPN, No DPN and Healthy Volunteers. Greater blood volume and longer recirculation time within the thalamus in patients with Painful DPN suggests that abnormal thalamic vascularity could be an important contributing factor in Painful DPN.

To quantify flow within the ROIs, we used two mathematical models to describe the concentration-time curve of a bolus of gadolinium as it passes through the thalamus. The first (Model A), uses a gamma variate model to fit the data to produce two parameters that are often used: localised blood volume and flow. Blood volume and flow are related to each other by the transit time, according to the central volume principle: flow = volume/transit time. For a review of these concepts, see the article by Griffiths et al (Griffiths, Pandya et al. 2006). When purely intravascular tracers are imaged, the mean transit time cannot be calculated in a straightforward manner from the first moment of a concentration time curve, as the first moment is related to the second moment which has a dependence on vascular topology (Griffiths, Gaines et al. 2005). To highlight this concept, we denoted the first moment of the calculated concentration time curve as the TT_{FM} rather than the mean transit time. TT_{FM} has a high dependence on mean transit time, and therefore it does provide information about the temporal characteristics of tissue perfusion. No attempt was made to quantify CBV (based on knowledge of the arterial input), and thus, relative values were obtained and compared.

The second model (Model B) used to interrogate the concentration-time curve utilises mathematical principles commonly employed in pharmacokinetic studies. Unlike the first, this model incorporated the whole concentration time profile (0 – 100mins) until the concentration of gadolinium reaches a steady state. Hence it includes the recirculation of gadolinium through the body, thereby indirectly quantifying potential confounding factors such as body size, cardiac output, vascular disease and medication use.

Thalamic perfusion abnormalities demonstrated in Painful and Painless DPN were not found in the caudate nucleus, another deep brain nucleus which served as an internal control. Unlike the caudate, the thalamus plays a central role in modulating/processing somatosensory information that is relayed to the cerebral cortices and is therefore possibly more metabolically active in DPN. This greater metabolic capacity increases thalamic sensitivity to diabetic metabolic disturbances. Diabetes has been shown to alter endothelial

function and permeability of the blood brain barrier, thus affecting microcirculation and regional metabolism. This increased susceptibility to diabetic metabolic disturbance may then lead to thalamic neuronal dysfunction and eventually neuronal loss. Our finding of MR spectroscopic reduction in thalamic NA:Cho ratios in Painless DPN would seem to support this hypothesis (Selvarajah, Wilkinson et al. 2008). Other studies in type 1 diabetes have shown that the fronto-temporal cortex and periventricular white matter are also affected by diabetic metabolic disturbance. This is manifested by atrophy and the increased presence of leukoariorosis or white matter hyperintensities (van Harten, de Leeuw et al. 2006). It is possible, therefore, that regional differences in cerebral metabolic capacity may increase sensitivity to hyperglycaemia in these regions.

As we have previously demonstrated, thalamic sensory neuronal dysfunction accompanies Painless DPN and preservation of thalamic function maybe a prerequisite for perception of pain in this condition. Understanding the pathogenesis of thalamic dysfunction may lead to new insights into CNS involvement in diabetes. Increased thalamic vascularity demonstrated in this study has similarities to findings of increased blood flow in epineural vessels in sural nerve studies in Painful DPN (Eaton, Harris et al. 2003) and increased proliferation of new vessels in the diabetic retina. Various metabolic and vascular factors have been postulated to explain the pathogenesis of different diabetic microvascular complications (nephropathy, neuropathy and retinopathy); given the systemic nature of diabetes, we postulate similar pathophysiological changes may occur within the thalamus.

To our knowledge, this is the first report of the use of MR perfusion imaging to assess capillary bed perfusion in the thalamus in DPN. Limitations of the current study need to be considered. Group classification is prone to bias in small samples and requires cross validation for these results to be generalised beyond the current sample. In addition our study groups comprised an age spread of several years, and age is a factor in cerebral hypoperfusion, a larger study is needed to allow analysis of covariance with age as a covariate to account for age-related effects. Paradoxically, however, in this study patients

with Painful DPN comprised the oldest cohort but nonetheless possessed the highest thalamic rCBV.

Although these are clearly shortcomings of the current study, our goals were to assess whether: 1) MR perfusion imaging in patients with diabetes might detect changes in regional brain perfusion and 2) thalamic perfusion abnormalities are present in DPN. The data presented here at least preliminarily support these views. Several other areas need additional research. Evaluating a larger sample, including greater numbers of cases of Painful and Painless DPN; along with studying patients with Subclinical DPN will not only provide more information about the sensitivity and specificity of MR perfusion imaging but also may lead to new insight into the pathogenesis of CNS complications in DPN. Also important will be following up patients with DPN over time to help better define the relationship between clinical measures of progression, such as NCS, and regional thalamic haemodynamic function measured with MR perfusion imaging. In turn, this information (together with MR spectroscopic determined biochemical abnormalities) might provide a means to assess the efficacy of clinical interventions in slowing the pathophysiological changes associated with DPN.

Finally, MR perfusion is a promising technique for assessing regional brain haemodynamic function in DPN. The results of this study using semiquantitative analysis of MR perfusion imaging was effective in assessing thalamic haemodynamic dysfunction in patients with diabetes as a whole and patients with DPN. These novel findings suggest a pathogenic microvascular mechanism for thalamic neuronal dysfunction in DPN, which may be amenable to modifications of microvascular disease risk factors.

7 A RANDOMISED DOUBLE BLIND PLACEBO CONTROLLED TRIAL OF CANNABIS BASED MEDICINAL PRODUCT (SATIVEX) IN PAINFUL DPN: IS DEPRESSION A MAJOR CONFOUNDING FACTOR?

7.1 Introduction

Distal symmetrical diabetic polyneuropathy is one of the commonest long-term complications of diabetes mellitus and leads to significant morbidity and mortality, resulting in a huge economic burden for diabetes care (Vinik, Park et al. 2000). Painful DPN affects 15-26% of all patients with diabetes and about a third of patients with DPN (Daousi, Benbow et al. 2006; Davies, Brophy et al. 2006). The intractable symptoms experienced cause great distress and disability to sufferers and affect quality of life immensely (Quattrini and Tesfaye 2003). The cause of DPN, or indeed neuropathic pain, is not known although metabolic and vascular factors may be involved (Tefaye, Malik et al. 1994; Cameron, Eaton et al. 2001). Whilst the search for potential therapeutic agents to halt or reverse the neuropathic process continues (Cameron and Cotter 1993), the first step should be to improve glycaemic control, with additional drug treatments required to alleviate painful symptoms (Ziegler 2008). Unfortunately these drugs are not always effective and often complicated by unwanted side effects. A recent review of trials in painful DPN has shown that at best only a third of patients achieve 50% pain relief with conventional treatments (Jensen, Backonja et al. 2006). Thus, there is need for more effective treatments with fewer side effects.

In our centre we have had anecdotal reports by patients who gained relief of painful symptoms and had better nights' sleep after smoking cannabis. In several randomised controlled trials, cannabis and cannabis based medicinal products (CBME) have been shown to provide effective pain relief in a variety of mainly, non diabetic neuropathic pain conditions (Rog, Nurmikko et al. 2005; Abrams, Jay et al. 2007). A meta-analysis of these clinical trials concluded that further valid randomised controlled studies are required before cannabinoids can be considered for treating neuropathic pain (Campbell, Tramer et al.

2001). In this study we report the first randomised placebo controlled trial assessing the efficacy and safety of a CBME (Sativex) in the treatment of intractable painful DPN.

7.2 Methods

7.2.1 Patients

Forty diabetic patients from the Royal Hallamshire Hospital Diabetes Database (Sheffield, UK) with chronic painful DPN were screened to take part in this study. Patients with stable glycaemic control (HbA1c <11%) and over the age of 18 with painful DPN (Neuropathy Total Symptom Score - NTSS 6 >4 and <16) for at least six months were assessed. Only those with intractable neuropathic pain in whom symptoms persisted despite an adequate trial with a tricyclic antidepressant (minimum three months) were recruited. Exclusion criteria included history of previous cerebrovascular event or other neurological disorders, history or electrocardiograph evidence of cardiovascular disease, schizophrenia or any other psychiatric illnesses, epilepsy, current or past history of substance abuse, current or past history of consuming more than 20 units of alcohol a week, neuropathy caused by other aetiologies, known or suspected hypersensitivity/adverse reaction to cannabinoids and significant hepatic or renal impairment. All patients gave written informed consent prior to taking part in this study, which had ethical approval from the South Sheffield Regional Ethics Committee.

7.2.2 Study Design

A prospective, randomised, double-blind, placebo controlled trial design was employed. This study was divided into three phases with seven assessment visits at regular intervals (Figure 7.1). During the screening visit detailed clinical and neurophysiological assessments were performed to quantify the severity of neuropathy. The presence of depression and anxiety was assessed by completion of the Hospital Anxiety and Depression Scale (HADS) questionnaire. During the subsequent 2 week screening phase patients were given a study

diary to complete to assess baseline pain and sleep scores. Patients were asked to complete the diary each morning upon awakening and reflect upon the pain intensity and sleep quality over the preceding 24 hours. Three modalities of pain in the lower limbs (superficial, deep and muscular) were assessed daily using a 100 mm visual analogue scale (VAS). On the second visit patients were block randomised to receive either the active or placebo study medication. Over the subsequent two weeks the dose of the study medication was titrated up to reach a maximum tolerated level when either unacceptable side effects prevented further dose escalation or pain/symptom control was apparent. We used a predetermined dose titration regime to standardise the process across the study. Patients were then instructed to remain on their maximum tolerated dose for the remaining duration of the study (Maintenance Phase for 10 weeks).

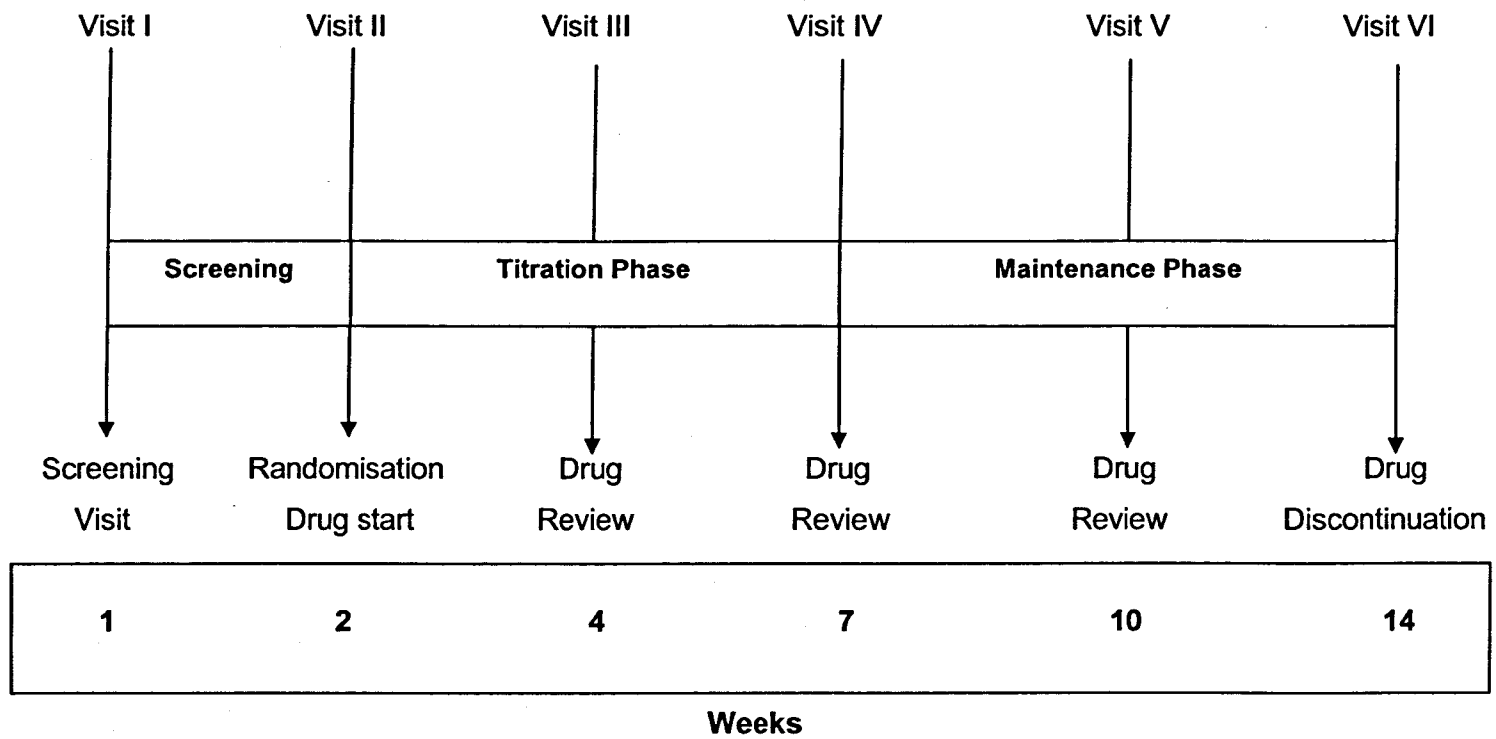


Figure 7.1: Treatment Phases and Study Visits.

Two health care professionals, a treating physician and a nurse assessor conducted the study. The physician was responsible for dose titrations and assessing adverse events, while the nurse assessor completed study questionnaires and case report forms at each visit. This measure was undertaken to prevent inadvertent unblinding which could occur if the responsibility of both these tasks were to fall on a single individual.

7.2.3 Assessment of Neuropathy

Detailed clinical and neurophysiological assessments were performed to quantify the severity of neuropathy at Visit 1 and Visit 6. The outcome of a detailed neurological examination was graded by defined criteria according to the standard NIS questionnaire.

All patients underwent: 1) vibration perception thresholds acquired from the dorsal aspect of the right foot using Computer Assisted Sensory Evaluation IV (CASE IV, W.R.Electronics, Stillwater, MN, USA) system employing standard techniques;^{16,17} 2) cardiac autonomic function tests performed with a computer assisted technique¹⁸ and 3) nerve conduction studies performed, at a stable skin temperature of 31°C and a room temperature of 24°C, using a Medelec electrophysiological system (Synergy Oxford Instruments, Oxford, UK). The following nerve attributes were measured: 1) sural sensory nerve action potential amplitude and conduction velocities and 2) common peroneal and tibial motor nerve distal latency, compound muscle action potential and conduction velocity.

7.2.4 Concomitant Medication Use

Patients were allowed to continue taking concurrent medications for neuropathic pain for the duration of the study. Additional analgesia, if required, was prescribed according to the following algorithm. Week 1: paracetamol or aspirin; Weeks 2 through 6 (if further therapy is needed and indicated): non-steroidal anti-inflammatory medication, co-proxamol or co-codamol. If Class 2 controlled substances were required (with the exception of codeine), then the patient was discontinued from the study.

7.2.5 Primary Outcome Measure

Improvements in pain symptoms (superficial, deep and muscular) as assessed by daily pain diary and Neuropathic Pain Scale (NPS) questionnaire (completed at each study visit) were used as the primary outcome measure. The endpoint chosen was each mean score for pain while taking the maximum tolerated dose of the assigned drug during the final week of Maintenance Phase 2 in both treatment arms. In addition the total pain score, which was the average score of all three pain modalities, was also calculated. The change in average baseline and endpoint (average final week scores prior to termination visit) scores for each modality of pain and the total pain score was compared between sativex and placebo. Similarly average change in NPS questionnaire scores from baseline (screening visit) to endpoint (termination visit) was compared.

7.2.6 Secondary Outcome Measure

McGill Pain and Quality of Life (Melzack 1975), SF-36 Health Survey (Jenkinson, Coulter et al. 1993) and EuroQOL (EQ-5D) (The EuroQol group 1990) questionnaires were employed to assess quality of life at each study visit. Average change from baseline (Visit 1) to endpoint (Visit 6) for these questionnaires was compared between study groups. Tolerability and side effect profile of the study medication was evaluated by completion of a standardised case report form at each study visit. To assess DPN disease progression over the study period, differences in neurophysiological assessment parameters between baseline (Visit 1) and endpoint (Visit 6) were compared between study groups.

7.2.7 Study Medication

Sativex and its matching placebo were presented in a pump action spray formulated with ethanol 50% and propylene glycol 50%. The active preparation comprised of approximately equal (1:1) concentrations of tetrahydrocannabinol (THC, 27 mg/ml) and cannabidiol (CBD, 25 mg/ml). One dose increment comprises one actuation of the spray which releases

100mcl, containing THC 2.7 mg and CBD 2.5 mg. Doses were administered sublingually in divided doses up to four times a day.

7.2.8 Data Analysis and Statistical Methods

Analyses were conducted using the intent-to-treat population, which included all randomised patients. Before the analysis was performed, we define the intensity of pain as an average of pain scores in the patient's diary if no more than 50 % of the scores were missing. If more than 50 % of the scores were missing, the mean daily score for pain intensity was considered to be missing. For patients missing post-baseline measurements, the last observation carried forward approach was applied by imputing the last non-missing post-baseline value.

The first analysis step was to examine differences in the baseline characteristics between the two treatment arms. Imbalances in the baseline characteristics that are strong predictors of the primary outcome were then assessed by sensitivity analysis. Subsequently adjustment for a baseline covariate was performed if the covariate is correlated to the outcome at a correlation coefficient >0.50 .

The second step was to assess the distribution of the primary outcome measure with each of the predictors (covariates). If the response is a continuous variable with a normal distribution then multiple linear regression was used. However a response with a skewed distribution will first be transformed.

As a sensitivity analysis, the level of change in the intensity of pain was calculated as the difference between the scores for pain at baseline (the mean score during the Screening Phase) and the scores for pain during the treatment (mean of the last week of Maintenance Phase 2). The percentage change was calculated as the change in pain divided by the score for pain at baseline times 100 percent. A significant improvement was defined as at least a 30 percent improvement in pain scores from baseline. These estimates was analysed by multiple linear regression analysis. Data on proportions was analysed with the use of Fisher's exact test.

To assess disease progression in both treatment groups, changes from baseline to endpoint were assessed using univariate analysis of covariance (ANCOVA) with baseline measures as covariates for the following neurophysiological parameters: vibration perception threshold, heart rate variation with respiration and each attribute of nerve electrophysiology (sural, peroneal and tibial nerves). For each parameter, patients with possible outlying changes (any value \leq the 5th or \geq 95th percentile) were identified for each treatment group and reviewed for clinical relevance.

To assess the impact of depression on the outcome of this study we performed a post hoc analysis. All patients were divided into two groups based on HADS [no depression (HADS<10) and depression (HADS \geq 10)]. Using ANCOVA we compared mean change from baseline of total pain score between depressed and non depressed patients after adjusting for study medication received.

7.3 Results

7.3.1 Baseline Characteristics

Of the 38 patients screened, 30 were randomised, of these 24 had type 2 diabetes. The average duration of diabetes was 12.5 years. Figure 2 displays participant flow for the duration of the study. None of the patients had previously used cannabis medicinally and 2 (7.1%) had used it recreationally. Baseline demographics of both groups are presented in Table 7.1. There was no significant difference in baseline characteristics between study groups. The mean number of spray taken during the analysis period is shown in Table 7.1. Neurophysiological assessments performed are displayed in Table 7.2. Concomitant analgesic medications taken during the study is shown in Table 7.3.

Table 7.1: Baseline Characteristics of Study Participants.

	Sativex	Placebo	P
N	15	15	
Age (Yr)	58.2 (8.8)	54.4 (11.6)	0.24
Sex (Female)	4	7	0.38
Weight (kg)	96.6 (18.4)	92.6 (24.7)	0.63
Height (m)	1.74 (0.1)	1.71 (0.10)	0.38
BMI (kg/M²)	31.9 (6.3)	31.6 (8.2)	0.92
Alcohol (U)	5.96 (7.4)	3.40 (3.7)	0.33
Cannabis	2	0	0.60
Smokers	4	3	0.75
HbA1c (%)	8.64 (1.7)	8.39 (1.6)	0.72
Type 2 Diabetes	13	11	0.23
Duration (Yr)	11.2 (8.4)	13.7 (6.0)	0.37
No. of Sprays	7.0 (3.8)	7.3 (3.8)	0.84

Table 7.2: Baseline and Endpoint Neurophysiological Assessments.

	BASELINE		ENDPOINT	
	Sativex	Placebo	Sativex	Placebo
Vib JND	23.4 (2.6)	23.7 (1.4)	23.4 (3.1)	23.6 (1.3)
HRDB	0.42 (0.8)	0.79 (1.0)	0.57 (0.9)	0.9 (1.0)
SA	2.7 (4.1)	4.4 (6.8)	5.1 (6.0)	1.4 (2.1)
SV	18.8 (20.1)	22.5 (21.3)	24.8 (20.8)	12.1 (18.4)
PV	29.1 (13.5)	31.6 (11.9)	31.4 (11.9)	34.6 (5.6)
PA	2.7 (2.7)	2.2 (1.9)	3.0 (3.0)	2.2 (2.0)
TV	22.0 (17.5)	27.5 (15.6)	29.9 (15.5)	29.6 (11.5)
TL	3.5 (2.7)	3.7 (2.2)	3.6 (2.1)	4.7 (2.0)
TFWL	21.2 (23.6)	33.1 (20.9)	18.7 (26.4)	21.4 (23.0)

Vibration “just noticeable difference” (**Vib JND**), R-R variability with heart rate deep breathing (**HRDB**), sural sensory nerve action potential (**SA, μV**), Sural sensory nerve conduction velocity (**SV, ms^{-1}**), common peroneal nerve conduction velocity (**PV, ms^{-1}**), common peroneal compound muscle action potential (**PA, μV**), tibial motor nerve velocity (**TV, ms^{-1}**), tibial motor nerve distal latency (**TL, msec**) and tibial motor nerve F-wave latency (**TFWL, msec**). Results are given as mean (SD).

Table 7.3: Concomitant Analgesic Medication.

	Sativex	Placebo		Sativex	Placebo
No Meds Taken	3 (20%)	1 (7%)	Ibuprofen	0	2 (13%)
Amitriptyline	1 (7%)	2 (13%)	Imipramine	2 (13%)	5 (33%)
Carbamazepine	2 (13%)	1 (7%)	MST	1 (7%)	0
Celebrex	1 (7%)	0	Naproxen	1 (7%)	0
Cocodamol	2 (13%)	4 (27%)	Panadol AF	0	1 (7%)
Codeine	1 (7%)	0	Paracetamol	3 (20%)	2 (13%)
Codydramol	2 (13%)	1 (7%)	Pregabalin	2 (13%)	0
Diclofenac	2 (13%)	0	Solpadol	1 (7%)	0
Dihydrocodeine	1 (7%)	2 (13%)	TENS	1 (7%)	0
Gabapentin	5 (33%)	7 (47%)	Temazepam	1 (7%)	0

7.3.2 Withdrawals

Of the 30 patients randomised, six withdrew before completing the study. Five patients were receiving sativex. In this group, there was one serious adverse event (confusion) and two adverse events (migraine and dysphoria), which led to withdrawal. Two other patients withdrew consent. One patient, receiving placebo, withdrew due to headaches and

listlessness. We excluded one placebo treated patient from the intention to treat analysis (n=29) because of a protocol violation (Figure 7.2).

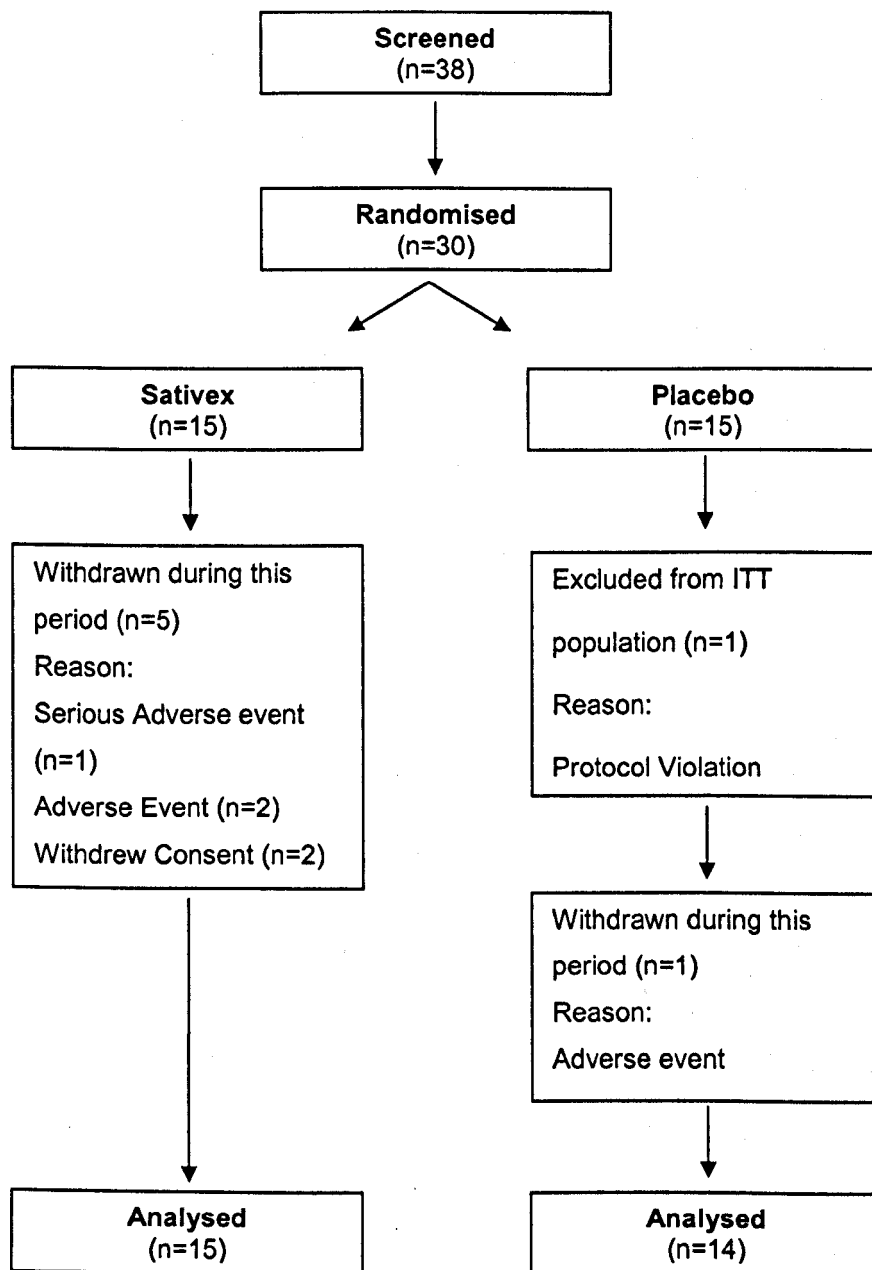


Figure 7.2: Participant Flow Plan.

7.3.3 Primary outcome measure

Covariates used in the analysis were duration of diabetes, baseline scores, age and sex. Figure 7.3 displays mean total pain scores and each individual pain component scores for the duration of the study. There was no significant difference in mean change in total pain VAS between placebo and sativex treatments [-13.7mm vs -21.9mm (sativex vs placebo); $p=0.40$ (SEM=9.5; 95%CI -11.3:27.8)]. Similarly there was no significant difference in mean change in superficial [-13.4mm vs -16.7mm; $p=0.72$ (9.1;-15.3:21.93)], deep [-16.0mm vs -25.3mm; $p=0.38$ (10.5;-12.2:30.8)] and muscular [-11.7mm vs -23.6mm; $p=0.26$ (10.3;-9.15:33.0)] pain VAS between study groups. Differences in NPS (Table 7.4) between study groups also did not reach statistical significance [-15.4 vs -11.5, $p=0.62$ (7.8; -20.1:12.1)].

Eight (53%) patients in the sativex arm responded to treatment (defined as at least a 30% improvement in total pain VAS) versus nine (64%) in the placebo arm ($p=0.55$, odds ratio 0.63, 95% CI 0.14:2.82).

7.3.4 Secondary outcome measures

McGill pain questionnaire did not show a significant difference in sensory scale ($p=0.65$, 3.3;-5.39-8.44), affective scale ($p=0.81$, 1.3;-3.0:2.4), VAS ($p=0.24$, 1.0;-0.91:3.4) and present pain intensity ($p=0.57$, 0.53;-0.79:1.4) (Table 5). Quality of life as assessed using the EQ-5D questionnaire showed improvement in both study groups (Table 5) but the differences between groups were not statistically significant (health state index $p=0.87$; SEM 0.09, 95% CI -0.19:0.16; health status VAS $p=0.92$; 7.2, -14.2:15.7). Similarly there was no significant difference in each of the quality of life measures assessed by SF-36 questionnaire between study groups (Table 7.5 and and 7.6).

Table 7.4: Primary Outcome Measures.

	BASELINE		ENDPOINT	
	Sativex	Placebo	Sativex	Placebo
PAIN DIARY SCORES				
Superficial Pain	52.3 (33.0)	45.9 (24.6)	37.9 (32.1)	30.2 (30.1)
Deep Pain	63.1 (29.4)	47.4 (21.4)	44.5 (32.7)	24.99 (29.5)
Muscular Pain	52.0 (34.2)	41.4 (28.3)	37.9 (32.9)	20.4 (29.9)
Total Pain	55.8 (26.7)	44.9 (21.5)	40.1 (28.5)	25.2 (28.8)
NPS				
Total Score	67.1 (19.4)	63.6 (14.0)	51.6 (21.9)	51.9 (24.1)

Pain diary scores derived from 100mm visual analogue scale (VAS) completed daily. Total pain score derived from average of superficial, deep and muscular pain scores. **NPS**, Neuropathic Pain Scale. Results expressed as Mean (SD).

Table 7.5: Secondary Outcome Measures (Part 1).

	BASELINE		ENDPOINT	
	Sativex	Placebo	Sativex	Placebo
McGill				
Sensory Scale	19.2 (6.9)	16.3 (6.3)	14.7 (7.2)	12.5 (8.7)
Affective Scale	4.6 (4.3)	5.0 (3.8)	3.1 (2.3)	3.6 (3.8)
VAS	7.6 (1.8)	6.9 (1.7)	5.1 (2.2)	3.8 (2.6)
PPI	2.5 (1.1)	2.0 (1.0)	2.1 (1.1)	1.4 (1.7)
EQ-5D				
Health Status Index	0.40 (0.21)	0.43 (0.21)	0.54 (0.22)	0.56 (0.22)
Health Status VAS	46.0 (20.4)	44.6 (21.8)	58.1 (20.5)	56.4 (11.7)

EQ-5D, EuroQOL questionnaire; **McGill**, McGill Pain Questionnaire, **VAS**, Visual Analogue Scale, **PPI**, Present Pain Intensity.. Results expressed as Mean (SD).

Table 7.6: Secondary Outcome Measures (Part 2).				
	BASELINE		ENDPOINT	
	Sativex	Placebo	Sativex	Placebo
SF-36				
Physical Func.	26.9 (15.1)	30.8 (22.7)	30.5 (16.6)	36.5 (27.9)
Role Physical	8.9 (27.1)	12.5 (23.5)	12.5 (32.1)	39.3 (47.7)
Bodily Pain	22.4 (15.5)	25.7 (11.3)	35.6 (16.6)	41.2 (24.6)
General Health	33.5 (18.7)	28.4 (20.8)	34.1 (18.2)	29.6 (19.5)
Vitality	28.3 (23.2)	30.8 (19.2)	33.9 (22.4)	39.6 (19.4)
Social Func.	50.8 (32.5)	48.2 (24.9)	55.4 (25.3)	67.0 (27.6)
Role Emotional	38.1 (41.1)	33.3 (40.8)	54.8 (46.4)	47.6 (48.4)
Mental Health	57.9 (22.6)	57.1 (19.9)	64.4 (20.3)	59.4 (20.6)

SF-36, Short Form-36 Health Survey questionnaire. Results expressed as Mean (SD).

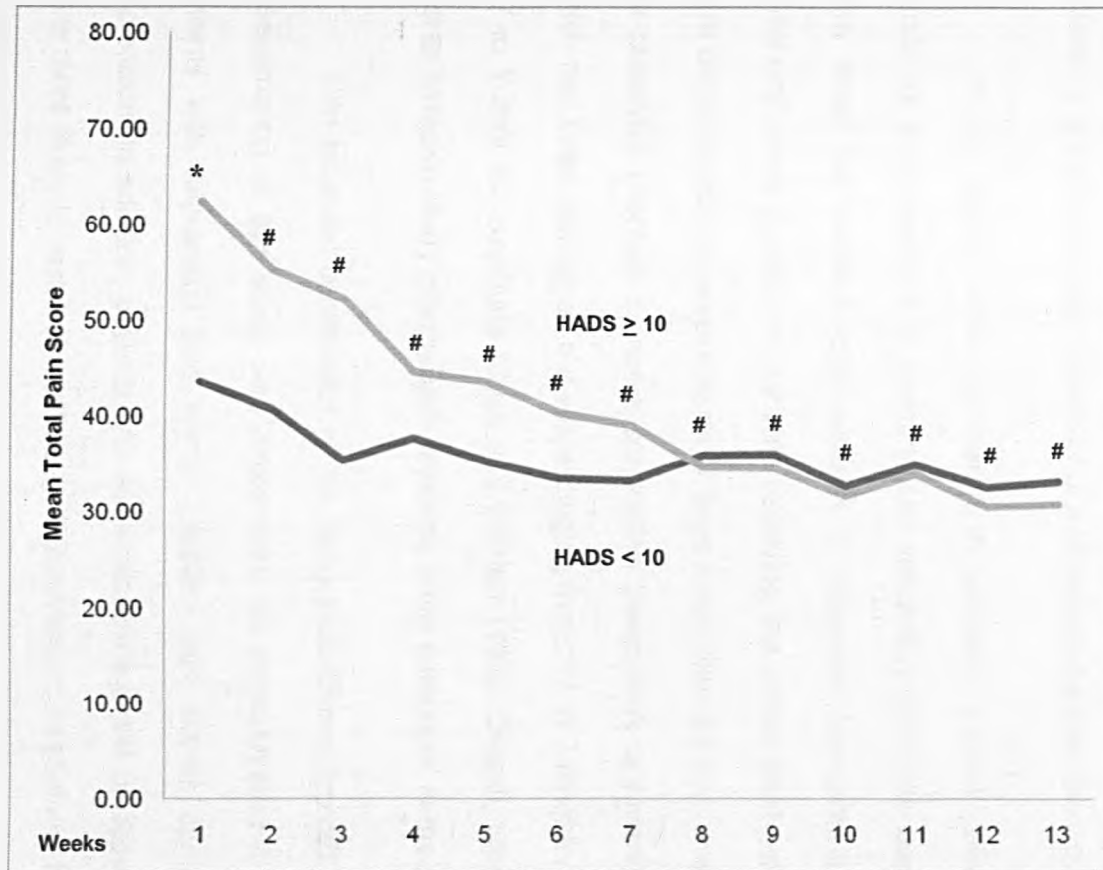
7.3.5 Neurophysiological assessments

Results of neurophysiological assessments that were performed are displayed in Table 7.2. Over the 13 week treatment period there were improvements in most parameters of nerve conduction studied in those receiving sativex compared with placebo. Mean differences nerve conduction velocity between sativex patients and placebo patients in change from baseline to endpoint ranged from 2.3 to 7.9 m/s and -10.4 to 3.0m/s respectively. The direction of change for nerve conduction amplitude from baseline also favoured the sativex

group (mean difference range: sativex 0.3 to 2.4 μ V vs placebo 0.0 to -3.0 μ V). Only sural amplitude (mean change from baseline 1.70 vs -2.5 $p=0.038$, SEM 1.7; 95%CI 0.23:7.5) reached statistical significance. There was no significant difference in vibration perception threshold ($p=0.81$, 2.6;-6.0:4.8) and heart rate variation during deep breathing ($p=0.62$, 0.32;-0.82:0.50) between groups.

7.3.6 Depression post hoc analysis

Of the 29 patients included in the intention to treat analysis, we excluded one patient (sativex arm) from this analysis because of a failure to complete the HADS questionnaire at baseline. Mean HADS for patients with depression ($n=10$) and no depression ($n=18$) were 13.4(SD 3.5) and 5.94(2.2) respectively. Patients with depression had significantly higher baseline total pain scores (mm) compared to those without depression [62.3(22.1) vs 43.4(24.3); $p=0.05$, Figure 7.3]. Patients with depression also had significant improvement in total pain score compared to those without depression, after adjusting for allocation to the active or placebo arm of the study [mean change in total pain scores (SD) -31.6(24.2) vs -10.7(25.0); $p=0.04$, SEM 9.8, 95%CI 0.54:41.1].



95%	-37.6:	-35.8:	-39.2:	-28.2:	-30.1:	-29.7:	-29.0:	-21.3:	-21.7:	-22.7:	-23.1:	-22.6:	-21.8:
CI	-0.001	7.00	5.67	14.2	13.6	15.9	17.4	23.9	24.5	24.7	25.0	26.6	26.8

Figure 7.3: Mean weekly total pain scores (visual analogue scale, mm) of patients categorised according to Hospital Anxiety and Depression Scores (HADS). * $p = 0.05$, # $p > 0.05$.

7.4 Discussion

Despite being a common illness, to date there exist few effective treatments that provide symptomatic relief for painful DPN. For centuries, cannabinoids have been consumed for their analgesic properties and more recently studied in other neuropathic conditions, namely multiple sclerosis (Kalant 2001). This is the first randomised, placebo-controlled trial assessing the efficacy and tolerability of a sativex in the treatment of painful DPN.

In this study, when compared to placebo, Sativex failed to show statistically significant improvements in primary and secondary outcome measures. However, there were small but modest improvements in objective neurophysiological assessments of peripheral nerve function in patients receiving the active treatment. These improvements were demonstrated in assessments of large nerve fibre but not small fibre function. Notably, the observed changes in nerve conduction parameters approached clinical significance, which has been defined as a d-value ranging from 2.2 to 2.9m/s for conduction velocity and 0.7 to 1.2mV for amplitude (Dyck and O'Brien 1989). Overall, however, the differences in scores between study groups were relatively small compared to the observed variance.

One potential confounder of the more patientive outcomes (pain and quality of life assessments) of this study was depression. As demonstrated in our post hoc analysis, patients with depression had higher baseline pain scores compared to those without depression. In addition, patients with even moderate levels of depression (HADS 13.4[3.5]) were more likely to respond favourably to intervention (regardless if active or placebo) with significant improvements in study end points.

Several studies have demonstrated that quality of life is significantly reduced in patients with painful DPN (Benbow, Wallymahmed et al. 1998; Galer, Gianas et al. 2000; Vinik, Hayes et al. 2005). These patients are also more likely than patients without DPN to have additional co-morbidities which include anxiety and pain associated interference with

sleep and activities of daily living (Gore, Brandenburg et al. 2005). It is not surprising, therefore, when these combinations lead to depression (Berger, Dukes et al. 2004). Most painful DPN clinical trials to date have not screened for depression and those that have use it as a reason to exclude patients from the study (Max, Lynch et al. 1992; Wernicke, Pritchett et al. 2006). Depression was a significant factor affecting the outcome of this study and could potentially be a major confounder in trials assessing the efficacy of therapeutic interventions in painful DPN. Future clinical trials should therefore consider screening for the presence of depression before recruiting patients to prevent its impact on outcomes.

Improvements in neurophysiological parameters observed in this study are novel, incidental findings and require confirmation in a larger, adequately powered study. There have previously been no human studies investigating the effects of cannabinoid agonists on nerve electrophysiology in DPN. Cannabinoid receptors have been identified throughout the nervous system and divided into two main classes: CB1 and CB2 (Grant and Cahn 2005). CB1 receptors have been demonstrated in both the PNS and CNS, including areas in the brain and spinal cord involved in pain transmission and modulation (Hohmann and Herkenham 1999) (Farquhar-Smith, Egertova et al. 2000). Electrophysiological and neurochemical studies in rats provide further evidence that cannabinoids suppress nociceptive transmission in vivo (Ahluwalia, Urban et al. 2000). This suppression is mediated by cannabinoid receptors and is generalised to different modalities of noxious stimulation (mechanical, thermal and chemical) (Iversen and Chapman 2002). Cameron et al. reported the antinociceptive property of cannabinoids in painful DPN in diabetic rats (Ellington, Cotter et al. 2002). They demonstrated that activation of peripheral CB1 receptors results in analgesia by inhibition of capsaicin evoked neuropeptide (calcitonin gene related peptide) release. None of these studies, however, assessed changes in nerve electrophysiology following the administration of cannabinoids. Recently, new non-CB1/CB2 cannabinoid receptors have been found in capsaicin sensitive sensory nerves in rats (Duncan, Kendall et al. 2004). These receptors modulate arterial vasoconstriction/relaxation. Hence we postulate

that improvements in nerve electrophysiology demonstrated in our study maybe the consequence of changes/improvements in nerve blood flow.

In addition to depression, the use of concurrent analgesic medications may also be another confounding factor. We allowed patients to continue these medications because of the proposed adjunctive use of Sativex for painful DPN. Also ethically we were reluctant to stop these medications as patients could have been benefiting from them. This may have attenuated the analgesic response to Sativex that was eventually observed.

In this study, as in a number of recent studies, there was a large placebo effect that led to a failure to show a difference in outcome measures. This study provides a unique insight into the placebo effect in a painful DPN trial. The patient population studied consisted of individuals with severe intractable neuropathic pain, causing significant morbidity and limitations to quality of life. The average duration of symptoms was long, over which period patients have tried and failed on a number of conventional medications. Hence, this group of individuals reflected the more severe end of the spectrum of DPN and were desperate for any intervention to improve their pain. These terribly disabled patients, not uncommon in clinical practice, were studied to see if adjunctive treatment with Sativex could provide additional pain relief. However, the high expectations of improvement upon entering the study, together with the psychological effects of taking medication combined with frequent interactions with a team of researchers interested in their well being and tendencies to please their doctors, may have resulted in the unexpectedly large placebo response. Notably there was no difference in the use of rescue medication between study groups to account for the improvements seen in the placebo group.

Recent clinical trials of pain relieving compounds in painful DPN have failed to show significant improvements in both clinical and neurophysiological surrogate neuropathy endpoints. The Ruboxistaurin Study Group recently reported on the placebo treated arms of two large, randomised, controlled trials (Tesfaye, Tandan et al. 2007). They found significant

improvements not only in symptoms but also in signs and quantitative vibration testing, in both the placebo and active arms even after one year follow-up. It was surprising that despite such a long follow-up period, there was (Boulton 2007) a strong placebo effect.

Given the strong interaction between chronic pain and depression and the findings of our study, concomitant depression could have been another confounding influence. This underlines the need for development of robust and objective clinical endpoints for use in clinical trials of painful neuropathy. Finally, whilst the search for therapeutic agents to halt or reverse the neuropathic process continues, more effective treatments are required which provided better symptom control with fewer side effects. In this first ever study assessing the efficacy of CBME in painful DPN, there was a large placebo response that led to negative findings. However, we identified depression as a major contributory factor influencing the outcome of this study. Therefore assessment of depression is important in future clinical trials into painful DPN.

8 FINAL DISCUSSION AND FURTHER WORK

A principal aim of this work was to investigate the involvement of the CNS in DPN. In chapter 3, I report that spinal cord atrophy is present not only in patients with established painless DPN, but appears to be an early phenomenon in patients with subclinical DPN. In addition, I also found significant correlations between spinal cord area and traditional neuropathy severity markers and Composite Neuropathy Score. Hence I postulate that spinal cord involvement may occur concomitantly with peripheral nerve damage. The findings of this study clearly demonstrate that the neuropathic process in man is not confined to the peripheral nerve and does involve the spinal cord. Worryingly, this occurs early in the neuropathic process. Even at the Subclinical DPN stage, extensive and perhaps even irreversible damage may have occurred. Prospective studies are now required to determine the natural history of spinal cord involvement in DPN.

As the spinal cord is the caudal portion of the CNS, its involvement made us question whether the brain too may be involved. Ascending sensory pathways traverse the spinal cord to terminate within the thalamus. The thalamus does not merely act as a sensory relay station but recently has been found to modulate/process information before it is transmitted to the sensory cortex. Using NAA resonance derived from H-MRS as a marker of neuronal integrity, I reported in chapter 4 the presence of thalamic neuronal dysfunction in patients with Painless DPN. This suggests the impact of diabetes on the nervous system appears to be far more generalised than previously thought. A larger study is required to confirm the findings of this preliminary study but also to investigate if neuronal dysfunction affects other regions of the brain responsible for sensory perception e.g. somatosensory cortex. A more thorough appreciation of the nature and extent of involvement of the thalamus a) at different stages of neuropathy and b) in painful neuropathy may be important to the understanding and hence treatment of this condition.

Little is known of the pathogenesis of thalamic neuronal impairment in DPN. In Chapter 5 I investigate a metabolic hypothesis involving abnormal glutamatergic homeostasis and astrocyte dysfunction. We report elevated Glx resonance and impaired thalamic astrocyte glial response to neuronal impairment in DPN. With evidence from histopathological studies that have shown glutamatergic dysfunction potentially results in neurotoxicity and subsequent neuronal dysfunction, our demonstration of elevated Glx suggests that glutamate mediated neuronal injury/cell death may be one of the mechanisms resulting in thalamic neuronal dysfunction in DPN. The diminished thalamic astrocyte response to neuronal impairment, as evidenced by lower ml resonance in patients with DPN, suggests that astrocyte dysfunction could be the underlying cause for abnormal thalamic glutamatergic homeostasis. Given the pathophysiological importance of glutamate further evaluation of the relationship between thalamic H-MRS abnormalities and DPN is needed.

In Chapter 6 I report on an MR perfusion study of the thalamus in DPN. Using exogenous intravenous contrast and fast acquisition sequence we were able to study the microvascular perfusion characteristics of the thalamus. Diabetic patients with Painful DPN had significantly higher thalamic rCBV and shorter recirculation time compared to the other study groups. This suggests that abnormal thalamic vascularity could be an important contributing factor to the development of pain in diabetes. These novel findings suggest a pathogenic microvascular mechanism for thalamic neuronal dysfunction in DN, which may be amenable to modifications of microvascular disease risk factors. MR perfusion is a promising technique providing biomarkers for assessing regional brain haemodynamic function in DN. Evaluating a larger sample of patients and including a subgroup with Subclinical DPN will not only provide more information on the reproducibility of MR perfusion imaging but also may lead to a new insight into the pathogenesis of CNS complications in DPN.

Painful diabetic neuropathy is a relatively common complication of diabetes that causes great distress and disability to sufferers and affects quality of life immensely.

Unfortunately current drugs are empirically given to sufferers of neuropathic pain of many origins and at best provide clinically significant (>50%) pain relief in only one third of patients, and this is achieved at the expense of often intolerable side-effect. Many patients turn to alternative therapies seeking adequate pain relief. In our clinical practice, we have had anecdotal reports of patients receiving significant pain relief from the use of cannabis. Hence we designed a randomised placebo controlled trial to assess the efficacy, tolerability and safety of sativex, in painful DPN. I reported the results of this study in Chapter 7. There was no significant difference in primary or secondary outcome measures of pain relief between patients randomised to sativex compared with placebo. In a subsequent post-hoc analysis, I reported that a potential major confounder to this trial and possibly future clinical trials in painful DPN is the presence of depression. Patients with depression had higher baseline pain scores and responded more favourably to the intervention regardless of receiving the active compound or placebo. A subsequent larger multi centre clinical trial based on this study showed similar results. Another factor which may have influenced the outcome of this trial was the unexpected large placebo response. Whilst the search for therapeutic agents to halt or reverse the neuropathic process continues, more effective treatments are required which provide better symptom control with fewer side effects. The assessment of depression may be important when designing future clinical trials into painful-DPN.

Our limited knowledge of the pathophysiological mechanisms involved in the onset and persistence of painful neuropathy possibly explains the lack of rationale (mechanism-based) treatment approaches that specifically target painful DPN. Recent evidence points to changes (central sensitisation) that occur at different levels within the CNS that may play an important role in the pathogenesis of painful DPN.

Future studies to address this will take advantage of recent advances in non-invasive, in-vivo neuroimaging techniques which allow investigation of regional brain activation in response to painful stimuli (BOLD fMRI). Utilising fMRI to study any disruption of the pain

matrix may provide sensitive and specific biomarkers of the sensation of DPNP. As well as clarifying disease mechanisms, identifying the neural correlates of pain-processing in DPN will result in opportunities to target specific components of the pain pathway pharmacologically. Combination of fMRI and drug administration, known as pharmacological fMRI (phMRI) may prove useful in the future development of new analgesic compounds. Experience from other chronic pain conditions strongly supports the view that neuro-imaging will aid our understanding of the basic mechanisms contributing to chronic pain states. These techniques might help diagnose a patient's pain condition in a more objective and robust way, enabling better targeting of therapies and rapid development of compounds to alleviate pain. A better understanding of these processes may lead to: i) a novel way of monitoring disease activity, natural history, response to treatment (quantify objectively the placebo effect) and ii) the identification of new targets for future therapies.

APPENDIX 1: Metabolic and Vascular Consequences of Diabetes on the Central Nervous System.

Diabetes mellitus is a common metabolic disorder that results in considerable morbidity, most of which is the results of target end-organ damage. The kidney, retina, peripheral nervous system (PNS), and small and large blood vessels are the predominant organs, which have been the focus of most clinical and experimental studies. The central nervous system (CNS), which is both functionally and structurally connected to the PNS, has been largely ignored. In recent years it has become evident that diabetes causes significant CNS complications, resulting in important functional impairments (Mijnhout, Scheltens et al. 2006). The CNS is affected by both the metabolic and vascular consequences of diabetes.

Cognitive Dysfunction

It is well established that the frequency of both diabetes and cognitive dysfunction increases with age (Brands, Henselmans et al. 2003). Epidemiologic studies have reported that diabetes is an independent risk factor for cognitive impairment (Fagot-Campagna, Bourdel-Marchasson et al. 2005; van den Berg, de Craen et al. 2006). Thus, it is not surprising then that both prospective and cross-sectional studies have suggested a link between diabetes and an increased risk of dementia (Lobo, Launer et al. 2000; Areosa and Grimley 2002; Allen, Frier et al. 2004). However, whether this refers to an increased risk of vascular dementia or Alzheimer's disease is still a cause of much debate. In most studies the definition of Alzheimer's disease is the presence of dementia in the absence of other systemic disorders (ie, vascular disease), which could account for the progressive decline (Stewart and Liolitsa 1999). It appears that categorising dementia into distinct subgroups limits etiologic research because this does not adequately recognize mixed, often overlapping disorders such as diabetes that may have effects on more than one pathologic process (Stewart and Liolitsa 1999).

Epidemiology

There have been a number of studies investigating the association between diabetes and cognitive decline and dementia (Heyman, Wilkinson et al. 1984; Broe, Henderson et al. 1990; Mortel, Wood et al. 1993; Ott, Stolk et al. 1996; Lindsay, Hebert et al. 1997). Two community-based prospective studies that have been performed are the Hisayama Study (Yoshitake, Kiyohara et al. 1995) and the Rotterdam Study (Ott, Stolk et al. 1996). The Hisayama Study reported a relative risk (RR) conferred by diabetes of 2.77 and 2.18 for vascular dementia and Alzheimer's disease, respectively. However, the diagnosis of diabetes was based only on history alone and, therefore, possibly overestimated its impact. The Rotterdam Study, on the other hand, used a more robust combination of history and random blood glucose, and if necessary an oral glucose tolerance test to diagnose diabetes. Here there was a significant increase in the RR of 1.9 in developing dementia and Alzheimer's disease. A nonsignificant increase in the RR of 1.0 was found for vascular dementia (Ott, Stolk et al. 1996).

Risk factors and pathogenesis

A number of factors coexist with diabetes that could explain its association with cognitive impairment and dementia.

Atherosclerotic disease

The Framingham Study demonstrated that hypertensive disease has clear modulating effects on cognitive dysfunction in diabetes (Elias, Elias et al. 1997). Diabetic hypertensive patients have low *N*-acetylaspartate (NAA) in deep white matter regions compared with hypertensive and normotensive individuals (Walker, Ben Salem et al. 2006). Cerebral NAA is an indicator of functional neuronal and axonal mass. Previous MRI studies showed that ischemic white matter lesions are associated with cognitive dysfunction, depression, gait disturbance, and future stroke (Sabri, Ringelstein et al. 1999; Firbank, Minett et al. 2003).

Thus, the decreased NAA in diabetic hypertensive patients indicates an increased risk for cognitive dysfunction.

In addition, raised triglyceride levels are also associated with worse performance on cognitive tests assessing attention, concentration, and psychomotor speed in patients with diabetes (Perlmutter, Nathan et al. 1988). These effects were independent of the cholesterol level, hypertension, and the rate of glycaemic control. Therefore, it is likely that when these risk factors combine, they mediate the development of cognitive dysfunction through acceleration of atherosclerotic disease.

Being overweight (body mass index ≥ 25 kg/m²) and obese (≥ 30 kg/m²) seems to be a risk factor for early dementia, whereas variation within the healthy range of body mass index (18.5–24.9 kg/m²) during midlife may not (Gustafson 2006). Because being overweight or obese may indicate a vascular burden increasing the risk for subsequent vascular disorders, these states may be an initial trigger eventually leading to Alzheimer's disease and vascular forms of dementia (Gustafson 2006). However, it is overlooked that adipose tissue is the largest endocrine organ in the human body and that the effects of being overweight or obese on brain health might be independent of vascular effects and instead be due to adipocyte hormones and cytokines (Gustafson 2006).

Glycaemic control

Glycaemic control may also be involved because elevated fasting plasma glucose and higher glycosylated hemoglobin (HbA_{1c}) have been found to increase error on the Mini-Mental State Examination and are associated with impairment in concentration and memory in patients with diabetes (Kalmijn, Feskens et al. 1995). Both the Rotterdam and Framingham studies found increased risk of dementia among patients with diabetes treated with insulin (Ott, Stolk et al. 1996; Elias, Elias et al. 1997)[5,8]. Patients on oral hypoglycaemic agents had an intermediate risk, suggesting that insulin treatment is not

causally related to the development of dementia but rather is a reflection of glycaemic control.

HbA_{1c} is also a risk factor for a greater risk of brain atrophy. In fact, the only significant correlate of the rate of brain atrophy other than age was greater HbA_{1c} in individuals without diabetes (Enzinger, Fazekas et al. 2005). The ARIC (Atherosclerosis Risk in Communities) study was a population-based magnetic resonance prospective investigation of atherosclerosis, cardiovascular risk factors, and cerebral atrophy as assessed by ventricular and sulcal size (Knopman, Mosley et al. 2005). Diabetes was found to be associated with increased ventricular size and, therefore, may contribute to pathologic reductions in brain volume. Using a more precise method, the Framingham Offspring Study reported that both diabetes and hypertension were associated with whole-brain volume reductions (Seshadri, Wolf et al. 2004). Another population-based study, in a larger European cohort (CASCADE [Cardiovascular Determinants of Dementia]), also reported a strong association between diabetes and cortical atrophy (Schmidt, Launer et al. 2004).

Insulin resistance

Investigators of the Rotterdam study observed that the association between diabetes and cognitive decline persisted despite adjustments for evidence of strokes and other cardiovascular risk factors (Ott, Stolk et al. 1996). In fact the impact of traditional risk factors for atherosclerotic disease on the RR of incident dementia was minimal (Ott, Stolk et al. 1996). This suggests that other nonvascular mechanisms may also play a major role in the pathogenesis of dementia (Stewart and Liolitsa 1999). One possible underlying mechanism may be the apparent similarity between the pathogenesis of insulin resistance and Alzheimer's disease. The structure of amylin, characteristically found in the pancreatic islets of Langerhans in type 2 diabetes, is similar to the β amyloid found in the neuronal plaques of Alzheimer's disease (Cooper, Leighton et al. 1988; Edgington 1994; Dore, Kar et al. 1997). They both are peptides made of β pleated sheets that induce toxic effects in both

Alzheimer's disease and diabetes. Defects in the insulin-like growth factor-1 signaling pathway may also be important in both amyloid plaque formation and neuronal loss in Alzheimer's disease and in amylin-induced toxicity in diabetes (Dore, Kar et al. 1997).

Acetylcholine levels, an important neurotransmitter thought to have a major role in mediating the effect of cognitive decline in Alzheimer's disease, have been found to be reduced in the brain of patients with diabetes (Coyle, Price et al. 1983). Other abnormalities related to diabetes that may have negative consequences on cognitive function are mitochondrial dysfunction (Odawara, Tada et al. 1995) and the neurotoxic processes of the aldose reductase pathway (Greene, Sima et al. 1992).

Advanced glycation end products

Advanced glycation end products (AGEs), the byproducts of chronic hyperglycemia, may also have a role in promoting cognitive decline. Postmortem studies have shown that AGEs are present within the matrix of neurofibrillary tangles and senile plaques of Alzheimer's disease (Dickson, Sinicropi et al. 1996; Horie, Miyata et al. 1997; Takeda, Wakai et al. 2001). This has led several investigators to postulate a role for glycation as a mechanism contributing to amyloidogenesis and the assembling of tau proteins, the building blocks of neurofibrillary tangles in Alzheimer's disease (Finch and Cohen 1997). In addition, the activation of the RAGE receptor by AGEs may also result in disturbances in vascular homeostasis, leading to further ischemic damage (Renard, Chappey et al. 1997). The binding of β amyloid to the RAGE receptor may also contribute to neuronal loss in Alzheimer's disease (Yan, Fu et al. 1997).

What remains uncertain, however, is whether these vascular and nonvascular abnormalities that have so far been demonstrated in patients with diabetes induce the development of Alzheimer's disease or simply exacerbate a pre-existing disposition to development of Alzheimer's disease. Only long-term prospective postmortem and magnetic resonance studies will be able to address this question.

Despite the wealth of evidence for the relationship between diabetes, dementia, and cognitive dysfunction, studies assessing the impact of diabetes management on modifying the rate or nature of cognitive decline have been few and far between. Gradman et al. (Gradman, Laws et al. 1993) assessed the impact of glycaemic control on cognitive function in patients with type 2 diabetes treated with glipizide for 7 months. These investigators concluded that poor glycaemic control in older patients with type 2 diabetes is associated with impaired cognitive functioning and that verbal learning and memory improved with better control. In a larger randomized controlled study, Ryan et al. (Ryan, Freed et al. 2006) found similar and statistically significant cognitive improvement with both rosiglitazone and glyburide following a 24-week treatment period. The magnitude of this effect was correlated with the degree to the improvement in fasting plasma glucose, although improvements in circulatory insulin and insulin sensitivity in the thiazolidinedione cohort did not impact on cognitive function.

Hypoglycaemia

Hypoglycemia and Cognitive Dysfunction

In healthy humans, brain glucose supply is maintained by an efficient homeostatic mechanism, which keeps blood glucose concentrations in a narrow range, sufficient to support normal brain function (Maran, Lomas et al. 1995). Falling blood glucose levels, unless corrected, would lead progressively from cognitive impairment to convulsion, coma, and eventually death. Using clamp techniques, moderate levels of hypoglycemia impair general cognitive abilities, including reaction time, analytical ability, verbal fluency, and verbal and visual memory (Frier and Fisher 1999). Cognitive performance as assessed by neurophysiologic methods, such as the P300 wave evoked potential (considered to represent stimulus processing and selection), are abnormal during moderate hypoglycemia indicating slower information processing (Picton 1992). Studies using the stepped glucose clamp have reported varying levels, between 2.3 to 3.1 mmol/L, at which cognitive

impairment occurs (Widom and Simonson 1990; Fanelli, Epifano et al. 1993; Veneman, Mitrakou et al. 1994).

There are also suggestions in the literature that some cognitive processes are more sensitive to hypoglycemia than others. For example, during moderate hypoglycemia, working memory is completely abolished, whereas short- and long-term memory only deteriorate in the same patients (Deary, Sommerfield et al. 2003). Simple reaction time was less affected than choice reaction time and finger tapping was insensitive to hypoglycemia (Cox, Gonder-Frederick et al. 1993). Using functional MRI to study regional changes in oxygenation status of the brain, Rosenthal et al. have shown that the effect of acute hypoglycemia on the human brain is both task and region specific (Rosenthal, Amiel et al. 2001). When the performance of simple tasks (finger tapping) is impaired by hypoglycemia, associated localized reduction in brain activation is seen. For more complex tasks (four choice reaction time), on the other hand, there is increased activation in planning areas compatible with recruitment of brain regions in an attempt to limit dysfunction. This may help explain the differences in sensitivity to hypoglycemia observed in different cortical functions.

The consequences of chronic recurrent severe hypoglycemia on the brain are not fully defined. The prospective DCCT (Diabetes Control and Complications Trial) and the Stockholm Diabetes Intervention Study have not shown any adverse effects of hypoglycemia on cognitive ability (DCCT research group 1996; Reichard, Pihl et al. 1996). However, these studies may not have been sufficiently long, and in the case of the DCCT there appeared to be a rather low rate of severe hypoglycemia (Warren and Frier 2005). Nonetheless, it is likely that hypoglycemia probably acts in conjunction with the other insults of diabetes (hyperglycemia, insulin resistance, hypertension, dyslipidemia, and so forth) to cause "diabetic encephalopathy" (Mijnhout, Scheltens et al. 2006).

Counterregulation

In healthy patients there are separate blood glucose thresholds for the sequence of events that occur during hypoglycemia. The triggering of autonomic activation occurs before significant cortical dysfunction develops. This allows an individual to remain able to take appropriate corrective action before coma ensues (Hepburn, Deary et al. 1991). Unfortunately, the adaptation of these thresholds to glycaemic exposure can subsequently lead to severe or life-threatening hypoglycaemic episodes (Cryer 1999).

In humans, Boyle et. al. demonstrated preservation of whole-brain glucose uptake in patients with strictly controlled diabetes during experimental hypoglycemia (3.0 mmol/L) compared with a 20% fall in glucose uptake in nondiabetic patients and patients with poorly controlled diabetes (Boyle, Kempers et al. 1995). A recent study using magnetic resonance spectroscopy demonstrated increased glucose content during hyperglycemia in patients who were hypoglycemia unaware compared with nondiabetic patients (Criego, Tkac et al. 2005). This suggests that hypoglycemia counterregulation is at least partly triggered by a glucose-sensitive brain region and so the brain must be responsible for adaptation to some degree.

One mechanism proposed for adaptation is brain upregulation of the GLUT1 transporter (Boyle, Nagy et al. 1994). Other authors have proposed the production of alternative brain fuels; however, neither the nature nor the existence of a second adaptive mechanism is clear (Mc 1953; Sloviter, Shimkin et al. 1966; Agardh, Westerberg et al. 1980). The adaptive mechanisms appear to be both dynamic and reversible. Mitrakou et al. demonstrated the preservation of cognitive function at blood glucose levels as low as 2.3 mmol/L in patients with insulinomas and that this effect was abolished after surgical resection (Mitrakou, Fanelli et al. 1993). Fanelli et al. showed that careful avoidance of hypoglycemia by hypoglycaemic-unaware patients led to an increase in the blood glucose level for cognitive impairment (Fanelli, Epifano et al. 1993).

Hypoglycemia and brain damage

Prolonged severe hypoglycemia eventually leads to coma and brain damage (Auer 2004). As the levels of blood glucose fall progressively to the range of 1 to 2 mmol/L, patients start to become stuporous and drowsy. Cerebral tissue energy failure results when blood glucose falls by more than 97% (Feise, Kogure et al. 1977). Before this period, alternative fuels (lactate, glycerol, and ketone bodies) can sustain energy requirements. Lactate alone can substitute roughly for a quarter of glucose utilization. As the duration of hypoglycemia increases, coma finally supervenes when the threshold of energy failure is reached (< 1 mmol/L). Over time, this is accompanied electroencephalographically by isoelectricity. Once the electroencephalogram goes flat, neuronal necrosis appears over the ensuing minutes as the neurotransmitter aspartate is released into the extracellular space and floods excitatory amino acid receptors located on neuronal dendrites (Agardh, Folbergrova et al. 1978; Sandberg, Butcher et al. 1986)]. Calcium fluxes occur and resultant membrane breaks lead rapidly to neuronal necrosis. Significant neuronal necrosis occurs after 30 minutes of electrocerebral silence.

Hypoglycaemic brain damage is unique in its distribution. Unlike ischemia, the neuropathologic distribution of hypoglycaemic brain damage has a predilection for superficial cortical layers and necrosis of the dentate nucleus of the hippocampus is often seen (Auer, Wieloch et al. 1984). These characteristic appearances are called cortical laminar necrosis and occur as a result of delayed selective neuronal necrosis of the cerebral cortex, with the cerebellum and brain stem universally spared (Agardh, Kalimo et al. 1981; Agardh and Siesjo 1981). Hypoglycemia also results in asymmetrical brain damage. This is because electrocerebral silence, which is a prerequisite for the initiation of neuronal necrosis, is asynchronous between the cerebral hemispheres (Harris, Wieloch et al. 1984).

Diffusion-weighted MRI is an established tool in the assessment of patients with hypoglycaemic coma (Fujioka, Okuchi et al. 1997; Barkovich, Ali et al. 1998; Bakshi, Morcos et al. 2000; Finelli 2001; Chan, Erbay et al. 2003; Aoki, Sato et al. 2004). It is sensitive for detecting neuronal energy depletion and subsequent cytotoxic edema, visualizing such

changes as a high intensity area. Within minutes, images can demonstrate abnormal signal and only fresh lesions are defined. The clinical outcome after a severe hypoglycaemic coma correlates with the basal ganglia involvement. Patients with basal ganglia involvement tend to survive in a persistent vegetative state, whereas those with the basal ganglia spared survive with lesser degrees of neurologic impairment.

Cerebrovascular Disease

Numerous prospective studies, mainly in type 2 diabetes, have shown a two- to fivefold increase in the RR of ischemic strokes (Abbott, Donahue et al. 1987; Davis, Dambrosia et al. 1987; Barrett-Connor and Khaw 1988; Stamler, Vaccaro et al. 1993; D'Agostino, Wolf et al. 1994; Kuusisto, Mykkanen et al. 1994; Tuomilehto, Rastenyte et al. 1996; Folsom, Rasmussen et al. 1999). In the Framingham Study, the increased risk attributable to diabetes was independent of other risk factors such as systolic blood pressure, atrial fibrillation, antihypertensive treatment, smoking, cardiovascular disease, and left ventricular hypertrophy (Elias, Elias et al. 1997).

Risk factors

Nonmodifiable risk factors

Sex appears to be an important modulator for stroke risk. In women, diabetes is as potent a risk factor as cigarette smoking; compared with men, diabetes is associated with a higher stroke case fatality with an odds ratio of 2.33 (Rothwell, Coull et al. 2004). Age is a risk factor that increases the incidence of stroke as the population ages (You, McNeil et al. 1997). Race and ethnicity also impact on the risk of stroke. The NOMAS (Northern Manhattan Stroke Study) found that blacks and Hispanics have a 2.4- and twofold increased risk of stroke, respectively, compared with whites (Sacco, Benson et al. 2001). This may be attributed to a greater role for diabetes and hypertension, which have a higher prevalence in these ethnic populations.

Modifiable risk factors

Given the strong association between diabetes and stroke, it is perhaps not surprising that both fasting and random plasma glucose were among the baseline characteristics in a variety of prospective studies that most strongly predict stroke in patients with type 2 diabetes mellitus. The UKPDS (United Kingdom Prospective Diabetes Study) reported that each 1% rise in HbA_{1c} resulted in an increase in case fatality odds ratio by 1.37 (Stevens, Coleman et al. 2004). Hypertension is a frequent complication in patients with diabetes compounding the risk of stroke in this population. As in the general population, well-controlled blood pressure can reduce the risk of stroke significantly among patients with diabetes. Data from the UKPDS showed a 44% RR reduction for stroke in the groups where blood pressure was well controlled (mean blood pressure 144/82 mm Hg) (UK Prospective Diabetes Study group 1998). In fact, tight blood pressure control has a greater impact in reducing the risk of any diabetes-related end point than intensive glucose control (24% RR in tight blood pressure vs 12% RR in intensive glucose control group, $P = 0.026$).

The relationship between hypercholesterolemia and stroke risk, on the other hand, is more tenuous. Meta-analysis of epidemiologic studies has not shown a clear relationship between cholesterol levels and stroke (Atkins, Psaty et al. 1993). This possibly reflects the more heterogeneous nature of cerebrovascular disease compared with coronary artery disease, with fewer strokes attributable to large-vessel atherosclerotic disease. Nonetheless, two prospective intervention studies (LIPID [Long-term Intervention with Pravastatin in Ischaemic Disease] and CARE [Cholesterol and Recurrent Events]), which had cerebrovascular events as a primary end point, showed a big RR reduction with statin treatment compared with placebo (1998; LIPID Study Group 1998; Plehn, Davis et al. 1999). More recently, the CARDS (Collaborative Atorvastatin Diabetes Study) trial randomized 2338 patients with type 2 diabetes without high low-density lipoprotein cholesterol, but with one other risk factor to receive either 10 mg of atorvastatin or placebo daily. Active treatment with atorvastatin significantly reduces the incidence of new stroke by 48%, independent of

patient's age, gender, baseline cholesterol, and blood pressure levels (Colhoun, Betteridge et al. 2004). In the NOMAS study, lower high-density lipoprotein (HDL) cholesterol has been shown to be associated with an increased risk of stroke (Sacco, Benson et al. 2001). This may suggest a link between diabetes through its association with metabolic syndrome and increased risk of stroke. This is possible because low HDL cholesterol levels are thought to contribute to the acceleration of atherosclerosis in many of these patients.

“Nontraditional” risk factors

In the ARIC study, the influence of diabetes on stroke incidence was not entirely explained by a set of traditional risk factors with which it is known to be associated (Folsom, Rasmussen et al. 1999). Similar outcomes were seen in the Physicians' Health Study, where clinical obesity increased stroke risk independent of blood pressure, cholesterol, and diabetes (Kurth, Gaziano et al. 2002). More recently, the Framingham Offspring Study found that the influence of the metabolic syndrome on stroke incidence persists after adjustment of its component risk factors (Najarian, Sullivan et al. 2006). This suggests that other factors of the metabolic syndrome, such as systemic inflammation, abnormal endothelial function, and vascular factors, may be operative. Thus, in addition to plasma glucose, the components of the metabolic syndrome both individually and collectively contribute to increased stroke risk.

Diabetic versus nondiabetic strokes

The distribution or pattern of ischemic stroke involvement differs with the presence or absence of diabetes. Autopsy studies suggest a higher rate of lacunae infarcts in the basal ganglia, paramedian basis pontis, thalamus, and cerebellum (Kane and Aronson 1968; Aronson 1973; Peress, Kane et al. 1973). These changes termed “encephalomalacia” have been seen in in vivo neuroimaging studies, where lacunar and subcortical infarcts are more common in patients with diabetes compared with complete middle cerebral artery territory infarcts (Karapanayiotides, Piechowski-Jozwiak et al. 2004). A prospective MRI study found that 41% of patients with type 2 diabetes who were initially free of lacunes developed them

over the 5-year follow-up period (Inoue, Fushimi et al. 1998). This association between diabetes, lacunar infarct, and subcortical infarct points to small-vessel disease of the deep penetrating vessels of the brain. In fact, histopathologic vascular abnormalities commonly found in diabetic retinopathy and nephropathy have also been shown to affect cerebral vessels (Fischer, Barner et al. 1979; Factor, Okun et al. 1980). These changes dubbed “nonatherosclerotic diabetic microangiopathy” are characterized by the accumulation of periodic acid-Schiff–positive material in the tunica media of vessels (Andresen, Rasmussen et al. 1996). Therefore, common pathogenic mechanisms causing both macro- and microvascular complications are implicated. Impaired cerebrovascular reactivity as demonstrated by transcranial Doppler ultrasound is a risk marker for first-ever lacuna infarct (Molina, Sabin et al. 1999). Cerebrovascular reserve capacity is lower in patients with diabetes, suggesting that diabetic microangiopathy reduces the vasodilatory ability of cerebral arterioles, placing additional strain on already ischemic regions (Fulesdi, Limburg et al. 1999). Chronic hyperglycemia is also thought to increase anaerobic metabolism and acidotoxicity, which results in poorer prognosis after stroke (Fanelli, Epifano et al. 1993). All these factors in combination result in a higher mortality rate (Stamler, Vaccaro et al. 1993) and slower recovery (Megherbi, Milan et al. 2003) after stroke in patients with diabetes compared to patients without diabetes.

Poststroke hyperglycemia

Poststroke hyperglycemia is a frequent finding in patients presenting with acute stroke and is an established predictor of poor outcome in both diabetic and nondiabetic patients (Capes, Hunt et al. 2001). It is not clear if this is simply a reflection of the catabolic stress response to stroke and thus an association by proxy to stroke severity, or if hyperglycemia could have a detrimental affect by inducing serum hyperviscosity and promoting acidosis by acting as a substrate for anaerobic lactic production in an ischemic milieu (Idris, Thomson et al. 2006). Trials are currently underway to assess the benefits of maintaining euglycemia in patients with acute stroke (Gray, Scott et al. 2004). Another possible explanation for the greater

prevalence of hyperglycemia in the acute stroke setting may be the unmasking of undiagnosed diabetes in this select group of patients who are already at high risk. A recent study estimated the prevalence of undiagnosed diabetes to range between 16% to 24% (confirmed by an oral glucose tolerance test 12 weeks after acute stroke)(Gray, Scott et al. 2004). Two thirds of patients with poststroke hyperglycemia had either impaired glucose tolerance or diabetes in this study.

Intracerebral hemorrhage

Intracerebral hemorrhage (ICH) appears to be less common in diabetes. ICH was six times less frequent in diabetic patients in the Copenhagen Stroke Study (Jorgensen, Nakayama et al. 1994). In the Honolulu Heart Program, the risk of ischemic stroke increased across categories of increasing glucose intolerance, whereas the risk of ICH did not vary and was not significantly increased (Burchfiel, Curb et al. 1994). These findings from epidemiologic studies seem to support the pathologic evidence that diabetes may be a protective factor against ICH. In a series of consecutive autopsies, Kane and Aronson found that fibrinoid necrosis occurred infrequently in the arterioles and small arteries in the diabetic brain even in those with hypertension (Aronson 1973). Alex et al. postulated that basement membrane thickening and endothelial cell proliferation, commonly seen in diabetic brains, render the cerebral vessels less prone to rupture (Alex, Baron et al. 1962).

APPENDIX 2: Standard Operating Procedures

DPN is usually easily detected by careful history and simple clinical examination. Sophisticated investigations are often unnecessary. Shoes and socks should be removed and the feet examined. Very occasionally, further investigations, including neurophysiological and radiological tests (e.g. MRI of the lumbosacral spine) may be required. For clinical trials and research purposes, a more detailed clinical assessment is required.

Clinical Assessments

Neuropathy Symptoms Assessments

When examining diabetic patients, it is important to ascertain that symptoms are of neuropathic origin (Table A7.1). The distribution and character of the sensory disturbance needs to be carefully documented, in order to exclude other causes of similar symptoms, such as lumbar disc prolapse, peripheral vascular disease, or musculoskeletal disorder (e.g. arthritis).

Table A7.1: Symptoms of DPN.

Sensory symptoms

Numbness, paraesthesiae, pain (burning, stabbing, shooting, deep aching), unusual sensations ('tightly wrapped', 'swelling', etc), allodynia, inability to identify objects in hands

Motor symptoms

difficulty climbing stairs, difficulty lifting/handling small objects

Motor nerve fibres may be evaluated by measuring clinical strength. One may divide muscular weakness into ascendant degrees of severity, paralysis being the highest degree. The physician may ask the patient to perform a movement against a resistance. Reflex testing is an important part of this examination, although it may be normally reduced in a significant portion of the population older than 50.

Motor Assessment

Muscle strength is usually normal early during the course of the disease; however mild weakness may be found in toe extensors. However, with progressive disease there is significant generalised muscular wasting, particularly in the small muscles of the hands and feet. The fine movements of the fingers are then affected, and there is difficulty in handling small objects. The clawing of the toes is believed to be due to unopposed (because of wasting of the small muscles of the foot) pulling of the long extensor and flexor tendons.

Sensation Assessment

Sensation assessment is performed by looking at touch, vibration, temperature and pain sensations. Touch sensation can be tested by lightly rubbing a piece of cotton wool on the foot skin. More accurately, a nylon monofilament, which has been standardised to deliver a 10 gram force when it applied perpendicularly to the skin's surface and buckles, may be used. This is also known as Semmes-Weinsten monofilament. It has been validated for screening of patients at risk of developing ulcers. The test is positive if a patient fails to feel 2 out of 5 sites tested (NHS electronic library for diabetes, 2001). Vibration sensation can be evaluated by using a tuning fork. In severe cases, patients will not perceive the sensation at all but in milder cases, perception will be reduced but present. Some tuning forks have weighted ends with a printed Hertz scale on them, which allows a more accurate determination of vibration threshold.

Thermal sensations can be evaluated by using a metal rod, which induces a cold sensation in the normal patient but may not be perceived in those with small fibre DPN. Tubes filled with water can be warmed up to different temperatures so that sensitivity to warming may also be tested. Pain sensation is assessed by the pin prick test. The patient is asked whether he/she can distinguish between a blunt and a sharp object. Weighted needles can also be employed in order to establish a threshold (Chan, 1990, Bennett, 2001). The most common presenting abnormality is a reduction or absence of vibration sense in the toes. As the disease progresses there is sensory loss in a “stocking” and sometimes in a “glove” distribution involving all modalities. When there is severe sensory loss, proprioception may also be impaired, leading to a positive Romberg’s sign. Ankle tendon reflexes are lost and with more advanced neuropathy, knee reflexes are often reduced or absent.

Simple bed-side investigations

Vibration perception threshold may also be accurately determined using a biothesiometer (Figure A7.1). This device is relatively simple and provides a rapid and reproducible result. The stylus is placed on the great toe or any bony prominence and the patient asked to indicate when the vibratory sensation is first felt. The voltage at which this happens is the vibration perception threshold.



Figure A7.1: Biothesiometer

Autonomic fibre function can be impaired very early in the natural history of diabetic neuropathy, long before autonomic symptoms develop. Recognising such impairment is important because the affected patients may benefit from a tight glycaemic control. The simplest way to measure autonomic function is by studying the cardiovascular system. Orthostatic hypotension has been proposed to occur when small fibres in the splanchnic vascular bed are damaged in advanced diabetic neuropathy (McLeod and Tuck 1987). This can be done by assessing the postural fall in blood pressure. Systolic blood pressure is recorded at rest with the patient lying on the couch, and then pressure is measured again after approximately 60 seconds of the patient standing up. Normally, the autonomic nervous system is able to keep the level of blood pressure relatively constant in such a situation. If the level falls by at least 30 mmHg, sympathetic autonomic failure is diagnosed. Parasympathetic autonomic function may be assessed by recording the ECG for a few minutes and looking at heart rate (R-R) variations. Physiologically, heart rate variations should be present at rest. However, these variations are lost in the presence of parasympathetic denervation. To enhance the sensitivity of this technique, the patient may perform slower and deeper breathings (6 in a minute) while the ECG is recorded.

Heart rate normally changes according to breathing, orthostatism, increased intra-thoracic pressure and sympathetic activation. O'Brien et al. have standardised a number of simple tests to evaluate autonomic nerve function (O'Brien, O'Hare et al. 1986). Age-related normative values have been established. However, these tests should be performed by a well-trained health care professional and demand particular care in interpretation. The sympathetic nervous system is assessed by the Valsalva manoeuvre (especially late phase II and phase IV) and by the blood pressure response to standing or tilt. The parasympathetic cardio-vagal axis may be assessed by measuring the R-R variation during deep breathing and during the Valsalva manoeuvre. Computer assisted autonomic function tests, including lying to standing R-R variation, response to the Valsalva manoeuvre, response of diastolic blood pressure to sustained handgrip; evaluation of genitor-urinary function. Autonomic

neuropathy may also affect small fibres in the periphery, namely those innervating blood vessel and sweat glands. These are sympathetic fibres, either noradrenergic or cholinergic. Sweating may be quantified by droplets morphometry (Silastic imprint method) following iontophoresis of sympathetic agonist (Kennedy and Navarro 1989).

Assessment of neuropathic pain

Visual analogue and verbal descriptive scales

Neuropathic pain, which is variable in intensity, may be assessed by a visual analogue scale, which results have been shown to correlate with other measures of pain (Scott and Huskisson 1976). The patient is asked to indicate pain intensity on a line numbered from 0 to 100. A verbal descriptive scale may also be employed, the patient being asked to describe pain intensity in accordance with a series of descriptive statements (e.g., absent, slight, intense, very intense, etc). An inherent assumption of both devices is that equal intervals along the scales denote equal degrees of pain; the use of the two scales together may increase confidence in the observed changes.

Questionnaires for pain

Various questionnaires are being proposed for neuropathic pain, however there is no general agreement on which one should be used in diabetes:

- Neuropathy Total Symptom Score-6 (Bastyr, Price et al. 2005) is an instrument that assesses symptoms rather than pain per se. A symptom score for frequency times is estimated separately for each of deep aching, superficial burning, prickling, and lancinating pains, as well as for numbness and allodynia. "Tight", "compression", "boring", "squeezing" descriptions of pain are to be included with deep-aching pain. Each symptom is graded on intensity (mild, moderate, or severe) and on frequency (never or occasional, occasional but abnormal [$<33\%$ of the time], often [$>33\%$ - $>66\%$] or most continuous [$\geq 66\%$]). The total score possible for each symptom is 3.66.

- The Neuropathy Pain Scale (Galer and Jensen 1997) includes 10 items: intensity, sharp, hot, dull, cold, sensitive, itchy, unpleasant, deep, superficial. Patients are asked to answer on a 0 to 10 scale; according to the intensity of the category of pain they are experiencing. A score can then be calculated for each item.
- The LANSS (Leeds Assessment of Neuropathic Symptoms and Signs) pain scale includes a short questionnaire and a brief clinical examination. This assessment investigates the nature of pain. The higher the LANSS index, the more likely is the pathological the nature of the pain experienced. It may be used to distinguish neuropathic from nociceptive and psychogenic pain (Bennett 2001).
- The McGill Pain Questionnaire takes into account the quality and emotional aspects of pain. It consists of four classes of words describing pain, listed within each subclass in order of increasing intensity. The subclasses are grouped into four major categories: sensory (i.e., words that describe pain in terms of temporal, spatial, pressure, and other properties: subclasses 1-10), affective (i.e., words that describe pain in terms of tension, fear and autonomic symptoms: subclasses 11-15), evaluative (i.e., describing the overall intensity of the experience of pain: subgroup 16), and miscellaneous (subgroups 17-20). A score can be calculated for each category. The questionnaire also asks for additional information such as exacerbating or relieving factors, and anatomical location. Patients are asked to choose one word from each group, or to omit the group entirely. Responses are scored using the rank values of words chosen (Melzack 1975). The McGill Pain questionnaire is a quick and simple tool and is very helpful in the global evaluation of chronic pain syndromes. It may be potentially useful to adapt it to address the specific problem of differential diagnosis of the neuropathic leg (Masson, Hunt et al. 1989).
- ***Quantifying pain in diabetic neuropathy***

- Attempts to quantify this very patientive entity have led to the development of numerous questionnaires (Table A3.1). These employ verbal rating scales, self completed forms and visual analogue scales. It is also beneficial to use a quality of life assessment tool as quality of life is often affected in chronic pain states. Some of the more commonly used questionnaires are shown in the box below. They are most frequently used in pain clinics and in clinical trials setting.

Table A3.1: Questionnaires used to quantify and assess pain in diabetic neuropathy.

Pain generic

McGill Pain Questionnaire (Melzack 1975)

Neuropathic Pain Scale (Galer and Jensen 1997)

Quality of life generic

EuroQOL Questionnaire (The EuroQol group 1990)

Modified SF-36 (Jenkinson, Coulter et al. 1993)

Nottingham QOL questionnaire (Keinanen-Kiukaanniemi, Ohinmaa et al. 1996)

Quality of life diabetes specific

Norfolk QOL questionnaire (Vinik, Hayes et al. 2005)

ADDQOL questionnaire (Bradley, Todd et al. 1999)

Quality of Life assessment

Although diagnosis can readily be made on clinical examination, measuring the full impact of painful neuropathy is more difficult. Chronic pain affects many aspects of the sufferer's life. It is important to appreciate that there is little relationship between symptoms and signs. Some patients may experience severe symptoms with relatively normal examination and vice-versa. Thus, symptoms should not be dismissed, simply because there are no abnormal signs. Clinical assessment must also include examination of the mental state of the patient. An empathic approach to history taking is recommended, especially with regard to positive symptoms, as patients are often worried and distressed. Some patients with painful neuropathy are very anxious, as they mistakenly believe that their condition will inevitably result in amputation. Clearly, such unwarranted fears need to be dismissed by health care professionals. Others may feel guilty, as they perceive that a period of poor compliance with their diabetes control may have led to their predicament. Some may be intensely angry, as they have to cope with yet another diabetic complication.

Unfortunately, there are few instruments specifically designed to assess quality of life in diabetic neuropathy. Clearly, any such instrument must take into account the presence and impact of other diabetic complications too, as these may influence overall well-being. Specifically developed questionnaires may allow patients to describe their experience in a more standardised manner, and this is particularly important in monitoring response to therapy. A number of questionnaires have been developed, in order to assess quality of life.

Health-related quality of life can be measured either with generic measures, such as the Nottingham Health Profile or the Medical Outcomes Study Short Form 36 (SF-36) (Hunt, McKenna et al. 1981; Jenkinson, Coulter et al. 1993) or with specific instruments for pain. Recently, a theoretically based, clinically relevant neuropathy-specific quality of life scale has been developed and validated. This instrument, called Neuropathy Quality of Life (NeuroQoL), includes a neuropathy symptoms checklist and psychosocial domains. Such an

approach, being patient-centred, should increase physician's understanding as to how diabetic patients experience and deal with their neuropathy (Vileikyte, Peyrot et al. 2003).

Severity of neuropathy

Although attention should be paid to symptoms, these per se are not a good measure of severity, since they tend to come and go and do not correlate well with severity of impairments (Dyck and Thomas 1999). Overall severity is rather the sum of all sensory, autonomic and motor symptoms and impairments caused by DPN. To help categorising patients more accurately within the context of a progressive disease, it is recommended (Grant et al., 1999) to take into account abnormalities in the following:

- Neuropathic symptoms: positive and negative symptoms, symptoms of weakness and autonomic symptoms.
- Neurological examination: sensory and motor examination
- Quantitative motor, sensory or autonomic examination: measurement of vibration, warmth and cool detection thresholds, heat as pain threshold, cardiac autonomic function tests etc.
- Nerve conduction: abnormality of nerve conduction of one or more attributes (amplitude, velocity or distal latency of motor nerves) in two or more nerves.

Staging of neuropathy has been developed by (Dyck and Thomas 1999). This staging depends upon the severity of neuropathic symptoms and great emphasis is given to neuropathic pain. Stages range from 0 (no neuropathy) to 3 (disabling neuropathy). Provided that minimal criteria for diabetic polyneuropathy are met, the presence of pain places a patient in stages 2 or 3. More precisely, pain is graded as being "disabling" (stage 3) when the patient has previously attended a physician for pain relief; work and recreational activities have been curtailed by at least 25% because of pain; and medication for pain relief

has been taken on a continuing basis (>50% of days) for at least 6 weeks. When this is not the case, then a patient would be placed in stage 2 (pain of a lesser severity).

Clinical neuropathy assessment

Objective

To grade, score and report neuropathic dysfunction, impairment and symptoms using standard and reproducible approaches so that results are quantitative and useful for subdividing patients into different stages of diabetic neuropathy (DPN).

Key points

- Assessments were performed by investigator:
- Who is experienced in neurological examination.
- Uses the same basis for grading abnormality over time.
- Assume that the basis of abnormality is the 5th or 95th percentile (whichever applies) based on study of healthy patients (without neurologic disease, systemic disease or other exposure or deficiency predisposing patients to neurologic disease) taking into account age, sex, anthropomorphic factors (height, weight, body mass index) and physical fitness.
- Who washed his hands before and after examining patients and never re-used pins.
- Clinical assessments were performed in the following order: Neuropathy Symptom Score, Neuropathy Symptom Change questionnaire and upper and lower limb neurological examination (Neuropathy Impairment Score).

Neuropathy Symptoms Score (NSS)

NSS is a standard tally of neuropathic symptoms abstracted from a neurologic history that inquires about neuropathic symptoms. Recorded symptoms related to DPN, not from cranial, plexus, mononeuropathies, arthritis, carpal tunnel, aches and pains of living etc. Investigator decided whether it is from DPN. The Investigator decides whether a symptom is beyond (abnormal) from what occurs in healthy people of the age and sex of that patient. It is graded towards specificity not sensitivity. A general approach used was to ask direct questions such as "Do you have"

if the answer is "yes" and consistent with DPN, scored 1 recorded

if the answer is "no", scored 0 recorded

Neuropathy Symptom Change (NSC)

The NSC is a standard true and false questionnaire of neuropathic symptoms based on an interview of patients by a trained clinician.

Clinical neurological examination

Lower limb function

Standard proforma was completed. Ability to arise from kneeling and walk on toes and heels is quite a good indicator that major muscles of the lower limb are strong.

Neuropathy impairment score (NIS)

Cranial nerve involvement is atypical for DPN.

Muscle weakness (weakness graded was from DPN)

the muscle groups tested are listed

scored for age, sex, physical build, fitness and anthropomorphic features

graded using percentile, plus:

0 = normal; 1 = 25%; 2 = 50% and 3 = 75% weak; 3.25 = just able to move joint against gravity; 3.5 = able to move at joint with gravity eliminated; 3.75 = muscle flicker only; and 4 = paralysed

Reflexes (abnormality graded due to DPN).

scored for age, sex, physical attributes (build, fitness and anthropomorphic features)

grade as 0 = normal, 1 = decreased, 2 = absent

hyperreflexia not graded

limbs repositioned as needed

if all reflexes were sluggish, graded as normal

decreased ankle reflexes were taken in context of age

used reinforcement where appropriate (i.e. clasped hands or clenched teeth)

Sensation

tested on terminal phalanx of index finger and great toes (other sites tested to help decide abnormality at sites recorded)

assessed were touch-pressure, joint position and motion, vibration and pin-prick

used cotton wool balls, neurotip pins, 10g monofilament and 257mHz tuning fork (Figure A7.2)

scoring was performed in the context of age, height and skin condition

graded for specificity, not sensitivity.

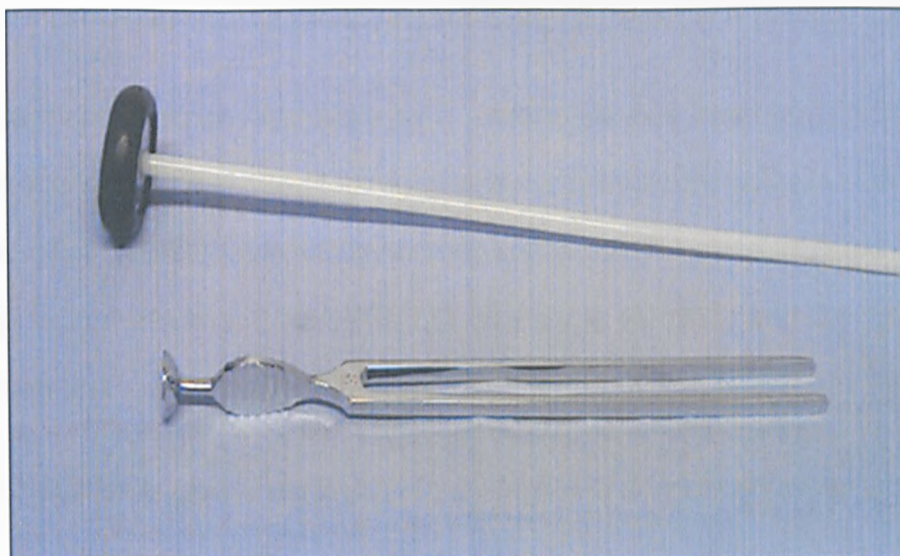


Figure A7.2: Tools for Clinical Examination

Recognition of concomitant neuropathy

Oculomotor neuropathy, median neuropathy at the wrist (from carpal tunnel syndrome) and ulnar neuropathy at the elbow (from compression neuropathy) are common in diabetes. Radiculoplexus neuropathies (cervical, thoracic or lumbosacral [proximal, amyotrophy]) may occur or multiple mononeuropathy may also occur in diabetic patients. For this study, impairments or symptoms from these associated neuropathies were not included

Cardiovascular autonomic function tests

Background information

This is probably the most important and reliable index of cardiovagal integrity. The afferent and efferent pathways are vagal. That vagal efferents are important have been experimentally demonstrated in both experimental animals and in humans. In the cat, heart rate variations are predictable on basis of recorded vagal efferent activity (Egbert and Katona 1980) and its variation is blocked by atropine or by freezing (Anrep and Segall 1926;

Koepchen, Klussendorf et al. 1981). In the human, atropine (a muscarinic antagonist) will totally abolish sinus arrhythmia (heart rate variation) (Wheeler and Watkins 1973).

The main origin of respiratory sinus arrhythmia has been suggested as being the medullary connection from the respiratory and cardioinhibitory centres. Evidence includes the preservation of heart rate variation with active but not passive breathing (Joels and Samueloff 1956; Katona and Jih 1975; Ott, Tarhan et al. 1975) and the loss of heart rate variation with brain stem infarction (Persson and Solders 1983).

Heart rate variation is greatly modulated by a number of peripheral inputs. These include:

1. Hering-Breuer respiratory reflex

When the lungs are stretched during inspiration pulmonary stretch receptors are activated and impulses travel up the vagus to cause inhibition of the respiratory centre.

2. Venoatrial mechanoreceptor sympathetic reflex

During inspiration there is increased filling and this reflex results in compensatory increased heart rate (Brooks, Lu et al. 1966; Koizumi, Ishikawa et al. 1975)

3. Bainbridge reflex

Inspiration results in increased venous return and an increase in pressure in the right atrium and great veins which in turn stimulate an increase in heart rate. The pathway is likely right atrium – vagus nerve – nucleus of tractus solitarius – vagus – sinoatrial node. The normal function of this reflex is probably a way of handling the increased venous return. This reflex is assessed by the Valsalva manoeuvre, which results in an increase in venous return (Bainbridge 1915; Bainbridge 1920).

4. Baroreceptors

Blood pressure (pulse pressure) changes occur during respiration. It has been suggested that breathing mechanically influences the left ventricle output activating arterial baroreflexes (Manzotti 1958; Davies and Neilson 1967). Also during inspiration there is an increase in negative intra thoracic pressure resulting in an increase in venous return – increase atrial filling – increase stroke volume and cardiac output (since $CO = SV \times HR$) and blood pressure (since $BP = CO \times PR$). The increase in blood pressure activates the baroreceptors and reflex variations of heart rate occur. Melcher suggested that sinus arrhythmia was due to a cardio-cardiac reflex with central resetting of the baroreflex (Melcher 1976). Standing produces venous pooling due to a gravitational effect, and hence a fall in venous return. Under normal circumstances, reflex activation of an intact sympathetic nervous system occurs, resulting in an increase in the force and rate of cardiac contraction, and peripheral vasoconstriction.

In conclusion, the efferent pathway is clearly vagal, since efferent blockade will abolish the reflex. However, the afferent and central mechanisms are problematical in humans. Based on present information, distension of the right atrium appears to be important. However, all of the above mentioned pathways could be important under different circumstances.

Factors that affect heart rate variation in autonomic function tests

1. Position

Heart rate variation varies with the position of the patient. The response is larger supine than sitting or erect (standing; (Davies and Neilson 1967; Bennett, Fentem et al. 1977; Mackay, Page et al. 1980). For this reason the patient is supine for assessments.

2. Depth of breathing

A standardised tidal volume was used since depth of breathing above about two litres causes insignificant changes in heart rate variation (Freyschuss and Melcher 1975). Bennet et al and Eckberg found little or no difference in heart rate variation for different depths of respiration (Bennett, Farquhar et al. 1978; Eckberg 1983).

3. Rate of breathing

Maximum heart rate variation with deep breathing occurs at a breathing frequency of 5-6 rpm (Angelone and Coulter 1964; Bennett, Farquhar et al. 1978; Mackay, Page et al. 1980; Pfeifer, Cook et al. 1982) in normal patients and this observation forms the basis for the standard test for deep breathing (Wheeler and Watkins 1973). Each patient breathes at six breaths per minute (five seconds in / five seconds out).

4. Effect of age

All studies to date have found a progressive reduction in the response with increasing age. Results were compared with age related normal ranges

5. Effect of sex of the patient

No sex difference have been observed (Hilsted and Jensen 1979)

6. Amount of rest

It has been demonstrated that following five minutes of rest, another 25 minutes of supine rest does not alter the heart rate variation (Hilsted and Jensen 1979). For this reason patients were asked to rest supine for five minutes before starting the test.

7. Influence of sympathetic activity

There is a sympathetic modulation of heart rate variation being inhibited by stress and sympathetic activation (Coker, Koziell et al. 1984) and during severe tachycardia (Pfeifer, Cook et al. 1982). For this reason patients were relaxed and comfortable during the duration of the study.

8. Time of day

No significant difference has been found in heart rate variation performed in the same patients in the morning and afternoon (Bennett, Farquhar et al. 1978).

9. Hypocapnia

One indirect effect of prolonged hyperventilation is the reduction of pCO₂ resulting in a depression in heart rate variation (Borgdorff 1975). For this reason patients were asked to breathe deeply for eight breaths only.

Autonomic Function Tests procedures

A standard battery of cardiac autonomic function tests was used, with age related reference ranges (O'Brien, O'Hare et al. 1986). These tests consisted of:

i) Resting heart rate

Basal heart rate was obtained from the mean of three consecutive R-R intervals obtained from a 12 – lead ECG recorded after the patient had rested supine for five minutes (Burdick Eclipse LE Electrocardiograph, Burdick Inc., Milton, Wisconsin USA). A value of 100bpm or higher was taken as abnormal.

ii) Heart rate response to the valsalva manoeuvre

A forced expiration against a closed glottis was performed. At the onset of straining arterial pressure rises due to an increased intrathoracic pressure. Since high intrathoracic pressure results in venous compression, there is a decrease in venous return and hence cardiac output. A resulting fall in arterial pressure inhibits baroreceptor firing, leading to a reflex tachycardia and a rise in peripheral resistance. When the glottis is opened, on relaxation, intrathoracic pressure normalises and cardiac output is restored. However, residual peripheral vasoconstriction produces a blood pressure 'overshoot'. Arterial baroreceptor stimulation then leads to a fall in heart rate and blood pressure returns to basal levels.

Participants were asked to exhale into a tube connected to a mercury sphygmomanometer and maintain a pressure of 40mmHg for 15 seconds. On relaxation ECG recording continued for a further 45 seconds. The valsalva ratio was calculated as the ratio of the highest heart rate achieved during the manoeuvre to the lowest heart rate during the relaxation period (i.e. the ratio of the longest R-R interval after the manoeuvre to the shortest R-R interval during it) (Ewing and Clarke 1982).

iii) Heart rate response to deep breathing

The rhythmic fluctuations in heart rate in response to inspiration (rise) and expiration (fall) can be expressed as the E:I ratio (ratio of R-R interval during maximal expiration to R-R interval during maximal inspiration). Patients were asked to take slow deep breaths at a rate of six cycles/min. Results were expressed as the ratio of the peak inspiratory heart rate to the lowest expiratory heart rate during a single cycle (O'Brien, O'Hare et al. 1986).

iv) Systolic blood pressure response to standing

Blood pressure was measured using a mercury sphygmomanometer 60 seconds after standing. A defect in this reflex arc is conventionally defined as a fall in systolic blood pressure of at least 30mmHg (Ewing and Clarke 1982), age-related adjustment is generally regarded as unnecessary (Ziegler, Laux et al. 1992).

v) Lying – standing heart rate response (30:15 ratio)

This index is based on the physiological mechanisms discussed above. The peak heart rate (due to sympathetic activation) occurs at approximately the 15th beat after standing; as vasoconstriction occurs and blood pressure rises towards baseline, heart rate falls, reaching a nadir at approximately the 30th beat (Ewing and Clarke 1982).

The maximum/minimum R-R (30:15) ratio is calculated as the ratio of the longest R-R interval on standing (usually around the 30th beat) to the shortest R-R interval after the change from the recumbent position (usually at about the 15th beat) (Ewing and Clarke 1982). It has been shown that the shortest R-R interval can occur between beats 6-24 and the longest R-R interval between beats 20-40 (Ziegler, Laux et al. 1992). Therefore, in these studies the max/min R-R ratio is defined as the longest R-R interval during beats 20-40 divided by the shortest R-R interval during beats 5-25.

For tests (ii) to (iv) heart rate was measured using a three lead surface ECG monitor connected to an ATARI 360S microprocessor (Royal Hallamshire Hospital) on an IBM 286 personal computer. Both were equipped with analogue to digital signal converters and QRS algorithm recognition software sampling at 600Hz.

Quantitative Sensory Testing Procedures

Quantitative sensory thresholds were assessed using the Computer Assisted Sensory Evaluation IV (CASE IV, Minnesota, USA, Figure A7.3). Figure A7.3 represents the apparatus through which a standard predicted vibratory stimulus could be applied by the computer to the part of the body being studied. The CASE IV system was also used to measure Cooling Detection (CDT) and Heat-Pain Detection Thresholds, by securing a porcelain block device, though, which predetermined temperature stimuli were applied, to the foot (Figure A7.3). This method eliminated the touch pressure artefact.

In my studies, quantitative sensory thresholds were only assessed in the lower limb. Values were expressed as percentiles, where >99th was considered to be abnormal. The CASE IV system uses 25 stimulation levels for patient testing. These 25 levels are termed 'just noticeable differences' or 'JNDs. Differences of less than one JND are difficult to distinguish, so that one JND step is the smallest difference that is presented to patients. The JNDs allow the minimum number of stimulus trials to be used. The test is started at a baseline level: for vibration stimuli, the baseline is 0 micrometers of displacement; for cooling stimuli, the baseline is set to 30°C; for heat-pain the baseline is set to 34°C. The thermal stimulator uses a 4-degrees-per second ramp up and down. For warming stimuli, the maximum temperature is limited to 50°C. For cooling stimuli, the minimum temperature is 8°C. With the CASE IV system, a statistically validated set of age adjusted normative data is provided.

One time period with 4,2,1 stepping is an algorithm for quick assessment of vibration and cooling detection thresholds. This is also the most used in practise (Figure A7.3). The testing is begun at a middle JND level (Dyck, O'Brien et al. 1993) . The system increases the next stimuli level if the patient cannot feel the stimulus. As the test progresses, the number of steps that the stimulus level increases or decreases changes, beginning with a JND value of 4, decreasing to 2, and finally to a step value of 1, until the threshold is defined.

During the test, a number of null stimuli are placed randomly among the others to prevent false results.

Several factors affect the length of testing: the time required to explain the test to the patient, the speed in which the patient makes decisions, the alertness of the patient and the number of stimuli needed to find the threshold. For thermal testing the time also depends on the level of sensitivity because larger stimulus magnitudes (longer times) are needed for insensitive patients. For one time period testing with 4, 2, 1 stepping, vibration and cooling tests take approximately ten minutes each. Heat pain Non-Repeating Ascending with Null stimuli only requires five minutes but should not be repeated on the same day if the patient fails to recognise any of the null stimuli.

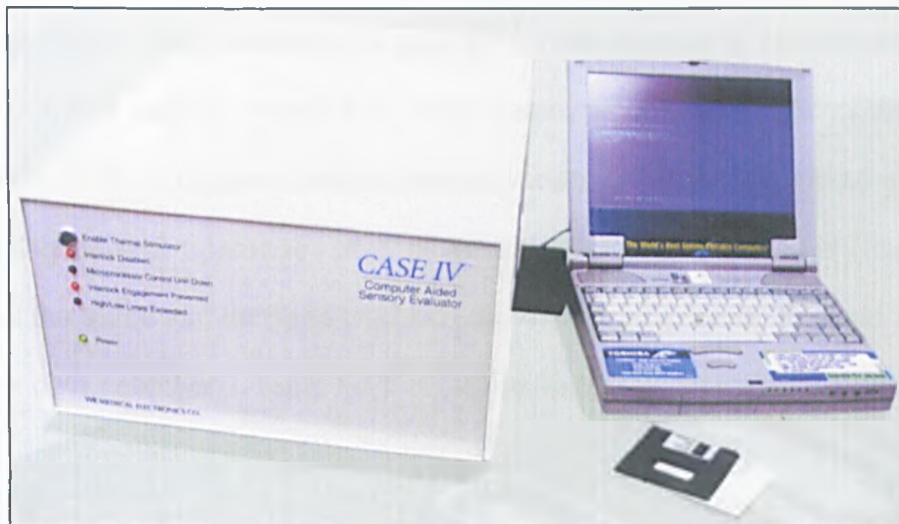


Figure A7.3: The CASE IV

Nerve Conduction Study Procedures

Nerve conduction studies (NCS) have long been the mainstay of measurement of DPN in clinical trials. NCS correlate well with the morphological changes seen in nerve biopsies (Veves, Malik et al. 1991) and with clinical testing and examination (Redmond, McKenna et al. 1992; Feki and Lefaucheur 2001). Conventional NCS, have a specificity of 58% and a sensitivity of 93% for the detection of DPN; with a specificity of 91% and a sensitivity of 81% for clinical worsening of DPN. This test is, therefore, ideally suited to clinical trials. However

NCS measure sensory and motor nerve function transmitted by impulses from large (myelinated) fibres that make up only 25% of the total nerve fibre population. Electrophysiological tests are therefore not sensitive to small fibre damage (Dyck, Litchy et al. 2003). Thus despite their reliability and high reproducibility, NCS cannot be used as the sole tests in the investigation of DPN (Valk, Grootenhuys et al. 2000).

In NCS, peripheral nerve conduction velocity (NCV), summated amplitude, conduction latency and motor nerve F-Wave latency are the parameters that are most commonly assessed. The median and ulnar (sensory and motor) nerves in the upper limb, are commonly tested. A reduction in peripheral nerve summated amplitudes is a measure of nerve fibre loss, whilst decreased latency and NCV are measures of the degree of nerve fibre demyelination. Reproducibility is poor for amplitudes but is satisfactory for all other parameters, and is particularly good for NCV measurement. For most of the parameters, total variance is mainly related to inter-examiner variability (Chaudhry, Corse et al. 1994). In trials involving several operators, sites of recording electrode placement must be strictly defined and the same standardised methodology strictly adhered to, to avoid inter-examiner variability in data collection. Table A7.2 highlights expected reasons for differences in NCS results.

Table A7.2: Factors affecting nerve conduction measurements

Factors attributable to the patient

Age, Height, Body mass index, Gender, The degree of DPN

Physiological factors

Skin temperature, hyperglycaemia

Operator dependant factors

Placement of recording and stimulating electrodes, Distance measurements

Failure to achieve supramaximal stimulation, Placement of cursors on the screen

The investigator (DS) performed all the nerve conduction studies attended formal and informal training sessions. Informal training sessions was a three week attachment with a clinical consultant neurophysiologist (Dr Jarrett) at the Royal Hallamshire hospital. A formal training session for technicians conducting a phase 2 clinical trial organised by Lily Research Laboratories was attended. Competency was established prior to assessing study patients by performing repeat nerve conduction measurements on 20 normal controls (medical students and willing members of staff) on two separate occasions to ensure reproducibility of methods precision of results and minimise intra examiner variability had been achieved.

Nerve conduction equipment

Nerve conduction machine: Medelec, Synergy Oxford Instruments, Oxford, UK

Recording electrodes: Standard commercially available electrodes was used for all nerve conduction studies.

Ground electrodes: Standard commercially available lead plate electrodes was used for this study.

Stimulating electrodes: Standard surface stimulating electrodes was used for this study.

Temperature control: A warming bath was used to raise limb temperature, which was maintained with a radiant heat lamp.

Temperature measurement

The temperature was measured continually during the nerve conduction study. The temperature at the time of collection of each waveform was automatically be entered into nerve conduction table. For the lower limb temperature measurements the thermistor was placed on the dorsum of the foot 2.0cm proximal to the first web space. The temperature of the lower limb was maintained to at least $31 + 1^{\circ}\text{C}$ during the entire nerve conduction study. If the temperature fell below the required temperature before the beginning of the study, the limb was rewarmed in a warming bath. Room temperature of $24 + 2^{\circ}\text{C}$.

Skin preparation

Skin preparation was performed before starting nerve conduction studies to minimise shock artefact, improve the quality of the study, and reduce the time taken to complete the study. Skin preparation procedure included cleaning the skin with alcohol and then abrading the skin with a mild dry abrasive material.

Ground electrode

The ground electrode will be placed between the active recording electrode and the stimulating electrode.

Nerve stimulation technique

All nerves were stimulated with an electrical stimulator. A constant current stimulator was used. The strength of the stimulus was increased in steps until a maximum response was obtained. When maximum response was obtained the stimulator was increased by 5-10% more to make sure the response was supramaximal. Sensory responses were averaged at least three times but no more that ten times. Motor responses were not averaged.

Measurements

All distances were measured to the nearest 1.0mm. Extreme care was taken to use the same distance for each subsequent test.

Waveform measurement

All waveform measurements were made at the sweep speed and display sensitivity at which the waveform was acquired.

Amplitude measurement

The motor amplitude was measured from the baseline to the negative peak. The amplitude was reported to the nearest 0.1mV. The amplitude of the sensory action potential was measured from the baseline to the negative peak. When there was a positivity preceding the negative component of the potential, the amplitude was measured from the base of the positive peak to the top of the negative peak. Sensory action potential was reported to the nearest whole number.

Latency measurement

The only latency measured for motor nerve conduction studies was the onset latency. The onset latency was measured at the take off of the negative component of the M-wave from the baseline. The latency was measured at the display sensitivity at which the waveform was acquired. Onset and peak latencies were measured for the sensory potentials. The onset latency was measured from the take off of the negative component of the sensory action potential from the baseline. If a positivity precedes the negative component the onset latency will be measured from the base of the positivity. The peak latency was measured at the peak of the negative component of the action potential.

Velocity measurement

The nerve conduction velocities were calculated using onset latencies. The distance used for calculation of conduction velocities were from 'long segment' distances. A long segment distance is the distance between the site of stimulation and the most distal stimulation site.

The composite score of NIS(LL) + 7 tests will be used to evaluate the severity and monitor neuropathic impairment in this study .

Sural Nerve Sensory Nerve Conduction Study

Anatomy

The sural nerve contributes to the sensory innervation of the leg and foot. This nerve, formed by the junction of the medial sural cutaneous with the peroneal anastomotic branch, passes downward near the lateral margin of the tendocalcaneus, lying close to the small saphenous vein, to the interval between the lateral malleolus and the calcaneus. The sural nerve runs forward below the lateral malleolus, and is continued as the lateral dorsal cutaneous nerve along the lateral side of the foot and little toe, supplying this portion of the foot with somatic sensation (Gray and Standring 2005).

The right sural nerve was examined first. If no response is obtainable then the left sural nerve will be evaluated.

Recording electrode

Disposable xbar electrodes were used

Active electrode: The active electrode was placed immediately behind the lateral malleolus. The electrode was placed over the sural nerve on a line that goes from the triceps sural tendon to the prominence of the lateral malleolus perpendicular to the sural nerve.

Reference Electrode: The reference electrode was placed 4.0cm distal to the active electrode, distal to the lateral malleolus along the course of the nerve.

Stimulating electrode

Bipolar, surface stimulating electrode

The sural nerve was stimulated 14.0cm from the active recording electrode. The nerve is located about 3.0cm lateral to the posterior midline.

Filter settings

30Hz LLF, 3.0 kHz HLF

Sweep speed

The sweep speed was set at 1.0ms/division. It was occasionally necessary to use longer sweep speeds to ensure that the entire waveform is recorded.

Amplifier gain

The amplifier gain/display sensitivity was set at 10 uV/division. It was occasionally necessary to use a different sensitivity to ensure that the response is a minimum of two divisions.

Procedure

The nerve was stimulated at a point 14.0cm proximal to the active recording electrode

When a supramaximal response was obtained, an average at least three but not more than ten responses was performed

Responses were marked with the cursors. If the instrument automatically marked the responses, I made sure they are marked correctly and changed the cursors if necessary

The distance between the active electrode and the site of the cathode stimulating electrode were measured and recorded

The skin temperature was recorded before and after nerve conduction was completed

Peroneal Motor Nerve conduction Study

Anatomy

The deep peroneal nerve begins at the bifurcation of the common peroneal nerve, between the fibula and upper part of the peroneus longus, passes obliquely forward beneath the extensor digitorum longus and comes into relation with the anterior tibial artery above the middle of the leg; it then descends with the artery to the front of the ankle joint, where it divides into a lateral and a medial terminal branch. It lies at first on the lateral side of the anterior tibial artery, then in front of it and again on its lateral side at the ankle joint. In the leg, the deep peroneal nerve supplies muscular branches to the tibialis anterior (ankle dorsiflexion), extensor digitorum longus (2nd and 5th toe extension), peroneus tertius and extensor hallucis proprius (big toe extension), and an articular branch to the ankle joint. The lateral terminal branch supplies the extensor digitorum brevis (2nd and 5th toe extension) and the tarsal joints, as well as the metatarsophalangeal joints of the second, third and fourth toes. The medial terminal branch divides into two sensory nerves that supply the adjacent sides of the great and second toes with sensation (Gray and Standring 2005).

Recording electrode

Disposable xbar electrodes were used

Active electrode: The active electrode was placed over the centre of the palpable portion of the extensor digitorum brevis muscle on the lateral aspect of the dorsum of the foot, 1.0cm distal to the calcaneous bone (bony prominence from which the muscle takes its origin)

Reference electrode: The reference electrode was placed over the fifth metatarsal-phalangeal joint on the lateral portion of the foot. It was placed lateral to the long extensor tendons of the small toe.

Stimulating electrode

Bipolar surface stimulating electrode

Distal stimulation site (ankle)

The cathode was placed on the anterior aspect of the ankle 2-5cm lateral to the tendon of the tibialis anterior muscle, approximately 5.0cm proximal to the lateral malleolus, and 8.5cm from the active electrode.

Proximal stimulation site (fibular head)

The cathode was placed over the nerve where it runs immediately below the fibula and enters the anterior compartment

Proximal stimulation site (knee)

The cathode was placed over the nerve in the popliteal fossa approximately 10.0cm proximal to the head of the fibula. The cathode was placed just medial to the tendon of the long head of the biceps femoris muscle.

Filter settings

2.0Hz LLF, 10.0 kHz HLF

Sweep speed

The sweep speed was set at 2.0ms/division. Occasionally it was necessary to use other sweep speeds (e.g. to 5.0 ms/division) to ensure that the entire waveform is recorded.

Amplifier gain

The amplifier gain/display sensitivity was set at 2.0mV/division. It was occasionally necessary to use a different sensitivity to ensure that the response is a minimum of two divisions.

Procedure

The nerve was stimulated at the ankle, fibula head and the knee.

When a supramaximal response was obtained, the response was recorded.

Responses were marked with cursors. If the instrument automatically marked the responses, I made sure they were marked correctly and changed the cursors if necessary

The appropriate distances were measured and recorded. The distance between the active recording electrode and the cathode of the stimulator was measured. The distance between the active electrode at the ankle and the site of the stimulating cathode electrode was measured. The distance between the ankle and fibula head was also measured. The distance between the ankle and the knee was measured.

The temperature was recorded before and after the nerve conduction is completed

Tibial motor nerve conduction study

Anatomy

The tibial nerve is the larger of the two divisions that form the sciatic nerve. It originates from L4 through S3 roots and travels as part of the sciatic through the posterior thigh under the long head of the bicep femoris. By the popliteal fossa, it has separated from the sciatic nerve.

It supplies the motor innervation of the posterior compartment of the leg and all foot muscles (except the extensor digitorum brevis). Its motor distribution in the leg includes the gastrocnemius, plantaris, soleus, popliteus, tibialis posterior, flexor digitorum longus and flexor hallucis longus.

The nerve is superficial as it passes vertically down the middle of the popliteal fossa. It lies lateral then crosses superficially to lie medial to the popliteal artery. As it leaves the fossa, it gives off the sural nerve supplying the posterior leg. Its course then becomes deep as it passes between the gastrocnemius and soleus muscles, under the tendinous arch formed by the soleus, lying on the posterior surface of the tibialis posterior muscle and then on the tibia. It again becomes superficial at the upper end of the medial malleolus where it passes behind it and in front of the calcaneus tendon. Its terminal course takes it under the flexor retinaculum (posterior tarsal tunnel). The medial calcaneus branches perforate the flexor retinaculum and supply the skin of the heel and medial side of the sole of the foot. Under the retinaculum the nerve divides into medial and lateral plantar branches and innervates the plantar aspect of the foot (Gray and Standring 2005).

Recording electrode

Disposable xbar electrodes were used

Active electrode: The active electrode was placed over the centre of the abductor hallucis muscle 1.0cm below and 1.0cm behind the prominence of the navicular bone

Reference electrode: The reference electrode was placed over the first metatarsal-phalangeal joint on the medial surface of the big toe

Stimulating electrode

Bipolar surface stimulating electrode

Distal stimulation site (ankle)

The cathode was placed on the tibial nerve on the medial aspect of the ankle 8.0cm proximal to the active recording electrode.

Proximal stimulation site (fibular head)

The cathode was placed over the nerve in the middle of the popliteal fossa.

Filter settings

3.0Hz LLF, 10.0 kHz HLF

Sweep speed

The sweep speed was set at 2.0ms/division. It was occasionally necessary to use other sweep speeds (e.g. to 5.0 ms/division) to ensure that the entire waveform was recorded.

Amplifier gain

The amplifier gain/display sensitivity was set at 5.0mV/division. It was occasionally necessary to use a different sensitivity to ensure that the response was a minimum of two divisions high.

Procedure

The nerve was stimulated at the ankle then at the knee.

When a supramaximal response was obtained, response was recorded.

Responses were marked with cursors. If the instrument automatically marked the responses, I made sure they were marked correctly and changed the cursors if necessary

The appropriate distances were measured and recorded. The distance between the active recording electrode at the ankle and the site of the stimulating electrode were measured. The distance between the ankle and the knee were also measured

The temperature was recorded before and after the nerve conduction was completed

Measurement of DPN

The neuropathy staging tool used in these studies was defined by P.J.Dyck and developed from the Rochester Diabetic Neuropathy Study (RDNS) (Dyck, Karnes et al. 1992). It takes into account results obtained from five neuropathy measures to calculate a composite score used detect and measure severity of neuropathy. The Neuropathy Impairment Score for the Lower Limbs plus Seven Tests or NIS(LL)+7 is considered by many to be the gold standard for the detection of DPN and change of DPN (following intervention) in clinical trials (Table A7.3, (Dyck and Thomas 1999). It includes measures of muscle weakness, reflex loss, sensory loss and nerve conduction abnormalities in the lower limbs, plus autonomic heart rate abnormalities during deep breathing.

Calculating the NIS(LL) + 7 tests score:

Sum of individual scores of the NIS for the lower limbs, NIS(LL). Items 17-24, 28-29 and 34-37.

In NIS (LL), substitute transformed points for percentile abnormality * of VDT for each great toe (obtained with CASE IV) for the clinical vibration sensation point score of the great toes.

Add transformed points for percentile abnormality * of HB DB (one time only).

Summate transformed points for percentile abnormality * of the five attributes of nerve conduction of lower limb (peroneal nerve (CMAP, MNCV and MNDL), tibial nerve (MNDL) and sural nerve (SNAP), divided by the number of attributes with obtainable values **, Multiply by 5 (the number of attributes), and add this number to the global score.

* $< 95^{\text{th}} = 0$; $\geq 95^{\text{th}} - 99^{\text{th}} = 1$; $\geq 99^{\text{th}} - 99.9^{\text{th}} = 2$; $\geq 99.9^{\text{th}} = 3$

** MNCV and MNDL cannot be estimated when CMAP is 0

This composite score was found to be both highly sensitive and specific for the detection of DPN, as well as, for neurological progression. Based on this composite score, patients were divided into three groups based on the severity of neuropathy (Table A7.3):

No DPN – N0

Subclinical DPN – N1a and N1b

Established (Painless DPN) – N2 and N3 with negative symptoms

Established (Painful DPN) – N2 and N3 with symptoms of painful DPN for at least six months

Table A7.3: Dyck's staging of Diabetic Peripheral Neuropathy (Dyck 1988)

N0

No polyneuropathy. Less than N1a

N1

N1a: Asymptomatic polyneuropathy as recognised by nerve conduction abnormality in at least two nerves or heartbeat deep breathing abnormality, caused by DPN.

N1b: N1a criteria plus neurologic examination abnormality or abnormality on quantitative sensory testing of vibration detection threshold or cooling detection threshold.

N2

N2a: Symptomatic mild DPN. Sensory autonomic or motor symptoms caused by DPN. Neuropathic impairment is the same or more than N1a. Patients has less than 50% weakness of ankle dorsiflexor muscles (able to walk on heels)

N2b: Symptomatic severe DPN. Neuropathic impairment is the same or more than N1a. Patient has 50% or greater weakness of ankle dorsiflexor muscles (unable to walk on heels).

N3

Disabling DPN

9 RESEARCH OUTPUT

At the time of writing, the following publications/presentations have resulted from data accumulated during the course of this work.

1. Peer reviewed first author papers

- **Selvarajah D**, Wilkinson ID, Emery CJ, Harris ND, Shaw PJ, Witte DR, Griffiths PD, Tesfaye S. Early involvement of the spinal cord in diabetic peripheral neuropathy. *Diabetes Care*. 2006 Dec;29(12):2664-9.*

- **Selvarajah D**, Wilkinson ID, Emery CJ, Griffiths PD, Gandhi R, Tesfaye S. Thalamic neuronal involvement in type 1 diabetes mellitus. *Diabetologia* **51**(11): 2088-92.

- **Selvarajah D**, Tesfaye S. Central nervous system involvement in diabetes mellitus. *Curr Diab Rep*. 2006;6:431-8.

2. Peer review papers in preparation

-**Selvarajah D** et al A Randomised Controlled Double Blind Controlled Trial of Cannabis Based Medicinal Product (Sativex) in Painful Diabetic Neuropathy: Is Depression a Major Confounding Factor?

3. Books Chapters

-**Selvarajah D**, Tesfaye S. Diabetic Peripheral Neuropathy. *Diabetes in the Arab World*. Ed. G Gill.

- **Selvarajah D**, Wilkinson ID, Gandhi R, Tesfaye S. Central Nervous System Involvement in Diabetic Neuropathy.

4. Selected first author oral and poster presentations

-Selvarajah D, Gandhi R, Griffiths PD, Emery CJ, Wilkinson ID, Tesfaye S. Thalamic Sensory Neuronal Involvement in Diabetic Neuropathy. American Diabetes Association 67th Scientific Sessions 2007, Diabetes UK APC 2006, ISMRM 2004 (Poster) and University of Sheffield Research Open Day 2007 (Prize for best research poster).

-Selvarajah D, Wilkinson ID, Emery CJ, Harris ND, Shaw PJ, Witte DR, Griffiths PD, Tesfaye S. Early Involvement of the Spinal Cord in Diabetic Peripheral Neuropathy. International Diabetes Federation 18th World Diabetes Congress, Paris 2003 (Kuusisto, Mykkanen et al.), Diabetes UK Annual Professional Conference 2003 (Kuusisto, Mykkanen et al.), American Society of Neuroradiologists 2004 (Oral).

-Selvarajah D, Gandhi R, Emery CJ, Bowler H, Tesfaye S. Depression is a major confounder in clinical trials of painful diabetes neuropathy. 43rd Annual Meeting of the European Association for the Study of Diabetes 2007 (Poster).

-Selvarajah D, Wilkinson ID, Emery CJ, Gandhi R, Griffiths PD, Tesfaye S. Unique thalamic perfusion abnormalities in painful but not painless diabetic neuropathy. Diabetes UK Annual Professional Conference 2009. (Eli Lilly Poster Award – Clinical Science).

-Selvarajah D. Diabetic Neuropathy. Diabetes UK Local Patient Support Group, Barnsley 2002 (Invited lecture).

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