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**Multiple sclerosis; physiological, perceptive and
neural responses to exercise intensity**

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Abstract

The aim of this work was to investigate physiological, perceptive and neurological responses to exercise intensity in people with multiple sclerosis (PwMS). The thesis begins with reviews of Multiple Sclerosis (MS) and exercise followed by three main studies.

The first study explores the within session and test-retest reliability of motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) from the resting tibialis anterior (TA) muscle of people with multiple sclerosis (PwMS). MEPs were recorded from 10 PwMS (2male, 8female) in 5 blocks of 5 trials using stimulators configured to fire a single pulse. MEP peak amplitudes (mV) and MEP areas (mV.mS) were measured at 2 durations MEPshort (30ms) and MEPlong (mean 50ms). The size of the first MEP (T1) from each block (mean 5.1) was significantly different to subsequent trials (T2 -T5) for MEPlong (mean 4.5 $p < 0.05$). After T1 was discarded, repeated measures ANOVAs of blocks (averages of 4 trials) revealed no significant differences within-subjects for amplitudes (MEPpeak) and areas (MEParea) for both MEPshort and MEPlong. A repeat session 7-14 days later revealed no significant differences between sessions ($p > 0.05$). The test-retest intraclass coefficients of correlation (ICC) and their 95% confidence intervals indicated high (> 0.80) reliability for both MEParea and MEPpeak. The results showed that consistent, repeatable TMS measures were obtained from the resting TA of PwMS.

The second study compared physiological and perceptive measures of PwMS to a group of healthy individuals while performing a symptom-limited graded exercise test (GXT), and through the post-exercise time-course to recovery. 54 PwMS (MSG, mean age 52.8years \pm 9.0) and 17 healthy, age-matched controls (CG, mean age 48.9 years \pm 5.7) performed a symptom-limited graded exercise test (GXT). Expired air (VO_2), heart rate (HR), and differential ratings of perceived exertion (RPE breathing and RPE legs) were recorded during exercise, and HR and RPE (breathing and legs) during the recovery period. There were no significant differences in any baseline measure except RPEleg (MSG mean 1.1 ± 1.2 ; CG 0.2 ± 0.4 $p < 0.05$). During the GXT MS group means (\pm SD) failed to reach any criterion measure considered to represent peak performance. The control group mean exercise measures reached recognised criteria for peak testing on two measures; mean heart rate of within \pm 10 beats of age predicted HRmax, mean RER value greater than 1.10. Significant differences existed between groups in all peak measures (mean MSG VO_2 peak 20.1 ± 6.4 , mean CG VO_2 peak 27.8 ± 6.8 ; mean MSG HRpeak 140.1 ± 24.8 , mean CG HRpeak 167.7 ± 9.4 ; mean MSG RPE breathing 5.1 ± 1.7 , mean CG RPE breathing 6.8 ± 2.3 ; mean MS watts 97.4 ± 35.2 , mean CG watts 161.8 ± 43.4 $p < 0.05$) except for RPEleg (mean MSG 6.2 ± 1.7 , mean CG 7.0 ± 1.8 $p > 0.05$). There were no significant effects on the peak measures of variables when weighted by MS classification (RR-MS, SP-MS and PP- MS). There were differences between group recovery values for RPEleg at 10 mins (mean MSG 1.8 ± 1.2 , mean CG 1.0 ± 1.1 $p < 0.05$) and Temp $^{\circ}$ C at 3mins (mean MSG 36.5 ± 0.5 , mean CG 36.9 ± 0.6 $p < 0.05$) and 10mins (mean MSG 36.4 ± 0.4 ,

mean CG 37.0 ± 0.3 ($p < 0.05$) post-exercise. MS HR remained marginally above pre-exercise HR values at 10 minutes post-exercise. Differential measures of RPE for both groups recovered to pre-exercise values at 5 mins (\pm SD). During maximal exertion, it was observed that PwMS irrespective of disease classification, or years from onset were neither limited by their heart rate, nor their breathing, but that leg fatigue or lack of central drive to the lower limb was the reason for their inability to continue.

In the third study, the physiological perceptible and central responses of PwMS were explored during exercise at low and high intensity, and through the time-course to recovery. Participants performed 2 exercise training (ET) sessions where they performed 20 minutes of exercise on a cycle ergometer at 45% (ET45) and 60% (ET60) relative to peak watts determined during a GXT. 12 MSG and 9 CG completed the 2 exercise sessions. Repeated measures ANOVA revealed no significant differences in groups' baseline measures of HR, Temp°C, RPEbr, RPEleg or TMS measures between-groups or between-sessions. When comparing groups during ET45, measures of all variables were similar, except for RPE leg at 14mins, (mean MSG 3.3 ± 1.1 , CG 2.1 ± 1.2 $p < 0.05$). During ET60 MSG HR was higher from 14mins (mean $107.8 \text{ bpm} \pm 12.6$ bpm, CG $136.8 \text{ bpm} \pm 13.8$ $p < 0.05$). When comparing the results of MSG ET45 and ET60, during the 35 minute post-exercise phase MSG HR recovered to pre-exercise values at 10 mins (mean HR $71.4 \text{ bpm} \pm 12.7$, baseline $63.8 \text{ bpm} \pm 9.8$ $p > 0.05$) after ET45, while post-ET60 HR

failed to recover before session-end (mean HR 83 bpm \pm 11.3, baseline 64.6 bpm \pm 8.2, $p < 0.05$).

TMS measures were significantly depressed after both training sessions. Mean MEP size were 71% \pm 38% of pre-exercise levels at 30 secs post-ET45 ($p < 0.05$) and 52% \pm 17.8% post ET60 at 2 minutes ($p < 0.05$). Post-ET45 MEPs recovered to pre-exercise values at 10mins while post-ET-60 MEPs recovered at 20 mins. MEP latency and MEP_{periph} were unchanged. Following ET60 we observed a strong negative relationship between Temp°C and MEPs ($r = -.65$, $p = 0.023$).

The investigation revealed significant, intensity-dependent, physiological and perceptive differences, during exercise and through the time-course to recovery. Analysis of responses to post-exercise TMS revealed a significant depression in corticospinal excitability, with a clear intensity-dependent difference in the depth and duration of MEP depression. In addition, an inverse relationship was found between internal body temperature and corticospinal excitability. The results may offer further guidance to clinicians for the provision of safe, appropriate and effective exercise prescription to PwMS

Presentations and Publications

The work contained in this thesis, unless indicated by acknowledgement or reference to published literature is the work of the author. The following publications contain, in part, findings from the thesis or relevant findings discussed in the thesis that are the work of the author and collaborators.

Dawes H, Collett J, Meaney A, Sackley C, Barker K, Wade D, Izardi J, Duda, J. (2013) *Delayed recovery of leg fatigue symptoms from maximal exercise in people with multiple sclerosis* (in final review)

Meaney A, Collett J, Dawes H, Howells K, Izadi H (2011) *The reliability of responses to transcranial magnetic stimulation in people with multiple sclerosis* (submitted; in review)

Collett J, Dawes H, Meaney A, Sackley C, Barker K, Wade D, Izardi H, Bateman J, Duda J and Buckingham E. (2011) *Exercise for multiple sclerosis: a single-blind randomized trial comparing three exercise intensities* Multiple Sclerosis

Meaney A, Collett J, Dawes H, Izadi H, Howells K. (2011) *Physiological and perceptive responses to maximal exercise testing in people with multiple sclerosis* (in press)

Collett J, Dawes H, Meaney A, Sackley C, Cavey A, Wade, D, Howells K, (2011) *Hydration and independence in activities of daily living in people with multiple sclerosis: a pilot investigation* Disability and Rehabilitation 1-4

M Feltham, J Collect , H Izadi, M Morris, A Meaney, K Howells , D Wade H Dawes (2011) *Heart rate response in people with multiple sclerosis was absent after participation in exercise programmes* (Proceedings from the Society of Research in Rehabilitation)

Soundy, A., Benson, J., Dawes, H., Smith, B., Collett, J., Meaney, A (2011)
Understanding hope in individuals with multiple sclerosis. Physiotherapy

Collett J, Dawes, H, Sackley C, Barker, K, Duda, J, Wade, D, Meaney, A, Morris M.
Exercise for People with Multiple Sclerosis: a single blind randomised trial comparing continuous, intermittent and combined exercise., (2010) Clinical Rehabilitation (in press)

Meaney A, Collett J, Dawes NH, Sackley C, Barker K, Wade D, Centre, Duda J, Izadi H, Howells H. *Optimal exercise prescription; the acute response to exercise intensity in people with Multiple Sclerosis*. (2010) (Proceedings from the Society of Research in Rehabilitation)

Collett J, Meaney A, Dawes H, Godsiff D. *A pilot study investigating the appropriateness of an incremental exercise test to volitional exhaustion for people with Multiple Sclerosis* Journal of Sport Science (2008) 26(S2): S1–S143

Meaney A, Collett J, Sterling-Reed C, Howells K, Dawes H, Izadi H. *The effect of cycling intensity on corticospinal excitability in a person with Multiple Sclerosis; a pilot study* Journal of Sport Science. (2008) 26(S2): p 126–127

Meaney A, Nowicky A, Dawes H, Howells K, *Corticospinal excitability after supramaximal exercise; a transcranial magnetic stimulation study* (2007) Journal of Sport Science 25 (3) p271-272

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Abbreviations

2MTW	2 minute timed walk
AMPA	alpha-amino3-hydroxy-5methyl-4-isoxazole propionic acid
ANOVA	Analysis of variance
ANS	Autonomic nervous system
AP	Action potential
ATP	Adenosine tri-phosphate
BBB	Blood brain barrier
BP	Blood pressure
CA ⁺	Calcium
CD ⁺	Cluster of differentiation
CF	Cardio-respiratory fitness
CHO	Carbohydrate
CIS	Clinically isolated syndrome
CNS	Central nervous system
CO ₂	Carbon dioxide
CSF	Cerebrospinal fluid
CV	Cardio-vascular fitness
EAE	Experimental autoimmune encephalomyelitis
EAAT	Excitatory amino acid transport

EBV	Epstein Barr Virus
ET45	Exercise training at 45% peak
ET60	Exercise training at 60% peak
FDCB	Frequency dependent conduction block
GABA	Gamma-amino butyric acid
GXT	Graded exercise test
HLA	Human leukocyte antigen
HPA	Hypothalamic-Pituitary-Adrenal
HR	Heart rate
HRmax	Maximal heart rate
HRpeak	Peak heart rate
HRR	Heart rate recovery
ICC	Intra-class correlation
K+	Potassium
Kv	Potassium gated ion channels
MEP	Motor evoked potential
MET	Metabolic equivalent of task
MGLUR	Metabotropic glutamate receptors
MRI	Magnetic resonance imaging
MS	Multiple sclerosis

N ₂	Nitrogen gas
Na	Sodium
Nav	Sodium gated ion channels
NO	Nitric oxide
NMDA	N-methyl-D-aspartate
O ₂	Oxygen
OPC	Oligodendrocyte precursor cell
PNS	Peripheral nervous system
PP-MS	Primary progressive MS
PwMS	People with multiple sclerosis
QOL	Quality of life
RPE	Rating of perceived exertion
RPEbr	Rating of perceived exertion for breathing
RPEleg	Rating of perceived exertion for legs
RR-MS	Relapsing remitting MS
SP-MS	Secondary progressive MS
TMS	Transcranial magnetic stimulation
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_2$ max	Maximal oxygen uptake
$\dot{V}O_2$ peak	Peak oxygen uptake

Table of Contents

Abstract.....	2
Acknowledgements.....	8
Chapter 1 Multiple Sclerosis.....	20
1.1 Introduction.....	20
1.2 Historical context.....	21
1.5 Classification of MS.....	28
1.6 Aetiology.....	29
1.7 Demyelination.....	32
1.8 Re-myelination.....	37
1.10 Glutamate.....	40
1.11 GABA.....	42
1.12 Nitric oxide.....	43
1.13 Mitochondria.....	44
1.14 Axonal transport.....	45
1.16 Activity-related fatigue.....	49
1.17 Physical management.....	50
1.18 Medication.....	50
Chapter 2 Exercise.....	54
2.1 Introduction.....	54
2.2 Exercise for health and rehabilitation.....	55
2.3 Attitudes and barriers to exercise.....	57
2.4 General definitions and principles of exercise.....	58
2.6 Exercise intensity.....	62
2.9 Exercise recommendations for PwMS.....	69
Chapter 3 Methods and materials.....	77
3.1 Introduction.....	77
3.2 Research setting.....	79
3.3 Performance measures: Intensity.....	79
3.3.1 Measuring intensity; The mode of exercise.....	79

3.5 Measurement of Intensity; Oxygen consumption ($\dot{V}O_2$)	82
3.6 Measurement of intensity. Metabolic equivalents.....	87
3.7 Measurement of intensity. Heart rate (HR).....	88
3.8.4 Conclusion.....	91
3.9 Measures of Intensity, Blood lactate.....	92
3.10 Rating of Perceived Exertion (RPE) scale.....	93
3.10.1 Introduction	93
3.10.2 Borg’s Rating of Perceived Exertion Scale (RPE)	95
3.10.3 RPE in MS	96
3.11 Symptom-limited exercise testing.....	98
3.12.1 MS Functional tests	99
3.12.3 The ‘timed up and go’ test (TUG)	101
3.13 Pilot Study: The appropriateness of maximal exercise testing on people with multiple sclerosis	101
3.16.1 Introduction	101
3.16.2 Participants	102
3.16.3 Methods.....	102
3.16.4 Results.....	103
3.16.6 Conclusion.....	104
3.17 Neurophysiological Measures	105
3.18 Transcranial Magnetic Stimulation.....	105
3.19 Principles of TMS	105
3.20 Magnetic Stimulators	107
3.21 Stimulating coil.....	108
3.22 Safety	109
3.23 MEP variability	110
3.24 TMS measures; Motor threshold.....	111
3.26 The cortical silent period	113
3.27 TMS and exercise induced fatigue	114
3.28 TMS and multiple sclerosis.....	116

3.29 TMS Pilot Study	117
3.29.1 Introduction	117
3.29.2 Methods	117
3.29.3 TMS	118
3.29.4 Analysis	118
3.29.5 Results	118
3.29.6 Conclusion	118
4.1 Introduction	121
4.3 TMS data acquisition	125
4.4 Data Analysis	127
4.5 Results	129
4.6 Discussion	134
4.7 Conclusion	138
Chapter 5 Exercise at maximal intensity	140
5.1 Introduction	140
5.2 Hypothesis	142
5.3 Methods and materials	142
5.3.1 Participants	142
5.3.2 Procedures	143
5.3.3 Cycle ergometer	144
5.3.4 Measurement of oxygen uptake	144
5.3.6 Test termination	145
5.5 Results	147
Figure 5.7 RPE breathing to % peak power (watts) during a graded exercise test	158
5.6 Discussion	162
5.8 Conclusion	171
6.1 Introduction	172
6.2.1 Methods and materials	174
6.2.2 Setting	175
6.2.3 Participants	175

6.2.4 Exercise schedule	176
6.2.5 Preparation and TMS data acquisition.....	177
6.2.6 Peripheral stimulation.....	179
6.2.7 Pre-exercise.....	180
6.2.8 Exercise training.....	180
6.2.9 Post-exercise	181
6.3 Analysis	181
6.4 Results	183
Table 6.4 Pre-exercise resting measures	185
6.4.1 MSG and CG ET 45 and ET 60 exercise phase	185
6.4.2 MSG and CG ET 45 and ET 60 post-exercise recovery period	185
6.4.3 MSG comparisons for ET45 and ET60	186
6.5 Discussion.....	191
Chapter 7 General discussion	197
7.1 Final Remarks	204
8 APPENDICES	205
8.1 RPE instructions given participants	205
8.2 Instructions given to participants before a symptom-limited graded exercise test ..	206
8.3 Participant TMS Questionnaire.....	208
8.4 Physical Activity Readiness-Questionnaire (PAR-Q).....	209
8.5 Rating of Perceived Exertion (Category-Ratio10) Scale	210
8.6 Barthel Index	211
8.7 Classes of medication taken by participants with MS.....	212
References	214

List of Figures

Figure 3.1	Participant exercising during the single-case pilot study.....	120
Figure 3.2	Transcranial magnetic stimulation (TMS) of the participant.....	121
Figure 4.1	Measurement of a motor evoked potential (MEP)	129
Figure 5.1	Changes over time to heart rate during the graded exercise test	151
Figure 5.2	Changes over time to RPE breathing during the graded exercise test	152
Figure 5.3	Changes over time to RPE legs during the graded exercise test	153
Figure 5.4	Changes over time to oxygen uptake (VO_2) during the graded exercise test	154
Figure 5.5	Scatterplot of heart rate to % peak power(watts) for MS and control groups	155
Figure 5.6	Scatterplots of perceived leg exertion to % peak power (watts) for MS and control groups	156
Figure 5.7	RPE breathing to % peak power (watts) for MS and control groups.....	157
Figure 5.8	Maximal oxygen uptake (VO_2) to % peak power (watts) for MS and control groups during a graded exercise test (GXT)	158
Figure 5.9	Recovery profile as a percentage of peak heart rate	161
Figure 6.1	Mean HR during ET45 and ET60	188
Figure 6.2	Mean ratings of perceived exertion for breathing (RPEbr) during ET45 and ET60	187
Figure 6.3	Mean ratings of perceived exertion for legs (RPEleg) during ET45 and ET60	188
Figure 6.4	Post-exercise corticospinal excitability.....	189

List of Tables

Table 2.1	Recommendations for exercise programmes for people with MS	69
Table 3.1	Mean resting heart rates during 3 sessions (7-21 day apart).....	90
Table 4.1	Descriptive details of participants.....	129
Table 4.2	MEP characteristics.....	130
Table 4.3	Effect of trial order.....	131
Table 4.4	Within-session stability of blocks of 4 trials.....	131
Table 4.5	Test-retest of blocks of 4 trials.....	132
Table 5.1	Descriptive details and resting measures of participants	147
Table 5.2	Multiple sclerosis disability scores and functional test results	148
Table 5.3	Comparison of group peak performance values.....	149
Table 5.4	Measures of heart rate, RPE and temperature during recovery.....	160
Table 6.1	Peak measures during the graded exercise test.....	176
Table 6.2	Pre-exercise MEP characteristics recorded from the tibialis anterior	179
Table 6.3	Participant details.....	183
Table 6.4	Pre-exercise measures.....	184

Chapter 1 Multiple Sclerosis

This Chapter is a general introduction to the clinical aspects of Multiple Sclerosis, including its prevalence, pathology and symptoms, and the central mechanisms and functional deficits that can affect exercise performance and recovery.

1.1 Introduction

Multiple sclerosis (MS) is a chronic, degenerative, immune-mediated disease of the central nervous system (CNS) affecting both white and grey matter (Dutta and Trapp, 2011). Inflammatory processes cause demyelination, scar formation and variable neuronal destruction in the brain and spinal cord leading to irreversible neurological disability, autonomic dysfunction, cognitive deterioration and physical impairment (Noseworthy et al., 2000). The clinical course follows a variable pattern characterised by relapses and/or progressive deterioration of physical and cognitive function. At present there is no known cure.

Although in recent years the effects of physical exercise on PwMS have received increasing attention, the specific relationship between exercise intensity and health benefit remains unresolved. There is mounting evidence that physical exercise not only improves fitness levels, but can also enhance lost or diminished function in people with MS (Rietberg et al., 2005, Dalgas et al., 2008), offering individuals the

opportunity to minimise the effects of the disease and maximise their quality of life. However few neurological conditions present such a range and complexity of problems (Thompson, 2008) and few pose such a challenge for determining an effective and appropriate programme of physical activity. Adaptive changes are fundamental to the rehabilitation process (Kesselring and Beer, 2005). Thus an understanding of the highly unpredictable and changeable nature of MS, the variability of its aetiology and pathology, and the heterogeneity of functional and physical deficits is critical for the formulation of effective, appropriate exercise strategies (Schapiro, 2007). The following sections review the research relating to this complex disease.

1.2 Historical context

Although many people had observed and described the pathological changes and symptoms of MS previously, Jean-Martin Charcot (1825-1893) is generally accredited as the first to comprehensively characterise MS as a distinct disease. During a series of lectures entitled 'les sclérose en plaques disséminée' presented in the Salpêtrière Hospital, Paris, Charcot described a condition occurring in younger adults who at autopsy were noted to have greyish and reddish plaques of variable contours and size scattered through the CNS (Lehmann et al., 2007). Charcot gave an account of their clinical features delineating the cerebral, spinal and mixed cerebrospinal structures with vivid descriptions of the clinical pathogenesis and pathophysiology. He identified the discrepancy between lesions and symptoms, and established the link between axonal loss and clinical disability

(Compston, 2005, Clanet, 2008). However, during the ensuing 150 years the complexity of MS pathology, its changeable nature and the significant heterogeneity of symptoms contributed to the wide diversity of perspectives regarding its pathology and treatment. Indeed until comparatively recently, MS was known by many different names suggesting that each of them described a separate clinical condition. Throughout the first part of the 20th Century 'disseminated sclerosis' was the commonly used term in England while 'multiple sclerosis' was widely used in the United States. The World Health Organisation continued to use the term 'cerebral sclerosis' until the mid-1950's (Jock- Murray, 2005). It was only after the rapid development of the newly formed MS Societies in New York and the publication of McAlpine, Compston and Lumsden's classic book in 1955 that the term 'Multiple Sclerosis' finally became universally accepted (Jock-Murray, 2005).

1.3 Prevalence and incidence

MS affects between 1 million and 2.5 million people worldwide. Prevalence and incidence rates vary considerably across the globe, with a strong north to south gradient defined by an increasing disease frequency with distance from the equator (Sellner et al., 2011). Northern Europe, in particular the British Isles and Scandinavia, countries of the former Soviet Union, Australia, and regions in north America defined as those above the 37th parallel (Kurtzke, 1993) have prevalence rates higher than 100 per 100,000 population (Pugliatti et al., 2002, Pugliatti and Rosati, 2008). The lowest rates (<5 per 100,000 population) are found in equatorial

regions of Asia, South America and Africa (Pugliatti et al., 2002). Paradoxically, some of the lowest rates of incidence are also found among isolated communities of Scandinavia in areas where national prevalence rates are among the highest in the world. Interestingly, dietary differences between communities living in close proximity have been proposed to explain the clear exception to MS risk in certain parts of Scandinavia (Ebers, 2008), to some extent supporting the view that genetic and environmental factors in association with the condition of age (Compston and Coles, 2008) can play distinct and inter-active roles in the geographical distribution of MS (Eliasdottir et al., 2011).

In England and Wales epidemiological studies have provided a range of MS prevalence rates varying from 74 to 112 per 100,000 population (Richards et al., 2002, Pugliatti et al., 2006), equivalent to about 58,000-66,000 people, with a clear association between northerliness and hospital admission rates (Ramagopalan et al., 2011). Scotland and its islands, in particular Orkney and the Shetland Isles have the highest prevalence rates of MS in the world, ranging from 145 to 196 per 100,000 (Pugliatti et al., 2006). Scotland also reported the highest incidence rate ever recorded at 12 per 100,000 (Pugliatti et al., 2002).

The highest incidence rates of MS occur in the 35-64 age range with typical onset early in the third decade (Schwendimann and Alekseeva, 2007). As a consequence MS is customarily depicted as a neurological disease of young adults (Noseworthy, 1999, Dutta et al., 2006). The female to male ratio is generally

reported at 2:1 to 3.1 (Pugliatti et al., 2006, Ahlgren et al., 2011). Although clinical presentation of MS before adulthood is rare, hospital and population studies show that 1.7% to 5% of people with MS (PwMS) experience their first clinical symptoms before the age of 16 (Deryck et al., 2006, Ness et al., 2007). A review of over 1000 MS patients in the London (Ontario) Natural History Cohort found that 10 to 15% of patients were diagnosed with MS before the age of 20 (Deshpande et al., 2006).

Late-onset MS (diagnosis after the age of 50 years) is uncommon (Noseworthy et al., 1983), and incidences of MS after 60 years of age occur very rarely (Hooge and Redekop, 1992). The effects of ageing on the clinical course of MS are not well understood and the detection of MS may be made more difficult by the existence of co-pathologies. There is strong evidence that incidence of MS at an advanced age indicate a shorter interval to higher rates of motor disability (Trojano et al., 2002, Confavreux and Vukusic, 2006, Kis et al., 2008).

MS mortality rates are difficult to estimate as the cause of death can be attributed to a primary co-pathology such as chronic heart disease, or to a secondary complication such as pneumonia or renal failure (Sadovnick et al., 1991). Nevertheless death is attributed to MS in two-thirds of cases (Compston and Coles, 2008). Death by suicide is significantly elevated when compared to the healthy population, accounting for up to 15% of deaths (Stenager et al., 1996) with men who contract MS before the age of 30 at highest risk (Stenager et al., 1996).

In general the median time to death (about 30 years from disease onset) represents a 5-10 year reduction in life expectancy (Bronnum-Hansen et al., 2004).

The high national prevalence rate and the long-term nature of the disease place a significant financial burden on the UK economy. In 2002 the direct yearly cost of MS to the National Health Service was estimated to be between £1.2 and £1.4 billion (Richards et al., 2002). A more recent report which included non-medical costs, informal care and other indirect financial outgoings estimated that the total annual MS related expenditure in the UK exceeded £2.9 billion (MS International Federation, 2010).

Although MS prevalence has remained stable in some geographical areas, the world-wide incidence of MS is continuing to grow, the latitude gradient is disappearing and the female-to-male ratio among patients with MS is on the increase (Orton et al., 2006, Alonso and Hernan, 2008, Benito-Leon, 2011); (Elhami et al., 2011, Hirst et al., 2009). The precise reason for the rise in MS incidence is unclear. Genetic influences take relatively long periods to affect prevalence rates, hence the increase is more likely a reflection of changing environmental factors, though these remain unidentified. The transcontinental migration of large numbers of people may have contributed to the global distribution of MS (Compston and Coles, 2008). Additionally, factors such as improvements in epidemiological study methods, improved detection of MS resulting from the international adoption of standard diagnostic criteria and/or

greater access to diagnostic technology may also in part explain the increase in world-wide incidence rates (Pugliatti and Rosati, 2008).

1.4 Diagnosis

For most people, the clinical course of MS begins with episodes of neurological dysfunction followed by complete recovery (Confavreux et al., 2000). As the disease progresses, clinical disability increases with each attack but the frequency of attacks decreases. Transition between the clinical stages of MS is usually gradual and indistinct, making for many intermediate forms of the disease (Compston, 2004b) and complicating both its diagnosis and treatment.

Magnetic resonance imaging (MRI) is the 'gold standard' for diagnosing MS and monitoring the disease course (Frohman et al., 2003). However, although MRI scans can reveal multiple lesions distributed through the white and grey matter of the CNS (Filippi et al., 2010b, Filippi et al., 2010a), diagnosis of MS with MRI is frequently confounded by the differential-diagnosis of other diseases characterised by demyelination of the optic nerves (neuritis), severe myelinopathy with extensive spinal cord lesions, or even a normal MRI with abnormalities typical to MS (Polman et al., 2011). Therefore, cerebrospinal fluid (CSF) analysis is often employed to confirm the diagnosis. A persistent and consistent presence of oligoclonal banding (OCB) exists in the CSF of MS patients, thus a positive CRF analysis requires only 2 lesions identified by MRI to confirm the diagnosis of MS (Giovannoni and Thompson, 2008).

85% of adult MS patients, 80% of pediatric MS cases and nearly all adolescent MS cases initially present with clinically isolated syndrome (CIS), or encephalomyelitis (Mikaeloff et al, 2004). CIS is an acute episode of demyelination affecting one or several sites in the CNS lasting for at least 24 hours. It typically involves a lesion affecting the optic nerve, brainstem/cerebellum, spinal cord or cerebral hemispheres (Polman et al., 2011). Not everyone who experiences CIS is diagnosed with MS. Indeed many patients with mild CIS recover without treatment. However, if white-matter abnormalities are detected by MRI at clinically unaffected sites, the chance of a future positive diagnosis increases from 50% within 2 years to 82% within 20 years (Fisniku et al., 2008). Although the probability of CIS converting to clinically defined MS (CDMS) increases over time, MS disease evolution is impossible to predict and not all patients with CIS progress to CDMS (Miller et al., 2005).

In practice, the initial diagnosis of MS continues to be based solely on clinical observation of both signs and symptoms (Tremlett et al., 2008), but the higher availability and reliability of advanced technology such as MRI has led it to become critical to the diagnostic process. Thus the standard definitions of clinical course patterns and outcomes in people with MS were revised in 2011 to take into account improvements in imaging techniques, thereby allowing a confirmed diagnosis of MS from a single MRI scan (Polman et al., 2011).

1.5 Classification of MS

A confirmed diagnosis of MS requires evidence of characteristic, neurological lesions in the CNS that have occurred in both time and space, and to exclude alternative diagnoses (Polman et al., 2011). PwMS are classified from clinical presentation in the following manner (Lublin and Reingold, 1996):

- relapsing-remitting (RR-MS): clearly defined disease relapses with full recovery or with sequelae and residual deficit upon recovery; periods between relapses characterised by a lack of disease progression
- secondary progressive (SP-MS): initial relapsing-remitting course followed by progression with or without occasional relapses, minor remissions and plateaux
- primary progressive (PP-MS): disease progression from onset with occasional plateaus and temporary minor improvements allowed
- benign: disease in which the patient remains fully functional in all neurological systems 15 years after onset
- malignant: disease with a rapid progressive course, leading to significant disability in multiple neurological systems or death in a relatively short time after disease onset

1.6 Aetiology

Epidemiological studies of MS indicate a complex aetiology in which unidentified environmental factors trigger the disease in genetically susceptible individuals (Compston, 1997). MS is linked to alleles of the major histocompatibility complex (MHC). Although the exact mechanisms are unknown, the human leukocyte antigen *HLA-DRB1*1501* gene is the strongest genetic factor identified so far influencing susceptibility to MS (Lang et al., 2002), and it may also determine the balance between disease susceptibility and resistance (Hauser and Oksenberg, 2006, De Jager et al., 2008). However, while some studies have reported associations between *HLA-DRB1*1501* and spinal cord abnormalities (Sombekke et al., 2009) and a possible relationship to a more severe disease course (DeLuca et al., 2007), others have reported no significant influence on disease severity, brain atrophy, or cognition (Van der Walt et al, 2010).

Using a dataset obtained from 98 multiple case families, Haines et al (1998) estimated that the HLA locus accounted for up to 62% of the inheritability of MS (Haines et al., 1998). MS has a familial recurrence rate of about 20% (Compston and Coles, 2008). When both parents have MS, the risk increases to about 30% (Schwendimann, 2007) but to date, investigations into the recurrence of familial MS and studies relating to monozygotic and dizygotic twins have provided no conclusive evidence of the presence of hereditary genetic traits (Oksenberg and Hauser, 2008, Ebers, 2008).

A current hot topic is the interaction of *HLA-DRB1*1501* with vitamin D binding proteins (vDBP), the major plasma carrier of vitamin D metabolites. Genetic differences in the vDBP gene have been found to influence vitamin D levels (Ramagopalan and Ebers, 2008, Agliardi et al., 2011). Vitamin D has long been implicated with MS. It is known to regulate neurotrophic growth factors (Taniura et al., 2006), halt the progression of experimental autoimmune encephalomyelitis (EAE), the animal model of MS (Cantorna et al., 1996), and may influence the anti-inflammatory immune response in PwMS (Ramagopalan et al., 2009).

Vitamin D can be synthesised either through exposure to sunlight or through the diet. The consumption of oily fish, rich in vitamin D, has been proposed as the major factor mediating the environmental effect of MS in some parts of Norway (Ebers, 2008). However, other north Atlantic populations (eg, those from Iceland, coastal Scotland, Orkney and Denmark) also follow diets that traditionally contain a relatively high consumption of oily fish, yet still present very high MS prevalence rates. Furthermore, although vDBP has been implicated in disease progression (Disanto et al., 2011), large-scale studies in Canada (Orton et al., 2011) and the United States of America (Simon et al., 2010) have failed to directly connect vitamin D metabolism to MS susceptibility. Moreover, recent studies examining the effects of high level vitamin D supplementation have found no significant effect on lesion load, nor have they been shown to produce a significant therapeutic advantage for PwMS (Stein et al., 2011).

Several studies have suggested a strong association between the Epstein-Barr virus (EBV) and *HLA-DRB1*1501* (Ascherio et al., 2001), (Lang et al., 2002, Lucas et al., 2011) The evidence shows that while people without EBV antibodies have an extremely low risk of developing MS (Levin et al., 2010), high-blood serum levels of EBV antibodies can signal an 8-fold stronger marker of MS risk in younger people (Munger et al., 2011) and those people infected by EBV in later life increase the risk of developing MS by up to 30-fold (Ascherio, 2008). However, while EBV may play a role in MS pathology in some individuals, there is no evidence to support the theory that EBV predisposes an individual to MS (Pender et al., 2011). Other viruses such as herpes (Martin, 1981, Sellner et al., 2010), measles (Fewster et al., 1979) and rubella (Horikawa et al., 1973), and various vaccines, in particular hepatitis B (Hernan et al., 2004) and influenza vaccines (Yahr and Lobo-Antunes, 1972) have been postulated but dismissed as possible causative agents that can increase the risk of disease acquisition. While It seems likely that vulnerability to environmental agents occurring at a crucial moment in an at-risk individual's maturation of the immune system can predispose development of the disease (Compston, 2004a, Bennett et al., 2008), and that those risks increase dramatically towards later life (Ascherio and Munger, 2007a, Ascherio and Munger, 2007b), a conclusive link between genetic predisposition and inheritability, geographical region and environment, or exposure to a specific causative trigger has yet to be demonstrated. In summary, unravelling the genomics of MS is at an early stage, the roles of environment and familial susceptibility are not fully

understood, and the contribution of genetics to disease risk, disease outcome and potential treatments for MS remains to be elucidated.

1.7 Demyelination

Myelin is formed from the extending plasma membrane of oligodendrocytes creating spiral segments of sheathing that wrap around axons and envelop bundles of axonal segments. The insulating and protective properties of the myelin sheath are largely due to its structure, thickness, low water content, and its richness in lipids. Myelin sheath thicknesses and internodal lengths vary according to axonal calibre (Baumann and Pham-Dinh, 2001). Axons in the CNS trigger myelination when they reach a diameter of about $0.2\mu\text{m}$ (Waxman, 2006). The number of wrappings around an axon can vary between 10 and 160 (Arbuthnott et al., 1980). Depending on the subtype of oligodendrocyte, 10 axons or more can be myelinated (Verkhatsky and Butt, 2007). As a consequence, inflammatory damage to a single oligodendrocyte has the potential to affect multiple axons.

Axonal geometry in association with the biophysiological properties of voltage gated channels determines the propagation and transport of action potentials. Conduction velocity is dependent on the diameter of the axon and the action of the oligodendrocyte. Each $1\mu\text{m}$ of outer diameter of the axon adds 6m/s to conduction velocity at 37°C . A single oligodendrocyte increases the conduction velocity of the axon it sheaths by $\sim 10\%$. Sodium gated ion channels (Nav) play a critical role in conducting action potentials (AP) along axons in the brain and spinal cord

(Waxman, 2006). Myelination concomitantly provokes a redistribution of Nav channels into clusters at the exposed (unmyelinated) segments of the axon called the nodes of Ranvier (Debanne et al., 2011). As myelination progresses, Nav expression switches from Nav1.2 to Nav 1.6 within the confines of the unmyelinated nodes, and axonal membrane concentrations of Nav change from less than 25 channels μm^2 under the myelin sheath, to $\sim 1,000 \mu\text{m}^2$ at the nodes of Ranvier (Waxman, 2006).

Multiple, voltage-insensitive potassium channels (Kv) determine the resting potential of the axon membrane, and play an indispensable role in repolarisation of the membrane after an AP. The Kv channels are expressed in relatively high density under the myelin, falling to about one-sixth of the density at the node of Ranvier (Debanne et al., 2011). The high level of Kv channels at the juxtapanodal region may prevent axonal hyper-excitability and help maintain the fidelity of AP propagation at the node of Ranvier (Kress and Mennerick, 2009). When activated, Kv channels produce varied waveforms, and have a significant impact on subsequent AP propagation characteristics (Kole et al., 2007). Voltage gated calcium channels (Cav) are expressed at a lower density than Nav and K⁺ channels and require relatively high depolarisations for activation ($> -30\text{mV}$) (Debanne et al., 2011). Although their role in AP propagation is less well understood than that of Nav and KV, Cav are known to regulate firing properties such as spike firing, burst firing and action potential threshold (Bender and Trussell, 2009).

In summary, the myelin sheath both increases membrane resistance, and reduces membrane capacitance by several orders of magnitude thus restricting current flow along the axon to the nodes of Ranvier. In the healthy neuron, an action potential (AP) initiates in the axonal initial segment (AIS) close to the soma, and propagates towards the terminal, jumping from node to node, thereby providing a saltatory pathway for the high-speed conduction of action potentials through the CNS. This method of conduction both increases conduction velocity, and significantly reduces the energy required to transport signals down an axon.

Demyelination is the hallmark of MS (Dutta and Trapp, 2011). Damage to the myelin sheath is driven by macrophages, and auto-reactive, myelin specific CD-4+ and CD-8+ T-cells (Bitsch et al., 2000, Beeton and Chandy, 2005). Functionally, CD-4+ cells are involved in delay-type hypersensitivity and are termed T helper (Th) cells. In contrast CD-8+ cells are involved in the destruction of antigen-specific targets and are termed cytotoxic cells (Friese and Fugger, 2005). CD-4+ cells are shown to predominate in acute lesions while CD-8+ cells are found to predominate almost tenfold in chronic MS lesions (Neumann et al., 2002).

During an inflammatory event, lymphocytes become attached to potassium (K+) receptors (Beeton and Chandy, 2005) in endothelial cells and pass through the blood-brain barrier (BBB) (Hauser and Oksenberg, 2006). Certain components of myelin have been found to share, or contain the same proteins found in viruses such as EBV, measles, herpes or influenza. These myelin proteins are thought to

provoke a misguided response in myelin-specific T-cells(Davidson and Diamond, 2001) setting up an inflammatory process that resembles a delayed-type hypersensitivity (Compston, 2004b). The consequential activation of pathogenic T-cells may result in the further opening of the BBB, and trigger the release of additional pro-inflammatory cytokines which then proceed to attack the myelin in the CNS (Lang et al., 2002). The inflammation is driven by a T-lymphocyte sub-unit that secretes interleukin (IL)-17 under IL-23 control (Jadidi-Niaragh and Mirshafiey, 2011) Th-17 cytokines generate intense inflammation and tissue injury by recruiting neutrophils to a site of inflammation in the CNS (Hussell et al., 2010). IL-17 and IL-22 disrupt the BBB allowing the efficient penetration of Th-17 into the brain where they can kill human neurons (Kebir et al., 2007). The inhibition of Th17 has been seen to substantially ameliorate MS disease severity in the EAE model (Du et al., 2009).

The different levels of demyelinating activity in lesions are characterised by the amount of cytotoxic T-cell (Traugott et al., 1983) and macrophage activation, and the phagocytosis of myelin proteins (Lucchinetti et al., 2004). In addition, the deficiency of regulatory T-cells in conjunction with the proliferation of B-cells, (Owens et al., 2003) and matrix metalloproteinase (MMP), an instigator of cell death (Rosenberg, 2009), is a strong indicator of on-going demyelinating activity. All three are markers commonly found in the CSF of people with MS (Leppert et al., 1996).

The general consensus is that pathogenesis of the lesions in PwMS is heterogeneous. The pattern of the lesions appears to be totally unpredictable (Frohman et al., 2006), indeed different types of lesions can be found in the same patient (Barnett and Prineas, 2004). Whilst MS symptoms are assumed to be directly caused by lesion damage to the white and grey matter in the CNS, the relationship between the location of lesions and the extent of clinical disability is weak (Bashir and Whitaker, 2002), making disability levels hard to predict. In contrast, the quantity of lesions, especially their development at an early stage of MS is associated with greater disability occurring in later years (Noseworthy et al., 2000). Brain atrophy appears to be widespread in MS, affecting both grey and white matter in all regions of the brain, the brainstem and the spinal cord. MRI studies have shown that brain atrophy commences at the very earliest stages of MS and accelerates rapidly as the disease develops (Bermel and Bakshi, 2006), with one study observing a volume loss of up to 7% over a two year period during the relapsing-remitting stage (Zivadinov et al., 2001). Brain volume continues to decrease during the progressive forms of the disease, but at a less aggressive rate (Bermel and Bakshi, 2006). Grey and white matter atrophy have been found correlate significantly with cognitive dysfunction (working memory and memory processing speed), and psychiatric symptoms (depression, anxiety, apathy) to a greater extent than whole-brain volume or lesion load in MS (Sanfilippo et al., 2006). The association between a decrease in whole-brain volume with an increase in physical disability is less clear, however spinal cord atrophy appears to have a particularly strong relationship with physical impairment (Lin et al., 2004).

1.8 Re-myelination

Remyelination is associated with functional recovery in MS (Miron et al., 2011) although some individuals have demonstrated extensive remyelination without any sign of functional improvement (Patrikios et al., 2006). The rapid recruitment of oligodendrocyte-precursor cells (OPCs) to areas of tissue injury appears to be normal in PwMS (Frohman et al., 2006). Indeed, remyelination occurs during the early stage of lesion development (Stadelmann and Bruck, 2008) and the initial stage of myelin destruction (Lassmann et al., 1997). However, the extent of remyelination between individuals, or even within specific MS lesions, is highly variable (Patrikios et al., 2006). Animal studies have shown the efficiency of remyelination to decline with age, more rapidly in males than females (Franklin and Kotter, 2008, Stadelmann et al., 2008). Complete remyelination can occur in both white, and grey matter lesions (Stadelmann and Bruck, 2008), however a number of growth-inhibiting substances have been identified within the scar of plaques that can arrest the development of the OPCs and prevent lesion repair (Frohman et al., 2006).

A strong indication of ongoing or past remyelination is the presence of shadow plaques, so-called because they are sharply demarcated plaques, clearly distinguishable from the surrounding white matter. The cause of this shadowy appearance is possibly the result of reduced myelin density, or to the loss of axonal fibres under the newly reformed sheath (Miron et al., 2011). During a systematic analysis of the incidence and distribution of shadow plaques in the autopsies of 51

PwMS of different clinical classifications and disease durations, Patrikios and co-workers (2006) observed that 20% of the patients showed extensive remyelination across 60% to 96% of the global lesion area. The remyelination appeared, in part, to be dependent upon the location of the lesion (remyelination appearing more commonly in the deep white matter or in subcortical locations). There was extensive remyelination in a considerable portion of MS patients, with the number of shadow plaques significantly associated both with older age at death, and longer disease duration (Patrikios et al., 2006). Hence the CNS displays a remarkable capability to trigger and maintain remyelinating mechanisms for a considerable period after disease commencement. Cycles of demyelination and remyelination may eventually exhaust the body's capacity to continue repairing the tissue (Kuhlmann et al., 2008). The reasons as to why the repair mechanisms ultimately fail in PwMS remains unresolved.

In animal models, glial cell transplantation in the CNS have been shown to enhance action potential conduction in myelin-deficient axons (Utzschneider et al., 1994), and the introduction of Schwann cells into the spinal cord of rats restored the conduction of trains of impulses through the lesion (Felts and Smith, 1992). More recently a cat model has provided compelling evidence that global remyelination of the CNS can provide complete restoration of clinical function in axons that have remained essentially intact (Duncan et al., 2009). However, studies exploring the stimulation, manipulation or implantation of OPCs in humans have found that OPCs are non-migratory, that engrafted OPCs rarely extended into

normal white matter and that the white matter tracts stay localised to the site of injection (Windrem et al., 2002).

In summary, even in the progressive forms of MS, compensatory mechanisms have been observed to respond to inflammatory injury to neural circuits which in some cases allow the partial or complete restoration of neural function (Sjostrom et al., 2008). However there are many disturbances to which the CNS cannot respond and where the insult and injury may be at such intensity that any natural response is inadequate (Kuhlmann et al., 2008).

1.9 Cellular abnormalities

Traditionally, axonal degeneration has been regarded as the major cause of neurological deficits and irreversible disability in PwMS (Dutta and Trapp, 2011), however, while it is acknowledged that the symptoms of MS are generally attributable to the interruption of myelinated tracts in the CNS (Hauser and Oksenberg, 2006), recent studies have shown that a certain proportion of neurodegeneration is independent of demyelination (Dutta et al., 2006, Waxman, 2008, Mahad et al., 2008, Dutta and Trapp, 2011). Indeed, the current view is that the whole CNS appears to be involved in the disease (Siffrin et al., 2010). Inflammatory processes within the CNS can trigger a cascade of events that profoundly affect synaptic density (Dutta et al., 2011), neurotransmitter concentrations (Werner et al., 2001), signal transmission mechanisms (Waxman,

2005), mitochondrial density (Mahad et al., 2008) and disrupt the microtubule transport systems critical to the normal functioning and survival of neurons (Dutta et al., 2011). The extent of MS related symptoms and disability is determined by the intrinsic ability of the CNS to retain the integrity and compliance of the central mechanisms and neural pathways (Waxman, 2006, Dutta and Trapp, 2011)

The ability of the CNS to adapt its circuitry in response to changing conditions is termed neuroplasticity (Konorski, 1948). It forms the structural and functional correlates for learning and memory, and is the mechanism by which adaptive changes are modulated by appropriate therapies (Kesselring et al., 2010a) Neuronal electrical properties can change in milliseconds, and the changes may endure for hours or days (Sjostrom et al., 2008). Short term adaptations occur continuously (Beck and Yaari, 2008), hence alterations to the intrinsic properties of synapses, dendrites or axons as the result of MS, have a profound effect on the ability of the CNS to make continual short term adaptations to such changing circumstances.

1.10 Glutamate

Glutamate is the principal fast excitatory neurotransmitter in the CNS. Glutamate dependant signalling is required for all sensory and motor processing, and glutamatergic receptors contribute significantly to synaptic plasticity, learning and memory formation (Bauer and Robinson, 2012). During inflammatory episodes, microglia and leukocytes release substantial amounts of glutamate into the extracellular space (Barger et al., 2007). In PwMS, glutamate concentrations have

been found to be significantly elevated, both in acute lesions and normal appearing white matter (Srinivasan et al., 2005), and doubled in cerebrospinal fluid (Stover et al., 1997).

Ionotropic glutamate receptor sub-types are classified into two groups; NMDA (N-methyl-D-aspartate), and non-NMDA (alpha-amino3-hydroxy-5methyl-4-isoxazole propionic acid (AMPA) and kainite). NMDA receptors consist of a complex pharmacology with multiple modulatory sites. They are key components in long-term potentiation (LTP) of neuronal pathways, memory formation and synaptic plasticity (Prybylowski and Wenthold, 2004), and cell survival (Sheng and Kim, 2002). AMPA receptors desensitize quickly, strongly influencing neuronal output, and are responsible for the majority of fast excitatory transmission in the human brain and enhanced synaptic plasticity (Lu et al., 2001). Metabotropic glutamate (MGLU) receptors do not conduct ionic flow, instead they activate intracellular enzymes through G-proteins, modulating ionic channel activity (Hassel and Dingledine, 2006). Recently, confocal microscopy has revealed that MS causes a significant decrease in excitatory synapses and mRNA proteins encoding AMPA, NMDA and MGLU receptors. Moreover, a significant reduction in their respective synaptic binding proteins within the hippocampi of post-mortem MS brains has been reported (Dutta et al., 2011).

Once released, glutamate needs to be cleared quickly from the extracellular space in order to terminate synaptic transmission (Kalloniatis and Tomisich, 1999). 95% of the extracellular glutamate is rapidly taken up by astrocytes via the excitatory

amino-acid transporters (EAAT) (Rothstein et al., 1996) where it is synthesized into glutamine, then shuttled back to neuronal vesicles where it re-synthesises into glutamate (Lehmann et al., 2009). Protein levels of the glutamate transporters EAAT-1 and EAAT-2 have been found to be significantly decreased in MS brains, suggesting a reduction in the ability to clear glutamate (Dutta et al., 2011).

The increase in extracellular glutamate is capable of precipitating excitotoxic cell death by over-stimulation of glutamate receptors (McDonald et al., 1998, Rothman and Olney, 1995). Indeed, in one EAE model, more than 60% of oligodendrocyte loss was attributed to glutamate excitotoxicity (Pitt et al., 2000). Moreover, excitotoxicity causes dysregulation of calcium homeostasis triggering calcium influx into surrounding cells, overloading the mitochondria and resulting in the significant dysfunction of the electron transport chain and inhibition of ATP production (Dutta et al., 2006).

1.11 GABA

Gama-aminobutyric acid (GABA) receptors and glycine are the principal inhibitory transmitters in the CNS that, when activated, can generate a membrane hyperpolarisation and a reduction in dendritic excitatory activity that strongly inhibits action potential firing (Ben-Ari et al., 2007). GABA and GABA-ergic receptors have been found to be significantly elevated in MS demyelinated hippocampi (Dutta et al., 2011) suggesting that GABA exerts a powerful inhibitory influence in MS.

1.12 Nitric oxide

When nitric oxide (NO) is released in excessive amounts it becomes neurotoxic and has been linked to the neurodegenerative process in MS (Svenningsson et al., 1999). Under normal conditions NO acts as a signalling molecule that regulates vasodilation, immune responses and neuronal function. Axons within the healthy CNS generally experience very low concentrations of NO, reportedly in the low nanomolar range. (Griffiths et al., 2003). During inflammatory episodes, activated microglia produce substantial amounts of NO to kill invading organisms or transformed cells (Merrill et al., 1993). Other cells in the CNS, such as astrocytes, can also be induced to express NO during an inflammatory attack. (Murphy et al., 1993). NO synthesis is significantly increased in acute MS lesions (Bo et al, 1994; Liu et al, 2001) and high concentrations of NO markers have been observed in the blood, urine and CSF of PwMS (Svenningsson et al., 1999, Giovannoni et al., 1999). In MS, NO released from activated microglia is known to have a deleterious effect on mitochondria, resulting in a reduction in ATP and an increase in glutamate release (Brown and Bal-Price, 2003). NO inhibits the axonal transport of synaptic vesicle precursors and may cause synaptic dysfunction (Stagi et al., 2005). Moreover, NO has been implicated in the disruption of the BBB (Boveri et al., 2006), demyelination and axonal injury (Hill et al., 2004), and is believed to contribute to physical impairment and loss of function through reversible and irreversible conduction block in demyelinated axons (Redford et al., 1997).

1.13 Mitochondria

Mitochondria have recently been shown play a role in the pathogenesis of MS (Dutta et al., 2006, Kalman et al., 2007, Mahad et al., 2008). The CNS accounts for 20% of oxygen inspired at rest, yet represents only 2% of body weight (Kann and Kovacs, 2007). Mitochondria produce high levels of metabolic energy for neurons in the form of ATP. Profound alterations to mitochondrial DNA have been found to be significant contributors to progressive neuronal degeneration in MS (Campbell et al., 2010) and brain atrophy. Post-mortem brain-weight in people with diverse mitochondrial diseases has been shown to be reduced by almost 20% (Lax and Jaros, 2012). Mitochondrial abnormalities have been found to occur in synapses and distal regions of axons long before the first signs of inflammatory swelling (Dutta et al., 2006) leading to axonal disruption and degeneration (Su et al., 2009). The high level of mitochondrial dysfunction in MS lesions may be the result of nitric oxide (NO) blocking the electron transport chain, thus disrupting mitochondrial respiration and reducing ATP synthesis (Su et al., 2009). Mitochondrial stress leads to persistent opening of the permeability transition pore of the inner membrane of the mitochondria, allowing larger solutes to enter, and further adding to the disruption of the electron transport chain and ATP synthesis. The resulting ATP deficiency results in the gradual loss of the Na^+/K^+ ATPase, which has a critical role in maintaining Na^+ and K^+ transmembrane gradients, membrane excitability and the conduction of APs (Benarroch, 2011).

1.14 Axonal transport

Axonal transport is critical for the rapid distribution and positioning of diverse cellular cargoes along the microtubules of neurons and are implicated in long term ,learning-related brain plasticity (Puthanveettil et al., 2008). Motor proteins are nanoscale transport vehicles which move molecular cargoes in cells (Gilbert, 2001). Kinesins are the principle carriers of organelles, mitochondria and vesicles along neurons in an anterograde direction. Each nanoscale step is coupled to hydrolysis of one molecule of ATP (Endow et al., 2010). Kinesins carry mitochondria for long distances to areas of high metabolic demand, such as the synapses, the axonal initial segment, nodes of Ranvier and to sites of neural inflammatory activity. Axonal transport is significantly impaired by inflammatory cytokines (Stagi et al., 2006) and blocked by NO (Stagi et al., 2005). In addition KIF1A, the major micro-tubule kinesin transporter and dynein, the retrograde axonal transporter have been found to be significantly decreased at both mRNA and protein levels in demyelinated MS hippocampi (Dutta et al., 2011).

1.15 MS disability, symptoms and management

The definition and concept of MS disability is complex. There are many historical, social, legal and philosophical influences in its interpretation. The experience of disability and its impact on quality of life is unique to each person. Hence the overall aim of the International Classification of Functioning, Disability and Health (ICF) is to provide a standard language and unified framework for the description of

health and health-related states described from the perspective of the 1) the body and its structures, and 2) activities and participation (World Health Organisation, 2001). According to the World Health Organisation, a physical impairment is a problem in body function or structure, such as a significant deviation or loss. Disability represents the restriction (resulting from an impairment), or an inability to perform certain tasks within the physical and social environment. Within the definitions of the Disability Discrimination Act (DDA) at least one of the following areas must be badly affected; mobility, manual dexterity, physical coordination, continence, ability to lift or carry everyday objects, memory or ability to concentrate, learn or understand; understanding the risk of physical danger.

Approximately 80% of people with MS initially present with relapsing and remitting MS (Noseworthy et al., 2000) . The remission of symptoms is possibly the result of remyelination. The early symptoms may appear trivial, occurring only once or twice a year. Typically patients will report sensory disturbances, optic neuritis, limb weakness and gait instability, clumsiness or facial weakness. As the disease progresses they may develop parathesias, spasticity, pain, heat intolerance, bladder/ bowel infections, incontinence and sexual dysfunction. 50% of MS patients remain ambulatory at 15.5 years from onset, however, almost 60% require assistance when walking and 70% are restricted in their ability to perform activities of daily living. Many are dependent on multiple mobility aids (Iezzoni et al., 2009). At any point in time approximately 30% of people with MS are restricted to a wheelchair (Richards et al., 2002).

Pain can occur as neuralgia, severe deforming spasticity or migraine, and may herald a relapse in MS (Kenner et al., 2007). Additional symptoms include sleep disorders (Tachibana et al., 1994, Stanton et al., 2006), depression (Voss et al., 2002), reduced activity levels (Ng and Kent-Braun, 1997), and reduced quality of life (Pittion-Vouyovitch et al., 2006). The prevalence of depression is estimated to range between 23 - 54% (Brown et al., 2009). Suicidal intent is higher than in the general population, with a Canadian study of 140 PwMS reporting that 6.4% had actually attempted suicide (Feinstein et al., 2002). Although anxiety disorders are less well reported, there is considerable overlap in the symptomology of anxiety, depression and fatigue reflecting a significant comorbidity in MS patients (Brown et al., 2009).

Fatigue affects almost 90% of MS patients (Krupp et al., 1988, Murray, 1985) with over 40% reporting it as their most serious complaint (Murray, 1985, Schwid et al., 2002). The symptoms of fatigue can be severely debilitating and significantly affect mood-state, cognitive performance and executive control (Holtzer and Foley, 2009). Chronic fatigue is critically linked to patients' perceptions of their general well-being (Egner et al., 2003). It has a significant impact on the ability to work and participation in social activities (Forbes et al., 2006). However, despite its ubiquitous presence, fatigue as the direct result of MS remains difficult to quantify and very hard to measure. Indeed, the exact aetiology of fatigue is unknown. It seems to be triggered by multiple factors, is influenced by central, peripheral and

autonomic mechanisms, and presents multidimensional challenges to PwMS (Rietberg et al., 2011).

Previous studies have reported conflicting results when relating levels of fatigue to physical disability (Mills and Young, 2011, Iriarte et al., 2000, Kroencke et al., 2000) but clear relationships exist between fatigue and disease impact (Mills and Young, 2011) and disease progress (Bakshi, 2003, Kos et al., 2007). Lassitude is perhaps the most common and hardest type of fatigue to understand. It occurs spontaneously, disables significantly (Schapiro, 2007) and is typified by an overwhelming sense of exhaustion or tiredness lasting from just a few hours to several days.

Surprisingly there is no universally accepted definition for MS related fatigue. In 1998 the MS Council for Clinical Practice proposed a description of fatigue as “ a subjective lack of physical and/ or mental energy that is perceived by the individual or caregiver to interfere with usual and desired activities”. However, this definition fails to convey the sense of overwhelming tiredness, lack of energy and the constant feeling of exhaustion characteristic of the malaise reported by PwMS . Non-activity related fatigue may be caused either by multiple factors relating to the disease process, or by the pathological consequence of the disease. The reasons why PwMS experience severe fatigue while at rest are as yet unknown.

1.16 Activity-related fatigue

Exertional fatigue, muscle fatiguability and exercise intolerance are shared symptoms of many neurological and non-neurological pathologies that may originate in the CNS, the peripheral nervous system (PNS), or both (Chaudhuri and Behan, 2004). Activity related fatigue can be categorised into a further three different entities, fatigue at rest, fatiguability (due to physiological, neurological, or psychological stress), and the impact of fatigue on other symptoms (Iriarte et al., 2000).

Fatigue has been shown to respond well to physical exercise (Mostert and Kesselring, 2002), however the desire to participate in activities may be compromised by an inability to tolerate sensations of effort, and post-exercise fatigue. Indeed, anxieties associated with ability to recover may lead PwMS to compensate, or even overcompensate their daily routines rather than yield to the feelings of fatigue (Rietberg et al., 2011).

There is a high correlation between dysfunction of the pyramidal tract and fatigue in MS (Iriarte et al., 2000, Samii et al., 1996a). Other abnormalities in the CNS may play an important role (Andreasen et al., 2009). A physiological explanation may involve the dysfunction of the motor system or its neural activation that arises due to altered properties within the muscle, or because the central nervous system fails to drive the motor neurons sufficiently (Gandevia, 2001). Central activation of skeletal muscle has been shown to be impaired in PwMS suffering from fatigue

(Andreasen et al., 2009). Some MS patients presenting with chronic fatigue exhibit dysfunction of the Hypothalamic-Pituitary-Adrenal (HPA) axis (Siegert and Abernethy, 2005). Studies have demonstrated that a hyperactive HPA axis is associated with neurological disability, cognitive impairment and brain atrophy, and may be used as a marker to predict future neuro-degeneration in MS (Heesen et al., 2007).

1.17 Physical management

Whilst there is general agreement that physical therapy plays an important role in maintaining and improving function, there is no evidence that physical therapy halts the primary symptoms of MS, and no consensus on the most effective approach of delivering therapeutic rehabilitation. Management factors relating to physical exercise PwMS are covered in greater depth in Chapter 2.

1.18 Medication

Disease modifying therapies for MS are still at a relatively early stage of development. Drugs have been shown to be effective for reducing relapse rates in the early forms of the disease with a 30% reduction in frequency of new episodes over 2 to 3 years, and to a certain extent slowing clinical progression during relapsing-remitting episodes (Noseworthy, 2008). However, studies have shown no useful effects of drug therapies on the secondary-progressive phase except in rare cases of progressive forms of the disease with continuing high relapse rates (El-Moslimany and Miller, 2008). Long-term observational studies assessing the

validity of drug interventions have suffered through the lack of patients' willingness to participate, or in some cases loss to follow-up, or death (Ebers et al., 2009). Initially, there appears to be a high level of compliance and adherence to medications, but discontinuation rates have been reported as high as 50% within 2 years of drug initiation (Wong et al., 2011). Classes of medication taken by participants in the experimental stages of the thesis can be found in Appendix 8.6

Overall, β -interferon has been shown to decrease clinical relapses and possibly slow-down the progression of disability, however the effects are inconsistent, and a high number of PwMS are non-responders (Oksenberg and Hauser, 2008). A Cochrane review examining the protective effects of interferon reported only a 20% reduction in the relative risk of an exacerbation of symptoms (Rice et al., 2001). Large clinical trials of interferons in progressive forms of MS have reported modest or no effect on disability outcomes (Group, 1998). A Cochrane review of the evidence relating to glatiramer acetate (Capaxone) found that disease progression was unaffected by its use. Indeed, there was no evidence to support a beneficial role of glatimere acetate for RR-MS patients over time, and its benefit on clinical relapses for RR-MS was also questionable (Munari et al., 2004). Nevertheless, early use of immuno-modulatory drugs suggest markedly better responses (Bates, 2011) within a narrow window of opportunity (Smith and Waxman, 2005), however, symptoms and functional deficits are not reversed. As the disease progresses, benefits from drugs appear to become minimal (Noseworthy, 2008), while deficits and disabilities continue to accumulate. Moreover, neither existing symptoms nor

functional disability are reversed. In general MS progression can be delayed but not completely stopped by available drug therapies (Goodin and Kappos, 2008).

1.19 Summary

MS presents a complex, unpredictable, changeable and heterogeneous disease that affects the CNS causing moderate to severe disability in the majority of those affected by it. Symptoms can change without warning leading to an unpredictable level of dysfunction and recovery (Thompson, 2008). The fluctuating, progressive nature of the disease can present confusing pictures to clinicians, complicating its management. An incomplete understanding of its pathophysiology and the ineffectiveness of available therapeutic and immune-modulatory treatments means that in general, PwMS experience a gradual decline in physical and cognitive function, well-being and quality of life.

Chapter 2 Exercise

This Chapter introduces the aims of the thesis describing the principles of exercise and the general health benefits to be gained from participation in it, the limiting factors affecting PwMS, and sets out the definitions and concepts on which the experimental parts of the thesis are based.

2.1 Introduction

The aim of this thesis is to examine acute physiological, central and perceptive responses of PwMS to exercise intensity during both the active phase, and immediately afterwards, through the post-exercise time-course to recovery. The short-term effects of exercise intensity in PwMS are unknown. As detailed in Chapter 1, PwMS are constantly required to adapt to an unpredictable and changeable disease that imposes considerable limitations on their daily activities (Stuifbergen et al., 2006, Soundy et al., 2011). Spasticity, weakness pain and fatigue are ubiquitous characteristics requiring constant management. Hence the intensity of any physical activity and the ability to recover from it are critical considerations. Increased levels of physical activity are proposed to limit the degree of disability experienced by an individual with MS while improving health, function, well-being and quality of life. However, despite its importance as a key component of an exercise prescription, little or no evidence exists relating to the

effects of exercise intensity on PwMS, either during the active phase or during the post-exercise time-course to recovery.

2.2 Exercise for health and rehabilitation

For the general population, evidence linking higher levels of physical activity to increased health benefits is unequivocal (Warburton et al., 2006, Lee et al., 2010). A higher level of physical fitness significantly delays all-cause mortality and reduces morbidity from the four main causes of death in developed countries; cardiovascular disease, cancer, diabetes and stroke (Haskell et al., 2007). Moreover, for people disabled by disease or injury, exercise is recommended as part of an active process that aims to improve their health, maximise participation, increase functional independence and enhance their quality of life (Beer, 2010). One of the primary aims of exercise for PwMS is to reduce disability and impairment, and increase levels of activity, participation and independence (Langdon and Thompson, 1999, Kesselring and Beer, 2005). Health-promoting behaviours such as regular physical exercise present an opportunity for PwMS to participate in the disease management process (Turner et al., 2009). Hence, interventions for PwMS need to be adaptable to the unpredictable changes in clinical status and appropriate to an individual's functional capacity (Kesselring and Beer, 2005, Beer, 2010).

The International Classification of Functioning, Disability and Health model (ICF) developed by the World Health Organisation as a framework for disability and health, acknowledges the complex interaction between an individual's health condition, and personal and environmental factors (Ustun et al., 2003). According to the model, outcomes should be measured at the following levels: body function and structure (impairment), activities and participation. Implicit to the success of a therapeutic programme is the understanding that treatment is fundamentally influenced by an individual's choice (Noseworthy, 2008). In the context of exercise prescription, the clear inference is that exercise programmes should deliver an effective physical, mental or social benefit that encourages participation and adherence. The setting of specific, measurable, realistic and attainable goals is an important issue in this respect (Kesselring and Beer, 2005).

There is a growing body of evidence that exercise is well tolerated by PwMS. Health benefits have been shown to be gained through cycling (Mostert and Kesselring, 2002), aquatic exercises (Gehlsen et al., 1984), aerobic and strength training (Romberg et al., 2004b) and walking (Newman et al., 2007). Exercise training has been shown to significantly improve aerobic fitness, (Petajan et al., 1996), mobility (Snook and Motl, 2009), muscle strength (Broekmans et al., 2011, Dalgas et al., 2009), mood-state and anxiety (Petruzzello et al., 2009). On the other hand, lower levels of physical activity have been associated with the worsening of MS symptoms (Motl et al., 2008).

2.3 Attitudes and barriers to exercise

For PwMS, participating in exercise remains somewhat controversial (Schapiro, 2010). It is not uncommon for PwMS to be advised to avoid participation in strenuous activities in case they provoke a worsening of existing symptoms (Smith et al., 2006), stimulate the onset of new symptoms such as fatigue (Sacco et al., 2011) or facilitate an exacerbation of the disease itself (Sutherland and Andersen, 2001, van Diemen et al., 1992, Rasova et al., 2006). This view remains prevalent in a high number of MS communities (Bjarnadottir et al., 2007), particularly within older age-groups where a high percentage advocate no exercise at all (Turner et al., 2009). As a consequence PwMS engage less in physical activity than non-diseased populations, to such an extent that their sedentary lifestyle has been described as “alarming” even in the context of the general prevalence of physical inactivity and sedentary lifestyle of adults in the USA (Motl et al., 2005). Sedentary behaviour refers to activities that fail to increase energy expenditure substantially above the resting level, such as sleeping, sitting, lying down, and watching television (Pate et al., 2008).

Chronic diseases can have a significant effect on patterns of sedentary behaviour (Chastin and Granat, 2010). A general lack of activity can precipitate a downward spiral leading to a further reduction in fitness and health affecting their function and mobility, independence, well-being and quality of life (QOL). PwMS who are detrained and unused to exercise face increased challenges and health risks when

starting an exercise prescription, especially if they also present with co-morbidities such as cardiovascular-pulmonary disorders or life-style related neuromuscular impairments. Moreover, unpredictable changes to components of fitness either due to the primary disease, or as secondary consequences of enforced physical inactivity can present confusing pictures to clinicians which further complicates the prescription of an appropriate exercise dose (Dawes, 2008b). The special considerations for this group includes spasticity, incoordination, ataxia, muscle weakness, foot drop and visual disturbances (Jackson and Mulcare, 2009a). Further barriers to exercise for PwMS include lack of provision of appropriate fitness facilities, and lack of exercise professionals with expertise in training people with specific neurological conditions (Rimmer et al., 2004).

2.4 General definitions and principles of exercise

Physical activity is traditionally defined as any bodily movement requiring energy metabolism above resting levels (Caspersen et al., 1985). Exercise is a subset of physical activity that is planned, structured and repetitive with the intention of improving or maintaining one or more of the components of health and fitness (Garber et al., 2011). The terms physical activity and exercise are often used interchangeably, although the characteristics of the tasks and resultant health benefits can differ widely.

Physical fitness describes a set of attributes relating to an individual's ability to perform physical activity (Holly and Shaffrath, 2001). It reflects the body's ability to

cope with the demands of activities of daily living, work or during leisure pursuits without experiencing undue fatigue (Caspersen et al., 1985). Each component of physical fitness, whether cardiorespiratory, muscle strength, endurance, flexibility, body-composition or neuro-motor fitness (balance, coordination and proprioception) has an influence on components of health and well-being (Garber et al., 2011). Increased volumes of physical activity are associated with higher levels of physical fitness (Garber et al., 2011), and small improvements in physical fitness are associated with significant reductions in health risks (Erikssen et al., 1998, Myers et al., 2002).

Physical fitness is based on the principle of adaptation, which states that if a specific physiological capacity is taxed by a physical training stimulus within a certain range and on a regular basis, this capacity usually expands (Holly and Shaffrath, 2001). Thus in order to elicit a training response, the body has to be challenged beyond a certain point, the training threshold. The gains and benefits of improved physical fitness are quickly reversed when the overload is withdrawn or training is reduced (Taivassalo and Haller, 2004). Training responses are generally specific to the tasks performed. Both central and physiological systems are involved. Hence, a training programme designed to improve aerobic power must overload the cardiovascular-pulmonary systems (Powers and Howley, 2004a). Anaerobic training normally involves working against resistive loads leading to an increase in muscle contractile proteins, neuromuscular efficiency, and ultimately, gains in strength and power (Kraemer et al., 1988).

Based on the specificity concept, the magnitude of adaptation can vary considerably depending on the mode of training. When considering the health and fitness benefits of exercise for PwMS, the training should provide a sufficient cardiovascular overload that stimulates improvements to optimal cardiac output, by using muscle groups that enhance local circulation and the metabolic machinery. Given that strength deficits may limit the ability of PwMS to exercise at sufficient intensity and duration to produce health benefits (Dalgas et al., 2009), endurance training produces adaptive changes to active muscle and greater improvements to cardiovascular-pulmonary systems. Cardiorespiratory fitness and endurance is essential to maintaining function and independence in most activities of daily living for PwMS. Therefore, this thesis will focus on the acute neurological, physiological and perceptive responses to cardio-respiratory, endurance training.

2.5 Exercise Prescription

To achieve health and fitness benefits, individuals are required to increase their physical activity by changing from a sedentary life-style to one that achieves recommended physical activity levels (Haskell et al., 2007). Health and fitness benefits from exercise are associated with the volume of activity performed (the sum of the frequency, intensity, duration and type of exercise), above the training threshold (Haskell et al., 2007). The relationship between an exercise dose (volume of exercise performed) and the response (health benefit) is generally linear (Powers and Howley, 2004a), suggesting that those PwMS who can tolerate

exercise at higher intensities for longer periods will achieve greater health benefits (Erikssen et al., 1998).

For clinical groups, optimal adaptations to exercise training require the development of, and adherence to a specific programme of activity, an exercise prescription. The prescription will define the potency of an exercise dose, and the frequency required to elicit and maintain a health benefit. However, an individual's response to an exercise dose is difficult to predict. Numerous factors such as age, clinical status, and environmental conditions will influence the response. For example, PwMS often require modifications to exercise intensity due to factors such as spasticity, weakness, pain or optical neuritis.

ACSM's Guidelines for Exercise Testing and Prescription (ACSM, 2010) lists several reasons for altering prescriptions:

- Variance in objective and subjective responses to an exercise session.
- Variance in the amount and rate of exercise training responses
- Differences in goals between individuals
- Variance in behavioural changes relative to the exercise prescription

With respect to PwMS, exercise professionals need to be aware of the various symptoms, and be aware of the effects that such symptoms may have on exercise performance (Jackson and Mulcare, 2009b).

2.6 Exercise intensity

Exercise intensity can be described objectively as the physiological response to physical activity, for example heart rate (Wasserman et al., 2005), or measured as the amount of work being produced, or described subjectively as an individual's perception of the magnitude of the work being performed. Measures of intensity can provide markers that reflect the integrity and compliance of neurological, physiological and perceptive mechanisms to produce and maintain physical exercise.

Intensity can be manipulated either by altering the time to produce a specific volume of exercise, or by altering the amount of exercise performed within a specific time. Regardless of the activity or mode of delivery, exercise intensity and duration are inversely related; the higher the exercise intensity, the shorter the duration that exercise can be sustained (Heyward, 2010). However, whilst intensity is a key component of an exercise prescription, the relationship between exercise intensity and health benefit for PwMS remain unknown.

2.7 Exercise in MS

The ability to work at intensities high enough to induce an adaptation is dependent on the functional state of the cardiovascular and respiratory processes to efficiently deliver oxygen, nutrients and cells via the blood stream, and the ability of skeletal muscle to utilise the oxygen and produce energy (Buckley and Hughes, 2008). An impairment or dysfunction in any of these processes reduces an individual's ability

to function normally. Respiratory dysfunction is a common problem in MS, mainly caused by respiratory muscle weakness (Buyse et al., 1997). In one study of 71 MS patients, respiratory impairment was observed in over 60% of the participants (Grasso et al., 2000), and pulmonary dysfunction in MS has been found present even in the absence of reduced respiratory muscle function (Altintas et al., 2007). Abnormal cardiovascular responses have been detected in a high proportion of PwMS (Anema et al., 1991, Acevedo et al., 2000) with clinical signs, such as atrial fibrillation, linked to the appearance of brain stem lesions (Chagnac et al., 1986, Schroth et al., 1992). Cardiovascular reflex testing of PwMS has revealed abnormalities of both sympathetic and parasympathetic mechanisms of the autonomic nervous system (ANS) (Nasseri et al., 1999). An abnormal disassociation between heart rate and blood pressure, with a profound attenuation of the pressor response, has been reported after a maximum voluntary contraction (Pepin et al., 1996), however differences in muscle strength and muscle mass between MS and healthy controls may explain the blunted pressor response (Ng et al., 2000). For an individual exercise prescription, an exercise dose relative to an individual's maximal exercise capacity is recommended, especially for clinical populations and deconditioned persons (Garber et al., 2011). In the Health and Fitness setting, the most commonly used method to set exercise intensity is to calculate the percentage of predicted maximal heart rate calculated from the formula '220 - age years' (Londeree and Moeschberger, 1984). The accuracy of age predicted maximal heart rate is not supported by all studies (Tanaka et al., 2001). Indeed, the ability to tolerate exercise intensity differs considerably between

individuals, and is dependent on multiple factors, such as resting heart rate, age, mode of exercise, fitness levels and clinical health status (ACSM, 2010).

The extent to which any level of exercise intensity contributes to fatigue or failure in the exercise task is thought to depend on the relative demand placed on each of the mechanisms and processes contributing to the force exerted by the muscle (Maluf et al., 2005). Gender and age are major contributing factors to muscle fatigue onset. For instance, when performing sustained voluntary contractions at low to moderate intensity, women have up to a 50% longer time to task failure, however at higher intensity, muscle fatiguability between the genders is less evident (Hunter and Enoka, 2001) and the difference in time to task failure between genders is reduced with age (Hunter et al., 2004). In PwMS, measurement of muscle function can be complicated by factors such as disease related weakness and spasticity. The precise central components and processes contributing to central drive deficits and muscle weakness in PwMS remain unknown, however the loss of ability to generate force, particularly in the lower limb is consistent with muscle impairment observed in other neurological conditions and spinal cord injury (Ng and Kent-Braun, 1997). In PwMS, long-term disuse has been found to result in lower bone density, atrophy of type I and type II fibres, and decreased enzyme content per fibre in lower-leg muscles similar to that found in hemiplegia (Kent-Braun et al., 1997). Muscle myosin heavy chain (MHC) and fibre-type characteristics in some upper- leg muscles of PwMS have been found to be more similar to those of age-matched sedentary controls with a significant

reduction in average muscle area (Carroll et al., 2005). The lower limb frequently tends to be affected before, and more than the upper limb (de Ruyter et al., 2001) with weakness in muscle groups directly relating to problems performing the activities they control (Iriarte, 1998) and in particular, difficulty generating fast powerful movements due to impaired central activation (Thoumie et al., 2005, Ng et al., 2004).

Two specific problems that may affect the ability of PwMS to generate and maintain a higher work-rate are fatigue and thermosensitivity (Beer, 2010). Exertional fatigue, muscle fatigue and exercise intolerance are shared symptoms of many neurological and non-neurological pathologies that originate in the CNS, the peripheral nervous system (PNS), or both (Chaudhuri and Behan, 2004). Activity related fatigue in MS can be categorised into three entities, fatigue at rest, fatigability (due to physiological, neurological, or psychological stress), and the impact of fatigue on MS symptoms (Iriarte et al., 2000).

There is a high correlation between dysfunction of the pyramidal tract and fatigue in MS (Iriarte et al., 2000, Samii et al., 1996a), although other abnormalities in the CNS may also play an important role (Andreassen et al., 2009). CNS deficits may cause a failure to sufficiently activate motor neurons sufficiently (Gandevia, 2001). Central activation of skeletal muscle has been shown to be impaired in PwMS suffering from fatigue (Andreassen et al., 2009). Some MS patients presenting with chronic fatigue exhibit dysfunction of the Hypothalamic-Pituitary-Adrenal (HPA) axis (Siegert and Abernethy, 2005). Studies have demonstrated that a hyperactive

HPA axis is associated with neurological disability, cognitive impairment and brain atrophy, and may be used as a marker to predict future neuro-degeneration in MS (Heesen et al., 2007).

Many PwMS exhibit a worsening of MS related symptoms with a rise in body heat. The effect is named after Wilhelm Uhthoff (1853-1927) who first identified and reported the phenomenon. In thermo-sensitive PwMS, exacerbations of symptoms can occur rapidly in response to any form of heat exposure, whether it be small changes in core body temperature or full body immersion in hot baths or showers (Guthrie and Nelson, 1995). In fact, the symptoms are so distinct that until 1983 the 'hot bath' test was accepted as a reliable clinical method to diagnose MS (Guthrie, 1951).

It has been postulated that an increase in core body temperature may negatively affect conduction properties of either partly-myelinated, or demyelinated axons in the CNS (Humm et al., 2004). Paradoxically, central conduction of evoked motor potentials in PwMS has been observed to be more affected by cold than by heat (Humm et al., 2004). However, when compared to non-cooled participants during a maximal exercise test, pre-exercise cooling produced both a lower heart rate, and reduced ratings of perceived exertion (White et al., 2000). Clearly, the process of thermoregulation in MS is not well understood. Although heat accumulation does not appear excessive, the inability to thermo-regulate may cause overheating, and

exacerbate deficits in central drive and symptoms of fatigue (Humm et al., 2004, Davis et al., 2005).

Dehydration in MS appears to be associated to disability levels, perhaps reflecting a strategy to manage bladder incontinence (Collett et al., 2011a), yet hydration status, and the effects of dehydration in exercising PwMS are rarely considered. Collett and colleagues (2011), recently reported that 42% of a MS test group (n=26) were inadequately hydrated. Hydration status profoundly affects blood pressure in people with autonomic failure, and substantially affects older people from the general population (Jordan et al., 2000). Even in healthy groups, mild dehydration provokes changes in cardiovascular, metabolic and central nervous system function (Murray, 2007). Higher core temperatures during exercise have been reported in dehydrated individuals (Greenleaf et al., 1971). Dehydration is also associated with higher ratings of fatigue and lower levels of cognitive function (Maughan, 2003). More importantly, during aerobic exercise even of short duration, cardiac output, blood pressure, sweating responsiveness and exercise performance are significantly impaired (Sawka and Coyle, 1999, Maughan, 2003, Montain et al., 1995), hence hydration status remains an important consideration for PwMS

2.8 Benefits of exercise

Cardiorespiratory fitness (CF) describes the ability to perform dynamic, moderate to high intensity activities for prolonged periods and is a strong, independent predictor of all-cause mortality (Blair et al., 1995). CF training in PwMS has been shown to lead to increased oxygen uptake (Petajan et al., 1996, Mostert and Kesselring, 2002), increased functional capacity (Schulz et al., 2004), greater mobility (Killeff and Ashburn, 2005, Romberg et al., 2004b), improved lung function (Mostert and Kesselring, 2002), and improved task performance and cerebrovascular function (Prakash et al., 2007). In addition, higher fitness levels have been linked to lower levels of emotional distress and depression, perhaps due to improved mobility and functionality offering a higher level of behavioural activation and social inclusion (Turner et al., 2009).

Exercise has been shown to induce considerable physiological change in the immune system (Pedersen and Hoffman-Goetz, 2000). CD4+ cells decline after intensive cycling (Pedersen et al., 1988), perhaps due to the increase in natural killer cells which are significant mediators of cytotoxic cells in the innate immune system (Mackinnon, 1989), and potentially raise resistance to viral infections (Pedersen and Hoffman-Goetz, 2000). In animal models, angiogenesis (the growth of new blood vessels) is the primary effect of endurance training in the motor cortex (Swain et al., 2003) and the hippocampus (Ding et al., 2006), perhaps in order to meet the increased metabolic demands of cortical neurons (Adkins et al., 2006). Voluntary wheel running increases concentrations of micro-glia and

astrocytes in mice, and may improve brain function (Ehninger and Kempermann, 2003). Animal models have also shown that exercise increases both brain derived neurotrophic factor (BDNF), and insulin like growth factor (IGF-1) in the CNS (Cotman et al., 2007), both mechanisms which may enhance learning and cortical plasticity (Berchtold et al., 2002). Rodent brains have shown enhanced neurogenesis in response to increased neural progenitor cells (van Praag et al., 1999). Furthermore, exercise accelerates both the growth, and enhances the density, of dendritic spines which support changes in synaptic strength (van Praag, 2009, van Praag et al., 1999), hence facilitating neuroplasticity and adaptation.

2.9 Exercise recommendations for PwMS

For many years, exercise recommendations for PwMS have followed the American College of Sports Medicine(ACSM) guidelines for exercise testing and prescription, sometimes with modifications based on the authors' experience, or others (White and Dressendorfer, 2004). However, when considering the heterogeneous and changeable nature of MS, exercise recommendations can serve only as basic guidelines. Table 2.1 shows the published exercise recommendations for PwMS, outlining the frequency, duration and intensity, and preferred mode.

Perhaps the most cited paper is that by Petajan and colleagues who randomly assigned 54 PwMS to an exercise programme consisting of 40 minute sessions of

Table 2.1 Recommendations for exercise programmes for people with MS

Author	Frequency (sessions per week)	Duration (minutes)	Exercise intensity	Mode	RPE
Wichmann, R; Hulme, J.B. (2008)	3	30-60	60-70% HRpeak	Treadmill, Exercise bike Rowing	N/A
Kraft, G.H. Catanzaro, M (2000)	3	20	N/A	Treadmill, Exercise bike Swimming	N/A
Mulcare, J. Jackson, K. (2004)	3	30	50-75% HRpredmax	Exercise bike	11-13 (6-20 scale)
Plow, M. Motl, R. (2013)	3	30	60-85% HRmax	Aerobic exercise	NA
Hutchinson, B. Hicks, R. (2005)	3-5	15-60	55-90% HRmax	Walking, Cycling Swimming	N/A
White, L. Dressendorfer, R. (2004)	2-3	20-30 (or 2x15)	65-75% HRpeak	Walking, Cycling Swimming	11-14 (6-20 scale)
Dalgas et al (2008)	2-3	10-40	60-80% HRmax	Walking, Cycling Swimming	N/A
Carroll, C. Lambert, C. (2009)	At least 3	30	60-75% HRpeak	Walking, Cycling Swimming	N/A
Note; N/A = not available					

combined arm and leg ergometry, 2-3 times a week for 15 weeks. Participants performed five minutes of exercise at 30% of $\dot{V}O_2$ max followed by 30 minutes at 60% of $\dot{V}O_2$ max. When compared against base-line measures, the exercise group displayed a significant improvement in maximal aerobic capacity, upper and lower body strength together with improvements in bladder and bowel function, fatigue, mood-state and factors relating to QOL (Petajan et al., 1996). Importantly, over the course of the intervention, 7 of the 54 participants experienced a 'noteworthy' exacerbation of MS symptoms requiring clinical treatment, and 2 participants' exacerbations were severe enough to preclude further participation (Petajan et al., 1996). Nevertheless, based on the results of the study, the authors recommended that PwMS should be encouraged to engage in regular aerobic activity.

Rietberg et al (2004) reviewed 2593 titles on behalf of the Cochrane Collaboration to formally assess exercise based rehabilitation programmes. Interestingly, although the Petajan paper was one of only 9 that fulfilled their inclusion criteria, only the study of Mostert and Kesselring (2002), was cited as describing evidence of deleterious effects after exercise testing, and no evidence of deleterious effects of exercise was found in the included studies. In the Mostert study, 13 PwMS trained for 3-4 weeks, for up to 5 sessions per week lasting 30 minutes performing aerobic exercise on a cycle ergometer at an unspecified intensity. Interestingly, although only 2 participants withdrew from the study, they reported a low adherence rate by the remaining subjects to the exercise programme. In contrast to Petajan et al, they found no measurable improvement in aerobic capacity.

However they concluded that an increased level of physical activity lead to an improved perception of health status, and recommended that regular aerobic exercise should be part of future inpatient rehabilitation programmes (Mostert and Kesselring, 2002).

In 2007, Rampello et al followed the same exercise protocol proposed by Petajan et al. 19 PwMS performed three supervised 60 minute training sessions per week for 8 weeks on a cycle ergometer at 60% maximal work rate (watts) calculated from a GXT. Intensity was progressively increased every week up to 80% of maximum work rate. There were significant increases in VO_2 peak and maximal work rate. However, eight participants (26%) failed to finish the study. The exercise programme induced a perception of breathlessness and fatigue in 2 PwMS, which persisted up to the beginning of the next exercise session, and they subsequently withdrew from the study. Two more participants withdrew because they felt the exercise programme was too stressful. Four subjects had a relapse of MS and could not complete the study, though whether this was also the result of the exercise programme is not discussed. Whilst the authors conceded that the high rate of participant loss implied a limited exercise tolerance in their MS group, and accepted that the exercise programme may have harmed some of their participants, they concluded that their results supported exercise training may be beneficial to those patients who do not experience an exacerbation of symptoms.

More recently, a systematic review by Asano et al, (2009) examined the role of exercise in MS. Specifically, the objective was to estimate the effect of prescribed exercise and to assess the methodological quality of interventions specifically prescribed exercise, exercise therapy and physical activity. Over two hundred publications were screened with eleven studies meeting the criteria (DeBolt and McCubbin, 2004, Oken et al., 2004, Romberg et al., 2004b, Schulz et al., 2004, Petajan et al., 1996, van den Berg et al., 2006, Surakka et al., 2004, Storr et al., 2006, Mostert and Kesselring, 2002, Harvey et al., 1999, Rampello et al., 2007). The methodological quality of the studies, assessed using the Physiotherapy Evidence Database (PEDro), scored from fair to good. The most common type of exercise intervention was aerobic (walking or cycling). The duration of the exercise interventions ranged from 30-90 minutes a session, with a frequency of 1-5 sessions a week for 3 weeks to six months. While there was some evidence to support the positive effects of exercise, the reviewers concluded there was insufficient evidence to guide a comprehensive exercise intervention or to provide an exercise recommendation for an individual with MS (Asano et al., 2009).

The Asano et al (2009) review was in broad agreement with the reviews of Rietberg et al (2004) and Dalgas et al, (2008). There was general consensus amongst the reviewers that future exercise interventions for PwMS needed to provide better evidence for the optimal dose of exercise prescriptions, in particular, exercise intensity (Rietberg et al., 2005, Dalgas et al., 2008, Asano et al., 2009).

Only recently has a study controlled for intensity as an independent component of exercise volume. Collett and colleagues (2011) conducted a clinical trial to evaluate the effect of exercise intensity over a three month period. They randomly allocated 54 PwMS to 3 different exercise groups. Individuals cycled for 20minutes at either 45% or 90% (30s cycling interspersed with 30s rest) of their peak work rate determined by a symptom-limited graded exercise test (GXT). A third group performed 10 minutes of the 90% protocol followed by 10 minutes at 45%. The cadence was set at 50 rpm for all interventions. Thus, although individuals performed exercise at different intensities, the total relative volume of exercise performed was the same across the 3 groups. Of note is that only 53% of participants in the combined exercise group completed the 12 week programme (against 95 % of the continual lighter work-rate). The investigators observed that when taking into account the number of adverse events, frequency of attendance and the number of withdrawals during the 12 week training programme, individuals tolerated the low-intensity exercise programme best, but remained cautionary about making recommendations on exercise intensity (Collett et al., 2011b).

In summary, PwMS live with uncertainty regarding their functional ability to perform basic activities of daily living. Exercise is an attractive, low-cost intervention that improves cardio-respiratory fitness levels, mobility, self-esteem and confidence, reduces depression and anxiety(Dawes, 2008a), and may lead to a reduction of symptoms that contribute to the functional deficits in PwMS (Motl and Goldman, 2011). However, compliance and adherence to exercise programmes at

recommended exercise intensities (Table 2.) are low (Mostert and Kesselring, 2002, Collett et al., 2011b) which imply that the recommended exercise intensity for PwMS may be set too high. There is a paucity of literature relating to exercise intensity for PwMS and the acute physiological, neurological and perceptive responses to intensity remain unreported. The implications are that little or no information exists to steer safe, effective and appropriate exercise strategies, to optimise training outcome, and engender participation.

2.10 Aim of the thesis

The aim of the thesis is to examine the acute physiological, central and perceptive responses of PwMS to exercise intensity, through both the exercise phase and the post-exercise time-course to recovery.

Specifically this work will

1. Compare maximal exercise capacity in PwMS and controls
2. Examine and compare the physiological and differential perceptive responses to exercise
3. Examine the central, physiological and perceptive responses during the post-exercise recovery period in PwMS
4. Compare 2 different exercise intensities

This thesis starts by detailing the methods used to measure exercise intensity and reviews the techniques used to assess physiological and perceptive responses. It

is important that the measures are reliable, responsive, and appropriate for PwMS. Chapter 4 explores responses to maximal exercise intensity during a graded exercise test. Chapter 5 describes the methodology of transcranial magnetic stimulation to explore central motor pathways. Chapter 6 explores the reliability of motor evoked potentials (MEPs) recorded from the lower limb of PwMS. Chapter 7 examines physiological, perceptive and corticospinal responses to intensity during 20 minutes of exercise, and for 35 minutes during the post-exercise time course to recovery.

Chapter 3 Methods and materials

The aim of this chapter is to describe and determine the suitability of the methods and materials used for the experimental chapters of this thesis.

3.1 Introduction

Evaluating the outcome of exercise interventions at any stage of MS is not only extremely challenging, but also of the greatest importance to improve the process and impact of exercise prescription and rehabilitation (Thompson, 2008). In view of the complex, unpredictable and heterogeneous nature of the disease, clinically and scientifically robust measurement tools are essential for assessing responses to exercise intensity in PwMS. The need to measure the outcome and effectiveness of therapeutic treatments and interventions is accepted as being essential to good practise in research. Reliability, validity and administrative burden are properties of measurement instruments that affect the credibility of the measurement process and the reporting of such research findings (Salter, 2005). Specifically, the methods used should be reliable, reproducible, responsive and appropriate.

Reliability refers to the quality of reproducibility and relates to the consistency and repeatability of a measure (Weir, 2005, Batterham and George, 2000).

Measurement error, whether random (eg noise or physiological adaptation), or systemic (eg bias resulting from learning through prior exposure to the measurement task) is present in all tests and measurements. The reliability of a measure is the degree to which it is free from random error. If no random error is present, the reliability is 1.0. The closer the number is to zero, the more random error increases (Cano and Thompson, 2010). A reliable measure provides confidence that changes in measurement parameters are due to natural changes within the subjects and not due to error in the test, the instrumentation or indeed error in the measures themselves (Weir, 2005).

Responsiveness reflects the ability to discriminate between values, and is a particularly important measure when evaluating change over time (Cano and Thompson, 2010). The two major components of responsiveness are 1) internal responsiveness; the ability of the method to detect change over time, and 2) external responsiveness, the extent to which a change in a measure relates to a reference measure that is regarded as an accepted indication of change (Cano and Thompson, 2010).

Determining the consistency and reproducibility of methods remains an essential part of methodological validation. Validity refers to the appropriateness and accuracy of the method to measure the desired parameter. Concurrent validity infers that a test correlates well to a previously validated measure. Conclusion validity relates to the utilisation of appropriate statistical tests, adequate sampling

and reliable measurement procedures. Standing procedures for each test must be rigorously adhered to in order to minimise inconsistencies and to ensure accuracy and reproducibility.

3.2 Research setting

The research was carried out at the Clinical Exercise and Rehabilitation (CLEAR) Unit, The Human Performance Centre, Oxford Brookes University, Oxford UK, and the Nuffield Centre of Enablement, Oxford.

3.3 Performance measures: Intensity

During exercise, work rate (or power) is the function of force and velocity (Enoka, 2002) describing the amount of work accomplished per unit of time. Exercise intensity can be described in absolute terms;` such as power output (watts), oxygen consumption per minute ($\dot{V}O_2$ max L.min⁻¹), heart rate (HR), blood lactate (Bla), or energy expenditure such as calories or metabolic equivalents (METs). It can also be described as a subjective measure that reflects a participant's rating of their perception of exertion (RPE). The following sections describe and determine the suitability of the methods and materials used for the experimental chapters of this thesis.

3.3.1 Measuring intensity; The mode of exercise

The treadmill and the cycle ergometer are the most commonly used devices in clinical exercise testing (ACSM, 2002). The principal difference between the two

test modes is that for most individuals, peak oxygen consumption ($\dot{V}O_2$) is approximately 5% to 20% lower on the cycle ergometer (Visich and Ehrman, 2009) even though maximum heart rate and blood lactate have been shown to be similar after tests on both (Wasserman et al., 2005).

The main disadvantage of using a treadmill for exercise testing is quantifying the work rate (watts). Moreover, a poor tolerance to treadmill exercise has been related to increased energy cost in PwMS (Olgiati et al., 1986). Ambulatory deficits in MS, such as foot-drop and other signs of paresis, can develop during walking. However, holding on to any part of the treadmill for support during a test, either due to impaired balance or gait problems, eliminates use of the arms causing a consequential reduction in work rate and interfering with the accurate determination of $\dot{V}O_2$.

The advantage of a cycle ergometer for PwMS includes the precise estimation of work rate, and greater safety (Wasserman, 2005), especially for those with orthopaedic, or neurological limitations that restrict weight bearing activity (Pina et al., 1995). On the cycle-ergometer, balance is not a factor since participants are seated in a fixed position on a stable construct and the upper body remains relatively quiet. The pedalling movement is complex enough to provide a functionally relevant test as the combined mechanical work of the legs has to be

sufficient to overcome the resistive load, therefore work production can be easily controlled and calculated.

Resistance is applied to the flywheel by a belt or cord. Adding weight to the weight-plate increases the friction on the flywheel. The work rate is calculated from the formula;

Power = weight (kg) x distance travelled by the flywheel (6m) x rpm =kgm.min⁻¹
(ACSM, 2000)

Arm ergometry offers an alternative mode for assessing maximal exercise capacity in people with gait deficits. However, a study of 24 MS patients, Foglio et al (1994) reported that one-third of the subjects were unable to participate in the arm ergometer exercise, either due to the lack of upper-limb coordination, or because of inadequate upper-body strength (Foglio et al., 1994). Moreover, peak $\dot{V}O_2$ achieved from an arm ergometer test has been reported to be about 70% that of cycle ergometer test, and 89% to 95% of the maximal values achieved during a treadmill walking test (Wasserman et al., 2005) .

A cycle ergometer can be calibrated by various methods. The standard method of calibrating a mechanically braked cycle ergometer is to attach a calibrated weight to the balance spring, and to check that the weight corresponds to the pendulum weight scale (Howley and Don-Franks, 2007). In the absence of calibration

devices, human 'biological' calibration can be performed (Cooper and Storer, 2001). When performed on a regular basis, dynamic 'human' calibration can indicate the reproducibility of physiological variables at standardised work rates, thus confirming a degree of ergometer calibration and reproducibility (Cooper and Storer, 2001). A limitation is that 'biological' calibration will not detect small changes in ergometer calibration, however it does indicate larger errors that can be dealt with subsequently.

Cycle ergometry has been used extensively to investigate the exercise capacity of PwMS (Petajan et al., 1996, Romberg et al., 2004a, Prakash et al., 2010). There is no specific test that can be considered to be a valid measure of both power and capacity because different protocols are required to measure different components of high intensity performance (Smith, 1987). In view of the broad range of physical and cognitive abilities of PwMS, a cycle ergometer was selected both as the most appropriate mode for testing maximal exercise capacity, and for conducting further exercise sessions at intensities relative to peak exercise values (White and Dressendorfer, 2004).

3.5 Measurement of Intensity; Oxygen consumption ($\dot{V}O_2$)

During steady state exercise the oxygen (O_2) consumed by all tissues is termed $\dot{V}O_2$ (Cooper and Storer, 2001). O_2 uptake changes linearly with incremental increases in workload. $\dot{V}O_{2\max}$ describes the highest oxygen uptake that an individual can obtain and consume during dynamic exercise (Astrand and Rodahl,

1986, Bassett and Howley, 2000) and is a criterion measure of the cardiovascular-pulmonary system's ability to increase heart rate and deliver oxygen to working muscle in both healthy and clinical groups (Wasserman et al., 2005, Heyward, 2010).

The value of a graded exercise test (GXT) to evaluate cardiovascular fitness and functional capacity is well established both in the general population (Holly and Shaffrath, 2001) and in PwMS (Jackson and Mulcare, 2009b). The results guide decisions relating to the delivery of medical and therapeutic treatment across a broad spectrum of clinical conditions. Traditionally, $\dot{V}O_2$ max is defined by the reaching of a plateau where oxygen consumption fails to increase, despite an increase in workload (ACSM, 2000). However, an individual's ability to reach the plateau during maximal exercise testing is highly variable (Day et al., 2003). Even in healthy subjects, about one-third exercising at maximal effort actually reach the $\dot{V}O_2$ plateau in oxygen consumption, mainly because of the inability to endure the physical discomfort (Wasserman et al., 2005). Therefore, the highest measure of oxygen consumption achieved during an exercise test is defined as $\dot{V}O_2$ peak regardless of whether or not a plateau is reached (Heyward, 2010). In reality, although $\dot{V}O_2$ peak does not fully satisfy the definition of $\dot{V}O_2$ max, it is usually equivalent to the $\dot{V}O_2$ max of healthy subjects (Wasserman et al., 2005) and the values can be construed as the same (Mulcare and Jackson, 2004).

In general, 70-85% of the limitation in $\dot{V}O_2$ max can be attributed to maximum cardiac output (Cerretelli and diPamprero, 1987) and skeletal muscle blood flow (Saltin and Calbet, 2006). The limiting factor for $\dot{V}O_2$ max in PwMS and other neurological populations is less clear. Some MS studies have reported that $\dot{V}O_2$ peak is reduced in PwMS with mild to moderate disability (Romberg et al., 2004a) while others have found that reduced $\dot{V}O_2$ peak is related to respiratory muscle function rather than to disability (Foglio et al., 1994). Most studies show that the expiratory muscles of PwMS suffer more weakness than inspiratory muscles (Buyse et al., 1997, Tantucci et al., 1994). Indeed, pulmonary dysfunction can be present in MS even in the absence of any respiratory symptoms (Altintas et al., 2007). Peripheral factors that limit $\dot{V}O_2$ max include reduced central drive, and decreased muscle diffusion capacity, mitochondrial enzyme levels and capillary density (Bassett & Howley 2000). Furthermore, clinical perturbations or complications as a result of co-pathologies may prevent PwMS from attaining the criteria for attaining $\dot{V}O_2$ max during a graded exercise test. Mono-paresis, leg or chest pain, dyspnea (shortage of breath) or lack of motivation may also cause PwMS to stop exercising (White and Dressendorfer, 2005). $\dot{V}O_2$ can also be prone to inherent variation due to anxiety and the psychological state of the individual (Sutherland, 2005). Anxiety may manifest as dyspnea (Gilman and Banzett, 2009) or panic disorder leading to hyperventilation (Sansone and

Sansone, 2009). These are important considerations when exercise testing PwMS as both anxiety and reduced cardiorespiratory fitness are frequently present.

For a given individual, measured oxygen consumption at a given work rate has been shown to be highly reproducible (ACSM, 2000). Hence a symptom-limited test to the limit of tolerance facilitates the identification of specific physiological limitations (Cooper and Storer, 2001) and is a valid index of $\dot{V}O_2$ peak. As $\dot{V}O_2$ depends to a large extent on body size, it is generally reported in terms relative to body weight ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$).

For the purpose of this study, O_2 and carbon dioxide (CO_2) content of the expired air was measured using a breath-by-breath gas analyser (Metamax 3B, Cortex Biophysik GmbH, Leipzig, Germany) via a face mask worn by the participant (Hans Rudolf, Kansas City, USA). The Metamax has been shown to be a reliable tool in healthy young adults (Meyer et al., 2001) and has been used widely for exercise testing in sports and clinical research settings. Prior to each test session the system was calibrated according to manufacturer's instructions using standard calibration gas (~15% O_2 and ~ 5% CO_2 , balance N_2). A three litre pump syringe was used to calibrate the flow sensor to determine the volume of expired air. Calculation of VO_2 consumed and carbon dioxide (VCO_2) produced per minute was through Haldane equations using the volume of expired air per minute (VE) and the

atmospheric pressure. The data was recorded and analysed via online telemetry to a personal computer.

3.7 Measurement of intensity. Respiratory exchange ratio

A product of $\dot{V}O_2$ max testing is the respiratory exchange ratio (RER). During exercise the amount of chemical energy released by each oxygen molecule is dependent on the fuel substrate used. Generally, protein plays a small role as a substrate during physical activity, thus the value of the respiratory quotient (RQ) reflects the relative amount of carbohydrate (CHO) and fat metabolised (Powers and Howley, 2004b). During aerobic exercise at submaximal levels an RQ of 0.7 indicates a reliance on fat metabolism, RQ \sim 0.85 represents a condition where fat and CHO are contributing to energy production in equal amounts and RQ of 1.0 indicates the metabolism of primarily CHO. The RER reflects the respiratory exchange of CO₂ and O₂ and commonly exceeds 1.0 during exercise (Ross, 2003).

Notwithstanding the altered muscle characteristics of PwMS, it has been identified that the high individual variability of muscle composition and their unique firing patterns during dynamic exercise affects O₂ kinetics independent of RER (Kushmerick et al., 1992). Indeed, even dietary-induced metabolic adaptations alter RQ kinetics. A high-fat diet increases the contribution of fat oxidation, while conversely, a predominantly CHO diet increases the range of CHO metabolism

(Mole and Hoffmann, 1999). Muscles are able to extract CHO even from a low-CHO diet, suggesting that muscle may utilise stored muscle glycogen as an energy source even at low intensity exercise (Sue et al., 1989). At 75% of $\dot{V}O_2$ max, it is quite possible for one individual to be reliant on entirely aerobic sources while another requires considerable anaerobic supplementation (Ekkekakis and Acevedo, 2006).

In order for RER to be used as an estimate of substrate utilisation, an individual must have reached a steady physiological state where blood and gas transport systems are keeping pace with tissue metabolism. During non-steady-state strenuous exercise, hyperventilation and the increased buffering of blood lactate causes the volume of VE to rise, thus altering the ratio of $\dot{V}CO_2$ to $\dot{V}O_2$. In practise, achieving a steady state may not be possible in impaired PwMS. Nevertheless, RER is considered to be a measurement parameter of maximal effort and a value of 1.15 remains a criterion for achieving true $\dot{V}O_2$ max (O'Connor et al., 2009).

3.6 Measurement of intensity. Metabolic equivalents

Metabolic equivalents (METs) are multiples of resting metabolic levels often used to quantify the intensity of physical activities in the clinical rehabilitation setting (Holly and Shaffrath, 2001). One MET is defined as the energy cost at rest. The higher the intensity of work, the greater the number of METs required to perform an activity. A major difficulty with using METs in the research setting is that individuals

vary widely in their functional capacity (Ferguson et al., 2001). The relative METs calculated for certain activities may be above the maximal capabilities of some individuals (Beale et al., 2010), especially when factors relating to skill, age, variations in fitness, cognitive and clinical status are taken into account. A recent position statement on exercise intensity terminology has suggested that absolute MET values are appropriate only for healthy adults up to the age of about 30 years (Norton et al., 2010) . Based on current evidence METs are not suitable measures for this study.

3.7 Measurement of intensity. Heart rate (HR)

The most frequently used method to measure exercise intensity in both clinical and exercise settings is heart rate (HR). It is well established that HR increases linearly with increases in oxygen uptake and exercise intensity, regardless of age and gender (Astrand et al, 2003). Because of the linear relationship between exercise intensity and HR, training intensities can be set by using HR values equivalent to a percentage of $\dot{V}O_2$ peak (ACSM, 2010). As with $\dot{V}O_2$ peak measurements the HR response is dependent to some extent on the mode of testing. Nevertheless, heart rate is widely used as a criterion for achieving peak exertion in the determination of maximal aerobic capacity (Wasserman et al., 2005)

Tachycardia is essential to maintain increased cardiac output. HR and stroke volume are responsible for the increase in cardiac output during exercise, however stroke volume does not increase beyond a work intensity equivalent to 40% of $\dot{V}O_2$

peak in untrained individuals and thereafter, the rise in cardiac output is achieved by increases in HR alone. As with VO_2max measures, HR may fail to increase despite an increase in work-load, in which case HR_{peak} values are recorded. Additionally, maximal cardiac output is not reached during sub-maximal exercise or during peak leg exercise on a recumbent cycle-ergometer (Stenberg et al., 1967). Submaximal exercise tests are often based on the assumption that age predicted HR_{max} values for individuals of a given age are accurate (Londeree and Moeschberger, 1984, Tanaka et al., 2001). However, Ponichtera-Mulcare et al, tested 20 ambulatory PwMS (MS1) and 8 semi-ambulatory (MS2) to volitional fatigue on a cycle ergometer and found mean maximal heart rates reflecting 87% (MS1) and 81% (MS2) of age predicted HR_{max} respectively for the two groups (Ponichtera-Mulcare et al., 1994). Even in the general population, up to 7% of men and women have an HR_{max} of 15bpm below age predicted HR_{max}, and up to 13% have HR_{max} that exceed age predicted max by 15bpm (Whaley et al., 1992).

In the absence of a maximal exercise test, exercise intensities are generally based on the Karvonen equation, or percentage of heart rate reserve (HRR) method, which takes into account an individual's resting HR (Karvonen and Vuorimaa, 1988). HRR is often recommended as a more appropriate method of setting exercise intensity for PwMS (ACSM) however, large errors may occur in age-predicted HRR. For that reason, intensities based solely on a percentage of HR_{max} or HRR are also likely to impose variable cardiovascular and metabolic demands in

PwMS, especially those with chronotropic incompetence and co-pathologies (Dwyer et al., 1994, Beale et al., 2010).

3.8 The Consistency of Resting HR in people with multiple sclerosis

One of the themes of this thesis is to assess the effects of exercise intensity on heart rate in PwMS. Resting HR reflects both the status of the cardiovascular system and acts as an indicator of autonomic tone (Thayer and Lane, 2007). Heart rate may increase in individuals who decrease their activity levels due to illness. Indeed, recent large scale epidemiological studies have shown that resting HR is an independent predictor of cardiovascular and all-cause mortality (Diaz et al., 2005) Due to the well documented inter-individual variability of HR in people with MS, the effects of their cognitive and emotional state on chronotropic competence, and taking into account environmental factors, there was a need to establish the validity and reliability of utilising measures of resting HR in this population.

3.8.1 Test Procedure

Testing was carried out in the Oxford Centre for Enablement, Oxford, UK. Participants were asked to refrain from the consumption of tea, caffeine, alcohol, cigarettes, for a period of at least 2 hours prior to testing, and not to take part in strenuous physical exercise for 24 hours prior to testing. The test session procedures were carried out by the same investigators, between 1200 and 1500hrs, in a quiet room at standard temperature (~21°C). They were required to lie semi-supine on a two-section medical examination plinth set so that their upper

bodies were angled to 30° of an up-right position. Resting HR was recorded with using HR monitor technology (Polar Electro, Finland) at 5 minute intervals for 20 minutes. The test was repeated 3 times within 21 days.

3.8.2 Analysis

Data was imported into SPSS V 17.0 (SPSS inc, Chicago USA) software for statistical analysis. Repeated measures analysis of variance (ANOVA) was used to determine differences in resting HR within, and between sessions

3.8.3 Results

Mean HR (\pm SD) for each session is shown in Table 3.1. Repeated measures ANOVAs revealed no differences in the resting HR in PwMS, either within or between sessions ($p>0.05$).

Table 3.1 Mean resting heart rates during 3 sessions (7-21 day apart)

<i>n</i> =12	Session 1	Session 2	Session 3
Heart rate (beats.min⁻¹)	64.3 \pm 9.3	64.7 \pm 8.0	64.3 \pm 9.0
Range	50.8 - 85.8	56.0 – 82.8	50.8 – 82.0

3.8.4 Conclusion

Resting HR is a stable and reliable measure in PwMS.

3.9 Measures of Intensity, Blood lactate

Although measures of blood lactate are commonly used as a means of calculating metabolic responses to exercise intensity, there is a paucity of literature relating to blood lactate in PwMS. The physiological basis for the use of blood lactate is based on muscle biochemistry and its part in blood lactate accumulation. Lactate concentrations increase as exercise intensity increases. The rise in blood lactate concentrations may occur either due to an increase in lactic acid production, or a decrease in lactic acid removal (Powers and Howley, 2004b) however considerable controversy exists over the precise mechanisms that lead to the rise in blood lactate levels.

Measurement of blood lactate is a useful aid for assessing exercise performance although the accumulation of lactate is not held to be a causative factor in fatigue. Measures between groups of PwMS and age and gender matched controls during maximal testing have shown little difference between the two groups (Morrison et al., 2008). Although a plateau in lactate values signifies the attainment of a true $\dot{V}O_2$ max, 8mmol. L^{-1} is accepted as a reasonable criterion value (O'Connor et al., 2009). The Lactate Pro is a portable blood lactate analyser that uses the electrochemical method of measuring enzymatic reactions on a small electrode by capillary action directly from a fingertip. It has been shown to be a reliable method of measuring blood lactate concentrations (Shimojo et al., 1993). Only a small

sample (5 µL) of blood is required, which is an advantage when multiple specimens are required from a single individual, but contains a greater risk of contamination from sweat in such a small sample. Therefore an alcohol-saturated tissue prior is used to remove oil, dirt and sweat from the skin before each sample. The skin should be allowed to dry prior to each specimen of blood being collected. The Lactate pro is supplied with calibration strips to ensure accurate function.

3.10 Rating of Perceived Exertion (RPE) scale

3.10.1 Introduction

In PwMS, the measurement of perceptual responses to exercise intensity provides a valuable and reliable indicator of exercise tolerance (Morrison et al., 2008) and physical functional (Fry and Pfalzer, 2008). Subjective measures are characterized by the fact that conscious awareness is indexed by a subject's self-reports of their perceptual experiences (Merikle, 1992). The conscious signal of exertion is neither purely muscular nor purely central but a combination of both that can reliably adapt behaviour to physiological capacity (Cabanac, 2006). Responses to exercise intensity are generally held to represent the complex interaction of multiple feedback signals from heart, lungs, muscle, joints and skin in response to effort, stress or discomfort interpreted from the body during physical exercise (Williamson et al., 2006). There is strong empirical evidence that perception of exertion grows exponentially with regard to increases in force, workload, O₂ consumption, HR and blood lactate levels across a variety of exercise modalities and conditions (Noble

and Robertson, 1996). More recently it has been proposed that corollary discharge pathways act at all levels of sensory processing (Poulet and Hedwig, 2007) and that all motor signals are generated in circuits engaged in sensory processing, whether or not they are directly engaged in generating a motor action or involved in coordinating different components of the motor pathways (Poulet and Hedwig, 2007).

Neurophysiological evidence for the role of corollary discharge in humans is lacking, however it is generally agreed that the central integration of sensory feedback is designed to support different types of physical activity, at different levels of intensity over a variety of time courses. It is plausible that both the central response to exercise and perceptions of exertion may share common motor pathways, but act independently in coordinating responses (Sommer and Wurtz, 2002, Williamson, 2010). For instance it has been shown that perception of effort can affect the cardiovascular response independent of exercise (Gandevia et al., 1993). It has been proposed that the CNS may anticipate the generated perception of effort involved, and displays a forward model of sensory- motor processing that predicts sensory feed-back (Wolpert and Flanagan, 2001). The issue of corollary discharge is a current 'hot topic' generating considerable debate, possibly indicating that perception of effort and sensory feedback is a highly complex process that is not fully understood.

Scales of perception are based on Steven's law of psychophysics which holds that the magnitude of sensation grows as a power function of the stimulus magnitude.

The general rule is that a constant percentage change, or a just noticeable difference in a stimulus produces a constant percentage change in a sensed effect (Stevens, 1986) . Magnitude estimation requires participants to assign numbers to stimuli of different intensities in such a way that the numbers match the perceived intensities and vary directly in proportion to them. Stevens proposed that the intensity of the sensation related to the physical magnitude of the stimulus proportional to the neural activity in the sensory pathway.

Gunnar Borg, recognising the importance of relating numbers to verbal descriptors and 'landmarks' (Fuhr et al., 2001), developed an interval (or ratio) scale that reflected individualised ratings of the magnitude of subjective intensity during physical exertion (Borg, 1982). The visual analogue scale uses verbal anchors corresponding to numerical values. Perceptions of exertion are separated into categories according to descriptors of intensity and a number assigned to each. The verbal anchors therefore determine the relationship between physical intensity and perception of exertion.

3.10.2 Borg's Rating of Perceived Exertion Scale (RPE)

The RPE scale was initially constructed as a 15 point scale from 6-20 to reflect the range of heart rate (HR) in a healthy group of young subjects (60 beats/min at rest to 200 beats/min) during maximal exercise on a cycle ergometer. The ratio scaling method showed that the perception of exertion grew with the physical work load (Borg, 1990). When it became clear that the CR-16 scale was not appropriate for

all participants, exercise modes or environmental conditions, the scale was revised to the CR-10 scale, making it applicable to a variety of physical functions and physiological responses (Borg, 1990). Differential ratings are distinct measures of perceived exertion relating to specific functions or parts of the body. For example, during a cycle test, RPE measures relating to the sensation of breathing and sensations relating to muscular in the legs can be recorded. The RPE CR10 scale has shown reasonable validity, sensitivity and reliability as a subjective measure in the healthy population, independent of the mode of exercise and fitness of participants.

3.10.3 RPE in MS

In PwMS, Morrison et al (2008) compared perceptive responses from a group of PwMS (n=12) to a group of age matched sedentary adults during an incremental cycle-ergometer maximal exercise test. RPE was recorded every 30 seconds using the modified 10-point Borg scale. There were no significant differences between the two groups in the means of RPE measured at during each stage or at 100% of VO_{2max} . Enjoyment of a physical activity has been shown to be determinant of physical behaviour, and greater adherence to an exercise prescription in PwMS (McAuley et al., 2007). RPE levels during 30 minutes of exercise at moderate intensity (60% VO_{2max}) have been shown to be significantly lower in PwMS if participants are pre-cooled, either in a cold bath (Wilson et al., 2002), or through

wearing cooling vests(Meyer-Heim et al., 2007) however, uncertainty remains whether the reduced RPE was due to lower heart-rates during the exercise, or the pre-exercise cooling having a placebo effect (White et al., 2000). A 20 minute cold bath prior to two 10-minute walks interspersed with a 30-minute rest did not reduce RPE during ambulation (Chiara et al., 1998).

RPE has been shown to be reliable measure for the 6 Minute Walk Test, Functional Stair Test, Static Standing Balance Test and Sit to Stand Test in PwMS (Fry and Pfalzer, 2008). However, subjects rated RPE consistently each visit, RPE did not always correlate well with performance (Fry and Pfalzer, 2008). The RPE scale been used to investigated central motor drive, as measured by transcranial magnetic stimulation (TMS) and perception of effort during sub-maximal finger abduction (Thickbroom et al., 2006).

The CR10's simplicity makes it appropriate for use by individuals with cognitive impairments such as brain injury. It enables determination of both relative and absolute subjective levels (Noble,1983). The CR10 has demonstrated reasonable inter-individual reliability and good correlation co-efficients ($r=0.91$) with HR Blood lactate and percentages of peak power. The scale has been shown to be sensitive in discriminating between different sensory descriptors in relation to percentages of peak power during cycling (Hamilton 1996). However, great care needs to be taken when recording RPE from neurological groups. People with cognitive impairment

find it difficult to 'dual task'. Thus, giving additional cognitive tasks to people with cognitive impairment while performing exercises, especially at higher intensity, may detrimentally affect their performance (Dawes et al, 2003).

To standardise the procedure and improve the accuracy of the RPE scale it is important that participants understand both the verbal anchors and the numerical value. The instructions given to each participant can be found in Appendix 8.1

3.11 Symptom-limited exercise testing

In view of the heterogeneous nature of MS related symptoms, physiological deconditioning, cognitive impairment and lower limb deficits, no single factor can be considered to be responsible for maximal exercise limitations. When an endpoint set on physiological maximal criteria is deemed to be unsuitable, a symptom limited peak exercise test can be performed. Individuals are instructed to pedal until discomfort, pain or fatigue prevents them from maintaining the required cadence. When the participant performs a test at maximal effort, the test is normally terminated due to volitional fatigue (Visich and Ehrman, 2009) or until discomfort, pain or fatigue prevents them from maintaining the required cadence (Wasserman et al., 2005). Explicit instructions given to a participant prior to exercise testing increases test validity and data accuracy (ACSM, 2010). The preliminary instructions given to each participant to standardise the procedure can be found in Appendix 8.2

3.12 Measuring disability

There are a wide range of measures tools for assessing impairment and disability, however care is required in selecting the appropriate test for PwMS. Functional outcome assessments can be confounded because much of the disability during the early phases of MS may be sub-clinical, and during the latter stages may be undetectable. Moreover, values of a specific measure or scale can fluctuate widely within the same patient as the disease progresses (Cofield and Cutter, 2008). The following sections review the functional tests used in the thesis.

3.12.1 MS Functional tests

Functional walk tests provide objective measures of functional status, capacity and mobility. The Barthel Index (BI) was developed by Mahoney and Barthel in 1965 as a 10 point scale that assesses functional status and independence in personal care and mobility. The BI has shown evidence of satisfactory psychometric properties, and predictive validity acceptable for outcome use (Christiansen, 2005). It is a reliable and valid instrument of activities of daily living and mobility (Collin et al., 1988), and is well suited to PwMS with moderate to severe disability (Coulthard-Morris, 2000). The BI relates significantly with other measures of a MS patients status, and has been shown to be better able at discriminating between individual PwMS on the basis of their disability than the Expanded Disability Status Scale (EDSS) (Polman et al., 2006). While, the BI has been found to be sensitive to change in the functional status of MS patients with moderate to severe disability, it may be less sensitive to improvements made by mildly impaired PwMS

(Coulthard-Morris, 2000). However, its psychometric validity and reliability make it an appropriate measure for this study.

3.12.2 Walking mobility tests.

Timed walking tests are simple to administer but limited in their ability to assess important aspects of gait, such as quality of movement, balance, the use of walking aids (walking sticks or crutches), and the amount of physical assistance required to complete the timed duration. Nevertheless, they are commonly used to assess both function and fitness. Traditionally, the 6 minute walk test (6MWT) has provided a reliable and responsive measure, however the burden it places on PwMS, especially those with ambulatory difficulties, remains a source of concern. The 2 minute walk test (2MWT) has been described as the fastest and most efficient measure among the timed tests (Brooks et al., 2001, Brooks et al., 2002). It has been found to be a good predictor of community mobility in PwMS and has been shown to be a reliable and valid measure of mobility in patients presenting with neurological impairment (Rossier and Wade, 2001). When compared with timed walks of longer duration (6 and 12 minutes) in stroke patients the 2MWT not only minimises the effects of fatigue, but correlates well to the longer distances (Kosak and Smith, 2005). More recently Connelly and colleagues (2009) reported high reliability of the 2MWT performed by a group of elderly subjects (mean age 87years) who averaged 77 metres and 80 metres during two test sessions (Connelly et al., 2009). Gijbels et al, (2011) compared the results of the 2MWT test to the more commonly used 6MWT in a group of people with MS reported that although they found a higher margin of error in the 2MWT in PwMS with mild

ambulatory dysfunction, the two timed walks captured the same aspects of mobility. Based on the 2MWT scores, the authors concluded that the last 4 minutes of the 6MWT test were unnecessary (Gijbels et al., 2011).

3.12.3 The ‘timed up and go’ test (TUG)

A timed ‘up and go’ (TUG) test performed once has been shown to be a clinically relevant and reliable measure for measuring walking mobility in PwMS (Nilsgard, 2007, Wade 1992) and correlates well with the Barthel Index (Podsiadlo and Richardson, 1991). The test requires the participant to stand up from a standard chair, to walk to a point 3 metres away, turn around a cone placed on the floor, walk back to the chair and sit down again as fast as possible (Podsiadlo and Richardson, 1991; Nilsagard, 2007). A time of more than 17 seconds is predictive of a decline in basic activities of daily living (Pandyan et al 1999, 2003). Participants received instructions regarding walking aids (sticks or wheeled walkers) and advised on how to manoeuvre around the cone in a safe manner.

3.13 Pilot Study: The appropriateness of maximal exercise testing on people with multiple sclerosis

3.16.1 Introduction

The purpose of this pilot study was to examine the appropriateness of a symptom-limited GXT for people with clinically confirmed MS. Responses to intensity were

recorded during the active phase and the post-exercise recovery period. In addition participants were contacted at home, 24 hours after completing the GXT.

3.16.2 Participants

.Six people with confirmed MS (5 women and 1 man, age mean 59 ± 0.01 years) agreed to participate. Prior to the GXT, baseline measures of tympanic temperature (Temp°C), heart rate (HR) and differential measures of RPE (CR10) for both breathing (RPEbr) and legs (RPEleg) (CR10) were recorded.

3.16.3 Methods

Participants performed the symptom limited GXT on a cycle ergometer. Feet were firmly supported by toe clips reinforced with straps and the handlebars adjusted for comfort. Following 2 minutes of unloaded pedalling RPE, HR, Temp°C, were recorded immediately prior to 500 grams being loaded onto the weight pan, increasing the resistance on the flywheel by 25 watts. Thereafter, the process was repeated at 2 minute intervals. As the test proceeded, participants were given verbal encouragement to achieve their best performance. The end-point was defined by the participants' inability to maintain the pre-determined cadence, for whatever reason.

Respiratory gas exchange variables were determined, breath-by-breath, on a Metamax mobile gas analyser. Peak measures of HR and VO₂ (L/min) were the average values recorded during the final 20 seconds of the last completed stage.

3.16.4 Results

The mean maximum work rate achieved during the exercise test was 133 ±13 Watts, $\dot{V}O_{2\text{ peak}}$ mean 20.6 ±1.1ml.kg⁻¹.min⁻¹, respiratory exchange rate (RER) mean 1.19 ±0.05, HR mean 143 ±14 b.min⁻¹; CR10_{breath} mean 5 ± 2; CR10_{legs} mean 6 ± 1; lactate mean 7.1 ±1.6 mmol.L⁻¹. Temperature increased by mean 0.1 ±0.1 °C. The difference between baseline and end rest period temperature was mean 0.0 ± 0.4 °C and HR was mean 19 ± 10 b.min⁻¹. The difference in next day vitality from baseline was mean 0.1 ±2.1.

3.16.5 Discussion

The incremental cycle ergometer test was well tolerated by the participants. However, it should be noted that not all physiological and perceptual markers indicated that participants were achieving maximal work rate. RPE and HR values were below what would be expected as maximal. In addition, no participant achieved a plateau in $\dot{V}O_2$ consumption during the final stage. The RER and lactate values indicated a pronounced anaerobic contribution to the exercise

performed. Perceptions of exertion in the legs were consistently higher than those for breathing. Post-exercise values of HR, BP and RPE were monitored for a further 25 minutes. Some parameters did not return to baseline levels during that period. No adverse events were reported, either during the exercise test or the post-exercise recovery period. All the participants were followed up the next day and answered the vitality questionnaire.

3.16.6 Conclusion

This study suggests that a symptom limited graded exercise test is an appropriate protocol for determining the absolute and relative exercise capacities of people with MS. No adverse events were reported, nor did it have a detrimental effect on feelings of vitality the day following maximal fitness testing.

3.17 Neurophysiological Measures

3.18 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a well-established, non-invasive technique used to examine the integrity and excitability of the corticospinal - neuromuscular pathway in healthy and neurologically impaired populations (Barker et al, 1986; Hess et al, 1986; 1997; Kobayashi and Pascual Leone, 2003; Hallett, 2007). The following sections describe the technical principles underlying TMS, the methodological considerations and safety issues that influence the design of the TMS experiments

3.19 Principles of TMS

TMS was developed in 1985 by Barker and co-workers in Sheffield (Barker et al., 1985), and was first used to test the integrity of the neural pathways during spinal cord, brainstem and cerebral cortical surgery. More recently TMS has been used to study central motor conduction disruption caused by neurological disease and disorders, rehabilitative stimulation and brain/ function mapping. TMS has also been used extensively, both in the general population and PwMS to monitor changes in cortico-spinal excitability after exercise,.

The technical principle of TMS is based on Faraday's law of induction. An electrical current flowing through a coil of wire generates a time varying magnetic field. The rate of change in the magnetic field determines the induction of a secondary current in a nearby conductor (Kobayashi and Pascual-Leone, 2003). In TMS the

stimulating coil acts as an electro-magnet and neural tissue acts as the nearby conductor. When triggered, a brief surge of current lasting $\sim 100\mu\text{s}$ passes through the coil inducing a rapidly changing magnetic field of up to 2.2 Tesla (Hallett, 2007). Although the stimulator capacitor releases a charge of up to 5000 amps, only a small fraction ($100,000^{\text{th}}$) of the coil's magnetic energy is actually transferred into the brain (Ruohonen and Risto, 2005). The pulse volume, estimated to be ~ 10 mm radius, ~ 15 -20mm depth (Wagner et al., 2008), is able to penetrate soft tissue and bone virtually unattenuated. The induced electrical field stimulates underlying nerve cells in a focused volume. The mechanism that drives active stimulation in cortical cells remains unclear and there remains considerable debate over the depth of the pulse, the neuronal populations it affects, and the pathways it takes. The characteristics of the response are suspected to be initiated at axonal initial segments, bends in neural fibres, or from synaptic boutons where the descending volleys have their maximal effect (Wagner et al., 2009).

The immediate measurable response to a pulse of TMS administered at the motor cortex is the direct activation of descending axons (D-wave) (Terao and Ugawa, 2002) closely followed by a series of indirect wave (I-waves) of about 1.5 - 2.0ms duration (Hanajima et al., 2002) that continue to fire for several milliseconds after the stimulus. The mechanisms that produce I- wave periodicity are unknown, but they are possibly due to the natural recurrent synaptic activation of the corticospinal neurons in response to the induced electrical field (Hallett, 2007, Gandevia, 1998), or to the intrinsic properties of the neuronal membranes in

response to the large synchronous excitatory input of the magnetic pulse (Priori et al., 1993).

A single stimulus evokes multiple descending volleys in corticospinal motor neurons producing a contralateral, synchronous muscle response, a motor evoked potential (MEP). Surface EMG electrodes are placed on the target muscles that are to be stimulated and once positioned, the magnetic coil is fired and MEPs recorded by surface EMG electrodes. The repetitive discharge of spinal motor neurons is considered to represent the natural excitability of the corticospinal-neuromuscular pathway in response to the magnetic pulse (Todd et al., 2007, Petersen et al., 2003, Taylor and Gandevia, 2004, Reis et al., 2008).

3.20 Magnetic Stimulators

TMS can be applied as a single pulse, in paired-pulses or as trains of pulses (rTMS). Repetitive (rTMS) stimulation of the motor cortex and other regions in the brain may induce effects that outlast the stimulation period. As well as being used as an investigational tool to explore cognitive processes and cortical physiology, rTMS has become an important method for the treatment of a variety of neurological and psychiatric disorders (Daskalakis and Chen, 2005) (Rothwell, 2007). Paired-pulse techniques involve the delivery of a conditioning stimulus to an area of the brain in order to assess the changes produced by the following test stimulus. Paired-pulse experiments are generally designed to explore the nature of cortical circuitry, and to examine connections within the motor cortex to other parts of the CNS (Rothwell, 2005). Paired-pulse systems consist of twin stimulators

connected by a module that controls pulse delivery. The timing of pulses can range from 1-999ms. Alternatively the stimulators can be fired simultaneously to generate a single pulse. The electrical field of a single pulse fired from two stimulators increases by ~113% when compared to a single stimulator. However, the length of the pulse increases by ~140% (Rothkegel et al., 2010). Recent research has shown that the longer pulses reduce resting motor thresholds by about 20%, and reduce MEP variability when muscle is under tonic contraction (Rothkegel et al., 2010) making the method an attractive proposition for exploring the central pathways in clinical groups with central conduction abnormalities.

3.21 Stimulating coil

The strength of the magnetic field is at its highest close to the coil surface however, the spatial distribution of the pulse is dependent on coil geometry and the anatomy of the region of the induced current. Circular coils deliver a diffuse field over the brain while figure of eight coils allow focal stimulation at a more limited and clearly defined location (Epstein, 2008), however, small movements of a focal coil substantially influences the size of the MEP. The cortical area or volume of brain stimulated depends on the location of the coil, the stimulator intensity needed to produce an effect, and direction of the induced electrical field. The depolarisation of the underlying neuronal cells is not due to the magnetic field but rather to the induced electrical field within the brain (Wagner et al., 2009)

3.22 Safety

Single pulse TMS is a safe procedure for examining corticospinal excitability if used with basic precautions (Rossi et al., 2009, Sandbrink, 2008). In general, contraindications to TMS are related to exposure to the magnetic field, and therefore similar to those for magnetic resonance imaging, applying to both the participant and the examiner (Sandbrink, 2008). Side effects are generally transient, but may include short term discomfort due to contracting scalp muscles near the site of stimulation, or a mild headache during or following TMS that is treatable with over-the-counter analgesics. There is no evidence of significant adverse effects to TMS in either the short or long term, on cognition, memory, sensory perception, hormone release or the cardiovascular system (Sandbrink, 2008, Chokroverty et al., 1995). While some participants have experienced transient hearing difficulties after TMS of deep brain regions (Zangen et al., 2005), others have reported no hearing-loss even after years of exposure to common TMS procedures (Pascual-Leone et al., 1992).

Seizures caused by single-pulse TMS are rare but possible in those people who are susceptible to intractable seizures (Rossi et al., 2009, Classen et al., 1995) (Anand and Hotson, 2002). The single report of a seizure during TMS of a person with MS was published as a single-case study. The investigators hypothesised that the seizure may have been the result of focal brain lesions detected by fMRI, or due to the use of olanzapine (Haupts et al., 2004), an anti-psychotic used for the treatment of schizophrenia and bi-polar disorder.

While the risk of TMS related adverse effects are rare, they cannot be completely excluded. The TMS experiments in this thesis conformed to the published safety guidelines (Wassermann, 1998), and the ethical and regulatory requirements pertaining to research and clinical applications of TMS (Rossi et al., 2009). The consent form included a safety questionnaire (Keel et al., 2001) commonly used by TMS investigators to screen subjects for various conditions that may increase risk.

3.23 MEP variability

The MEP is influenced by intrinsic factors relating to the excitability of the corticospinal pathway (Hess et al., 1987, Rothwell, 2008), such as the number of motor neurons recruited by the magnetic pulse, the number of motor neurons discharging more than once in response to the stimulus and the synchronisation of the motor neuronal discharges (Rosler and Magistris, 2008). The stimulus response relationship varies considerably between subjects, between muscles in the same individual, even those closely anatomically located, such as hand muscles or upper leg muscles (Ellaway et al., 1998). Corticospinal excitability can be affected by tonic muscle contractions, de-synchronisation of the discharges, phase cancellation and impedance mismatch. Changes in the repetitive discharges in response to the magnetic pulse are purported to cause much of the trial to trial variability (Magistris et al., 1998), but appear to be independent of age, gender, and height, or indeed the strength of the stimulus. Repetitive discharges, the indirect consequence of the magnetic pulse, occur with cortical stimulation but not

with stimulation at the level of the brainstem, suggesting a substantial contribution to variability from sub-cortical processes (Taylor et al., 2006). However, it remains unclear which neurophysiological mechanisms are involved in the varying levels of desynchronisation, and repetitive discharge of neurons. Moreover, whether the initial part of the stimulus or the repeat waves activates the motor neuron not only depends on the strength and number of the descending volleys, but also on the excitability of the cortico-spinal pathway (Taylor and Gandevia, 2008). Extrinsic factors that are implicated in MEP variability are the role of the coil, and its position on the scalp. Furthermore, MS related conduction block, conduction impedance and slowing of signal velocity may modify the MEP (Rosler and Hess, 2010).

3.24 TMS measures; Motor threshold

The measures most commonly used in the analysis of motor data are resting motor threshold (the minimal TMS intensity required to evoke an MEP), latency (the time from triggering the pulse to the start of the MEP), amplitude (base-line to peak or peak to peak value) and area (measured as root mean squared, or as the area under the line of the rectified signal). The absolute amplitude reflects both upper and lower motor neuron activity and is affected by peripheral nerve disorders (Sandbrink, 2008).

Motor threshold (MT) is the lowest stimulus intensity of TMS that gives a recordable MEP in the target muscle (Sandbrink, 2008). Obtaining accurate resting motor thresholds requires a systematic search for the optimal coil position (hotspot) above the motor area corresponding to the target limb or muscle (Conforto et al.,

2004, McDonnell et al., 2004). When the coil is directly over the hotspot it is general practise to obtain 5-10 MEPs of 50 μ V in at least 50% of successive trials (McDonnell et al., 2004). Extensive mapping of the motor cortex has shown that there are considerable differences between individuals and that the hotspot for stimulating a specific muscle may be several millimetres from any given point. Additional factors such as skull thickness, brain morphology, age or neuronal properties can also affect the MTs, and there are significant inter-hemispheric differences in 'hotspots' related to dominant handedness (Marchand-Pauvert et al., 1999)

Motor thresholds (MTs) are generally recognised as a measure of the integrity and excitability of the neural pathway at both the cortical and spinal level (Taylor and Gandevia, 2004). Studies investigating MTs have reported conflicting results. Wasserman (2002) found a wide variation in MTs over time (Wassermann, 2002). Other studies have reported consistent MTs over two sessions (Wheaton et al., 2009, Cacchio et al., 2009). Due to their variability, both within and across subjects, MTs cannot be recognised as suitable measurements in tests for individual subjects, or between groups, even with minor differences (Wassermann, 2002). Nevertheless, precise recordings of MTs are critical for determining the optimal experimental stimulator intensity as a % of maximal output.

3.25 Central motor conduction time (CMCT)

The latency of the evoked potential is the time taken from triggering the stimulus at the motor cortex to the start of the MEP. The time taken for a volley generated in the motor cortex to travel through the spinal cord, the 'central motor conduction time' (Olivier et al., 2002, Mesin et al., 2008, Sheean et al., 1997), can be determined by comparing the difference between the latency of a cortically stimulated MEP, and the latency of a TMS pulse at the spinal roots, or the latency of the response of electrical /magnetic stimulation at a point on the motor nerve. The physiological conduction velocity in the corticospinal tract has been measured between 65m/sec (Eisen and Shtybel, 1990) and 68.5m/sec (Ugawa et al., 1995). The CMCT of leg muscles is height dependent. Normative values for the tibialis anterior (TA) are 28.5 ± 2.5 milliseconds (ms) for people 150-174 cms tall, and 30.7 ± 1.8 ms for 175 -191 cms tall (Dvorak et al., 1991) with little change between men and women (Furby et al., 1992). People with MS generally have a prolonged CMCT represented by prolonged MEP latency (Sheean et al., 1997).

3.26 The cortical silent period

The 'silent period' (CSP) is characterised by an interruption of the EMG signal after TMS is delivered at the motor cortex during a tonic contraction (Orth and Rothwell, 2004). It is defined as the time between the end of the MEP and return of the EMG signal (Damron et al., 2008). CSP requires an individual to contract the target muscle at ~20-30% of MVC. The duration of the CSP is dependent on the % of stimulator output, yet appears to be unaffected by the intensity of MVC (Damron et

al., 2008). The resulting CSP lasts for only ~100ms with considerable variability of duration between subjects (Orth and Rothwell, 2004). The initial portion (40-50ms) of the CSP is generally believed to be a response to spinal inhibitory mechanisms that follow motorneuron excitation, namely hyperpolarization and recurrent inhibition. Inhibitory mechanisms at the spinal level are known to mediate the CSP during the latter stages (Orth and Rothwell, 2004).

The CSP is distributed unevenly between muscles and needs to be measured under the precise control of stimulus intensities relative to individual motor threshold values (Noordhooft, 2002), making it a difficult measure to control. Furthermore, the need to generate and maintain a tonic contraction within the specific parameters that precipitate a CSP may be difficult after lengthy physical exercise of moderate or vigorous intensity, especially in PwMS. Therefore, for the purpose of this thesis, we will not use CSPs as a measure of corticospinal excitability.

3.27 TMS and exercise induced fatigue

In the general population MEPs measured during, or within a few seconds of an isometric contraction, are reported to be increased in size relative to pre-exercise MEPs. This phenomenon is termed post-exercise MEP facilitation (PEF). Conversely, MEPs recorded from resting muscle after exercise display post-exercise depression relative to pre-exercise values (Samii et al., 1996b). Post exercise facilitation is short lived after a fatiguing contraction (~30seconds), but can increase the amplitudes of MEPs to higher than those measured from direct

electrical stimulation of the muscle. The associations between PEF and muscle fatigue remains unclear.

Previous studies examining corticospinal excitability after physical activity have reported a significant post-exercise depression in MEP amplitudes lasting several minutes, thought to be a marker of central fatigue (Petersen et al., 2003, Gandevia, 2001, Gandevia et al., 1996). Brasil-Neto et al (1994) first used TMS and EMG recordings to investigate fatigue and neuromuscular changes in the upper arm muscles after exhaustive exercise, reported a post-exercise depression of MEPs lasting several minutes (Brasil-Neto et al., 1994). Since then, TMS has been used to central responses to a broad range of anaerobic and aerobic activities in both trained and untrained subjects (Verin et al., 2004, Meaney et al., 2007). The longest duration of MEP depression was reported by Ross and colleagues, who observed a 67% depression in the tibialis anterior after 42.2 km treadmill running. At 4 hours post-exercise, MEPs were still depressed by 43% suggesting that the duration of exercise had a profound effect on corticospinal excitability (Ross et al., 2007)

Post-exercise depression was generally thought not to occur after exercise lasting less than 2 minutes (Samii et al., 1996b). In 2007, our group examined corticospinal responses to supramaximal exercise following a 30 second Wingate test (WAT) (Meaney et al., 2007). MEPs were recorded from the rectus femoris (RF) of 10 healthy participants. Immediately following the WAT, mean MEP amplitudes were depressed by 22.09% ($p > 0.05$) and proceeded to decrease to

31.9% ($p < 0.05$) at 8 minutes compared to pre-exercise values. MEP amplitudes remained depressed for 20 minutes post-exercise ($p < 0.05$). In view of the short duration (30 seconds) of the test, it was concluded that the post-exercise MEP depression was an effect of the intensity of exercise (Meaney et al., 2007).

3.28 TMS and multiple sclerosis

MS was the first neurological disease studied using TMS. Early trials on people with MS found that central motor conduction time (CMCT) was prolonged, motor thresholds were increased and MEP amplitudes were reduced (Hess et al., 1986). Since then, TMS has been used extensively in MS to study the lower limbs and functional motor disability (Ingram et al., 1988, Facchetti et al., 1997), disease classification (Humm et al., 2003, Rico et al., 2009), fatigue (Sandroni et al., 1992), drug treatment (Humm et al., 2006) and functional electrical stimulation (Everaert et al., 2010). There is a relationship between motor impairment and MEP threshold, latencies and MEP duration in people with MS (Thickbroom et al., 2005). Indeed, conduction deficits in the corticospinal-neuromuscular pathways can manifest as delayed, smaller MEPs making it difficult to both elicit and analyse responses to TMS (Rosler and Magistris, 2008).

There is a paucity of TMS literature relating to exercise induced fatigue in PwMS. Although MEP depression has been observed previously in PwMS (Petajan and White, 2000, Liepert et al., 2005) reports are rare. Indeed, many investigators have concluded that post-exercise MEP depression fails to occur in PwMS (Perretti et al., 2004, Jorgensen et al., 2005, Thickbroom et al., 2006) due to disease related

disinhibition in higher brain centres (Thickbroom et al., 2008, Teo et al., 2011). However, we postulated that perhaps the intensity at which the exercise had been performed in previous studies, rather than MS, may have limited the occurrence of post-exercise MEP depression

3.29 TMS Pilot Study

3.29.1 Introduction

We undertook a pilot study to investigate the corticospinal excitability of an individual with confirmed MS in response to dynamic exercise. We hypothesised that after exercise, the size of MEPs elicited by TMS would be depressed. We further hypothesised that if exercise intensity caused MEP depression, then exercise at higher intensity would have a greater effect on corticospinal excitability and would be reflected in the size of MEP.

3.29.2 Methods

Following institutional ethical approval a 59 year old male with confirmed MS agreed to participate. The investigation was conducted within the published safety guidelines for the application of TMS in clinical practice and research (Rossi et al, 2009). The participant was required to complete a TMS safety screening questionnaire (Keel et al, 2001) and a pre-exercise health questionnaire. Exclusion criteria were in accordance with published suggested safety guidelines (Wasserman 1998). A graded exercise tolerance test (GXT) performed to volitional fatigue was performed on a friction-braked cycle ergometer.

3.29.3 TMS

The participant attended 2 further sessions. On arrival, surface electrodes were placed over the rectus femoris of the dominant leg determined by dynamometer.. He lay on a medical examination plinth for 20mins while 5 pulses of TMS were administered at 5minute intervals. At each session he performed 20mins cycling exercise at either 45% (ET45) or 60% (ET60) of the peak watts achieved during the GXT before returning to the lying position. TMS was administered within 30 seconds of completion and repeated at 2, 4, 6, and 8 minutes. The experiment was conducted at 120% of pre-determined threshold.

3.29.4 Analysis

MEP amplitudes were visually examined for general trends. T-tests were used to determine differences between pre- and post- ET45 and ET60 data. Significance was accepted at $p < 0.05$.

3.29.5 Results

There was no difference in the pre-ET45 and pre-ET60 MEP amplitudes ($p>0.05$), or between the pre-exercise and post-exercise data for ET45 ($p>0.05$). However, post-ET60 MEP amplitudes were significantly depressed ($p<0.01$) and a significant difference existed between post-exercise ET60 and ET45 ($p<0.01$).

3.29.6 Conclusion

The pilot study found that MEP amplitudes were significantly depressed after 20 minutes of exercise at 60%, but not at 45% of the maximum GXT workload. The

results suggest that corticospinal responses to exercise at different intensities can be measured using TMS. In addition it found that exercising at 45% may be better tolerated in pwMS, however further tests incorporating more physiological markers are required before benefits established.

Figure 3.1 Participant exercising during the single-case pilot study



Note; The Bistim² and figure-of eight coil are at the forefront of the photograph. The cycle ergometer was used during all exercise test sessions.

Figure 3.2 Transcranial magnetic stimulation (TMS) of the participant



Chapter 4 TMS Reliability

This chapter examines the reliability of responses to transcranial magnetic stimulation in the resting tibialis anterior of people with multiple sclerosis

4.1 Introduction

Multiple sclerosis (MS) was the first neurological disease to be explored using transcranial magnetic stimulation (Hess et al., 1986, Hess et al., 1987). MS is a progressive, degenerative disease of the central nervous system (CNS) characterised by demyelination and neuro degeneration (Waxman, 2006, Siffrin et al., 2010, Dutta and Trapp, 2011). Progressive axonal loss is the major cause of permanent neurological disability (Frischer et al., 2009, Dutta and Trapp, 2011) with physical impairments particularly distinct in the lower body where incomplete motor unit activation and altered muscle characteristics are similar to those reported in hemiplegia and spinal cord injury (Kent-Braun et al., 1997, Carroll et al., 2005). Lower limb weakness and locomotor dysfunction are distinctive characteristics of MS (Kesselring and Beer, 2005) with the evaluation of lower limb function an important current focus (Everaert et al., 2010, Kesselring, 2010)

During the last 25 years transcranial magnetic stimulation (TMS) has become a well-established, non-invasive technique to examine the integrity and excitability of

the corticospinal-neuromuscular pathway in both healthy and neurologically impaired populations (Barker et al., 1986, Hess et al., 1986, Hess et al., 1987, Kobayashi and Pascual-Leone, 2003, Thickbroom et al., 2005, Thickbroom et al., 2006, Moosavi et al., 1999). The repetitive discharge of motor neurons and subsequent size of the motor evoked potential (MEP) in response to TMS is considered to represent the net inhibitory and excitatory influences on corticospinal cells, and the natural excitability of the corticospinal neuromuscular pathway (Taylor and Gandevia, 2004, Todd et al., 2007, Reis et al., 2008).

TMS studies have consistently reported prolonged central motor conduction times (CMCT) (Schmierer et al., 2002, Conte et al., 2009) higher motor thresholds (MT) (Caramia et al., 1991, Sheean et al., 1997) and reduced MEP size (Jorgensen et al., 2005, Thickbroom et al., 2005) when comparing MS patients to healthy controls. Abnormal MEPs may be the product of demyelination causing decreased central conduction velocities, frequency dependent conduction block (the inability to conduct trains of action potentials at high frequency), or complete conduction block (Kesselring et al., 2010a); Rosler and Hess, 2010). Temporal dispersion resulting from the desynchronisation of descending action potentials and phase cancellation further affects the MEP area in an unpredictable manner (Rosler et al., 2002, Wasserman et al., 2008). Nevertheless, TMS studies show that MEPs are sensitive to motor impairment and neurological dysfunction from the earliest stages of MS (Gagliardo et al., 2007, Rico et al., 2009), provide markers of motor function and post-exercise fatigue (Thickbroom et al., 2005, Thickbroom et al., 2006,

Thickbroom et al., 2008, Perretti et al., 2004), can be used for monitoring responses to treatment (Everaert et al., 2010) and may predict MS disease progression (Conte et al., 2009). However, the stability and repeatability of the TMS measure from resting muscle of the lower limb of people with MS (PwMS) remains unreported.

Evoking consistent responses in the lower limb with TMS, particularly in people with myelopathies or spinal cord injury can be difficult (Roy et al, 2010). The higher stimulus intensities required to stimulate skeletal muscle in the lower body generates repetitive corticospinal discharges leading to prolonged, complex polyphasic MEP forms (Rosler and Magistris, 2008) thus MEPs are generally delimited in order to reduce artefact and variability (Ellaway et al, 1998) however, the effect of MEP reduction on reliability has not been reported. In addition, subjects are commonly required to maintain a tonic contraction in order to both facilitate the MEP and reduce its variability, but this can prove problematic for patients with central motor conduction abnormalities (Everaert, 2010). Furthermore, active muscle contractions for PwMS may hide very small depressions in MEP size following exercise or therapeutic interventions. We therefore investigated the internal stability, consistency and reproducibility of blocks of trials from resting muscle over a 20 minute period and at a repeat session several days later.

4.2 Methods

Candidates for the study were referred by neurologists in Oxford or self-referred from regional MS societies. Participants were people with clinically definite MS (Poser 1983) as confirmed by a consultant neurologist however recently revised diagnostic criteria (Polman et al, 2011) were unavailable. The experiment was carried out at the Nuffield Orthopaedic Centre, Oxford. Ethical approval was obtained from Oxfordshire Research and Ethical Committee (07/H0604/84).

The investigation was conducted within the published safety guidelines for the application of TMS in clinical practice and research (Rossi et al, 2009). Participants were required to complete a TMS safety screening questionnaire (Keel et al, 2001). Exclusion criteria were in accordance with published suggested safety guidelines (Wasserman 1998). Participants were excluded if they had history of migraine or epilepsy, were fitted with cardiac pacemakers or cranial ferromagnetic implants, showed evidence of uncontrolled hypertension, pregnancy, or reported any occurrence of relapse or new neurological symptoms within 4 weeks of the investigation. Having been informed of the nature of the testing procedures, and advised that they could withdraw from the study at any time without giving reason, each participant signed an informed consent document in accordance with the Declaration of Helsinki. Details of medication were recorded. The 3 metre 'timed up and go', was used to assess mobility (Podsiadlo and Richardson, 1991), and the Barthel Index measured functional status, including mobility and independence in activities of daily living (Wade and Collin, 1988). Subjects were asked to refrain

from smoking or taking drinks containing caffeine or alcohol for at least 3hrs, and not to participate in strenuous exercise for 48 hours prior to the experiment.

4.3 TMS data acquisition

TMS was administered on two separate occasions (7-14 day interval). Participants attended test sessions at the same time of day performed under standard conditions at room temperature $\sim 21^{\circ}\text{C}$. They were required to lie semi-supine on a two-section medical examination plinth set so that their upper bodies were angled to 30° of an up-right position. Baseline body temperature was recorded with a tympanic thermometer and repeated every 5 minutes throughout the experiment. The target muscle from which MEPs were to be recorded was the tibialis anterior (TA) on the less affected, or previously dominant side of the body. The skin lying under the electrodes was cleaned with an alcohol swab. Paired silver-chloride surface electrodes (T3404, Thought Technology Ltd, Montreal, Canada) were positioned with the inter-electrode distance set at 20mm over the middle of the belly of the muscle in line with its longitudinal contour. The locations of electrodes were carefully noted for precise replacement during the next visit. The reference electrode was attached at the olecranon prominence of the ulna at the elbow. Twisted-paired electrode cables connected the electrodes to a four channel pre-amplifier (Neurolog 844, Digitimer Ltd, Letchworth, UK).

Single pulse TMS to the motor cortex was applied using two magnetic stimulators connected to a Bistim² module (Magstim Company, Dyfed, UK). The stimulators were set to fire simultaneously producing a single pulse from a double, cone

shaped coil with a diameter of 110mm, (Magstim Company, Dyfed, UK) specifically designed to elicit responses from relaxed muscles of the lower pelvic floor and lower limbs. Initial exploration determined the optimal stimulation point (hotspot) for the TA. The hand-held coil was placed on the head parallel to, and ~ 0.5-1.5 cm lateral to the mid-line (nasion to inion), with its mid-point aligned antero-posteriorly (auricular point to auricular point) against the vertex (Devanne, et al. 1997; Cacchio et al, 2009). The stimulators were set at 60% of the maximum combined output and the coil moved systematically around the location until the hotspot was identified. The exact coil position was carefully maintained throughout the experiment by aligning it to marks on the scalp made with an indelible ink pen.

The motor threshold (MT) of each subject (the lowest TMS intensity necessary to evoke MEPs in the target muscle) was determined by starting stimulation at 60% of maximal Bistim² output and reducing power in 5% increments until the stimulus artefact disappeared. The stimulator output was then increased in 1% increments +until threshold was reached. MT was defined as the intensity that elicited 5 or more MEPs with amplitudes of > 50µV in at least 5 of 10 successive stimuli from the relaxed target muscles (Rossini and Dal Forno, 2004). For the comfort of the participants we set an upper limit of 80% of maximal Bistim² output to elicit motor threshold. The experiment was conducted with the stimulator output set at 120-130% of the motor threshold value.

During the test procedure, the participants received a total of 25 TMS trials delivered in 5 blocks of 5 trials (T1-T5) with a delay of 7-10 s between each trial and a period of 5 minutes between each block. EMG signals were recorded through a NL844 (Neurolog 820) and relayed to a CED Micro1401 (Cambridge Electronic Design, Cambridge UK), amplified(x 1000) and filtered with a band-pass filter of 1000Hz to 10Hz. A 55Hz notch filter was applied. The data was digitised at 2000 Hz and stored for further analysis on a computer.

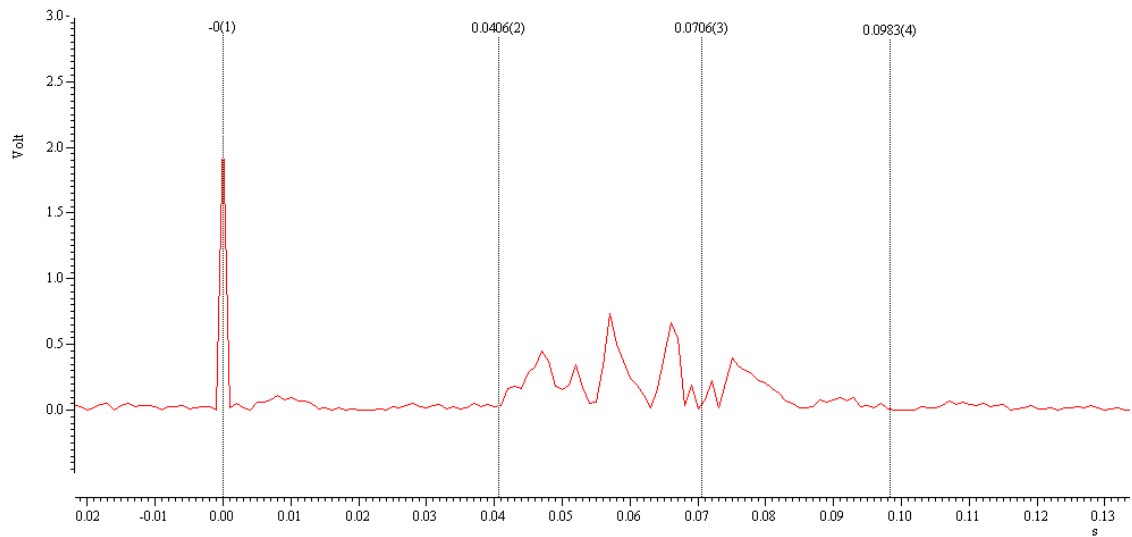
4.4 Data Analysis

The EMG signals were analysed using Signal v.3.11 (Cambridge Electronic Design, Cambridge UK). MEPs were full wave- rectified (Halliday and Farmer, 2010), and calculated as the product of the mean amplitude (Mv) multiplied by the duration (mS), taken as the area under the line delimited by vertical cursors (see Fig 1) (Ellaway et al, 1998). Two MEP durations, MEPshort and MEPlong were considered for analysis. The onset of rectified MEPs was defined as the deflection point of the leading edge of stimulus artefact above the background EMG and marked by a vertical cursor (C2). MEPshort was delimited at C2 with the second vertical cursor (C3) set at precisely 30ms from C2. MEPlong was delimited at C2, with the second vertical cursor (C4) set at the inflection point on the trailing edge of the longest waveform above background EMG of each participant (See Fig.1).

Areas were calculated for each trial (T1-T5) and then averaged into blocks of 5 trials. The data was imported into SPSS V 17.0 (SPSS inc, Chicago USA) software for statistical analysis. Demographic data was explored using descriptive statistics.

Repeated measures analysis of variance (ANOVA) was used to compare trials (T1-T5) and blocks of trials at both MEP durations.

Figure 4.1 Measurement of a motor evoked potential (MEP)



Note: MEP recorded from the TA. The rectified EMG signal displays the 20ms pre-stimulus window. Vertical lines denote cursor placements. Cursor (1) marks the TMS stimulus, (2) the onset of the MEP (3) shows the 30ms marker for MEPshort and (4) denotes the end point of MEPLong. MEP latency is calculated as the elapsed time (ms) between cursors 1 and 2. MEPpeak is the maximum amplitude, and MEP areas are calculated as amplitude (Mv) multiplied by duration (ms) measured under the line between the limits set by the cursors.

Areas were calculated for each trial (T1-T5) and then averaged into blocks of 5 trials. The data was imported into SPSS V 17.0 (SPSS inc, Chicago USA) software for statistical analysis. Demographic data was explored using descriptive statistics. Repeated measures analysis of variance (ANOVA) was used to compare trials (T1-T5) and blocks of trials at both MEP durations. If ANOVA revealed a significant main effect, tests of within-subjects, contrasts were used to determine different trials or blocks. To compare measures between sessions, paired two-tailed t-tests were used supported by ICC's and their 95% confidence intervals (CI).

4.5 Results

Fourteen subjects (11 female, 3 male, age 51.07 yrs \pm 9.76, 169cms \pm 9) with MS agreed to participate in the study. Participants' characteristics are detailed in Table 4.1. Mean tympanic temperatures were 36.6 °C \pm 0.4° and 36.5 °C \pm 0.3° for sessions 1 and 2 respectively. Responses to TMS were obtained from the TA of 10 participants (3 female participants failed to produce 50% MT before the pre-determined threshold limit was reached; 1 male participant was excluded due to excessive signal interference). MEPs contaminated by artefact or showing evidence of voluntary activation during the 20ms pre-stimulus 'window' and post-stimulus 'latency' period were excluded (<2% of trials per session). None of the participants reported adverse events in response to TMS. Table 4.2 displays the stability of motor threshold, MEP latency and MEPshort. MEPLong reflects high individual variability of amplitudes between sessions.

Table 4.1 Descriptive details of participants

Participants		
Age (yrs)	mean 51.07 ±9.8	range 31 - 67
Gender (m/f)	3male	11 female
MS Categorisation	RR n=7	SP n=7
Disease duration (yrs)	mean 12.8 ±9.6 yrs	range 2 - 36
Barthel Index (20 point)	median 20	range 14 - 20
TUG (s)	mean 11.1 ±3.7	range 5.8 - 18.3

Note; Clinical and self-reported characteristics; MS categorisation, RR =relapsing remitting SP=Secondary Progressive. TUG= ‘timed up and go’, a test of basic mobility. The Barthel Index is a scale of functional status, mobility and independence in activities of daily living.

Table 4.3 shows the effect from the repeated measures ANOVA of trial order from T1 to T5. The results of within blocks analysis revealed that trial 1 (T1) was significantly different to T2 -T5 for MEPlong ($F=4.674$; $p = 0.036$; partial $\eta^2 =.087$). T1 was discarded from all further analysis. Thereafter, the repeated measures ANOVAs of blocks constructed from the averages of 4 trials (Table 4.4) revealed no significant differences within-subjects, for either MEPshort or MEPlong ($p<0.05$). The results of the repeated experiment 7 days later (Table 4.5) revealed no significant differences between sessions ($p<0.05$) for blocks of 4 trials measured over both durations. The test-retest ICCs and 95% confidence intervals

further indicated good (<.70) to high (>.80) reliability for both MEPshort and MEPlong.

Table 4.2 MEP characteristics

TMS Measure	Session1	Session2	t-test	95% CI		ICC	95% CI	
				Lower	upper		Lower	upper
Motor threshold	48.4 ±4.8	48.7 ±4.7	.080	-.808	.058	.992	.947	.948
Latency (ms)	35.1 ±6.9	35.4 ±7.2	.306	-.933	.333	.993	.972	.988
MEP duration (ms)								
MEPshort	30	30	-	-	-	-	-	-
MEPlong	50.2 ±4.6	51.7 ±5.4	.405	-5.18	2.3	.601	-.81	.904
MEPpeak amplitude (mV)								
MEPshort	315 ±340	380 ±437	.270	-.192	.062	.911	.658	.979
MEPlong	398 ±347	396 ±449	.173	-.197	.042	.916	.683	.980
MEP Area (mV.ms)								
MEPshort	3.9 ±4.1	4.9 ±4.9	.114	-2.2	.292	.919	.668	.991
MEPlong	5.8 ±6.6	7.0 ±7.0	.058	-2.53	.560	.957	.765	.991

Note; Mean MEP characteristics, paired 2 tailed t-tests and intra-class correlation coefficients with respective 95% confidence intervals based on a two-way, mixed effect model with absolute values.

Table 4.3 Effect of trial order

MEP area (mV.ms)	F	Sig	Partial eta²
MEPshort			
trial 2 v trial 1	1.408	.241	.028
trial 3 v trial 2	1.990	.165	.039
trial 4 v trial 3	1.951	.169	.038
trial 5 v trial 4	.010	.920	.000
MEPlong			
trial 2 v trial 1	4.674	.036	.087
trial 3 v trial 2	.001	.974	.000
trial 4 v trial 3	.798	.376	.016
trial 5 v trial 4	.002	.964	.000

Note; Repeated measures one-way analysis of variance (ANOVA) determined the effect of trial order. Each group consisted of 49 trials in their respective order from each block.

Table 4.4 Within-session stability of MEP blocks (4 trials)

5 Blocks (4 trials)	F	sig	ICC	95% Confidence interval	
				Lower limit	Upper limit
Peak amplitude (mV)					
MEPshort	.990	.426	.899	.752	.965
MEPlong	1.001	.420	.894	.769	.986
Area (mV.ms)					
MEPshort	1.773	.156	.937	.856	.981
MEPlong	2.196	.089	.955	.934	.995

Note; Repeated measures ANOVAs based on the means of the MEP amplitudes and areas followed by Intra-class correlation coefficients (ICC's) and confidence intervals (CI) based on a two-way, mixed effect model with absolute values.

Table 4.5 Test-retest of Blocks (4 trials)

Block Id	Test 1	Test 2	T test <i>p</i>	95% confidence		ICC	95% Confidence		
				Lower	upper		Lower	upper	
MEPshort									
1	Area	3.36 ±3.84	4.55 ±5.38	.239	-3.37	0.99	.832	.418	.963
	Peak	295 ±301	418 ±512	.205	-321	081	.794	.365	.949
2	Area	2.49 ±2.91	2.91 ±3.19	.491	-2.09	1.13	.846	.377	.971
	Peak	248 ±260	253 ±223	.930	-.143	.085	.827	.334	.963
3	Area	3.81 ±4.13	5.28 ±5.19	.093	-3.24	3.10	.862	.479	.967
	Peak	330 ±419	393 ±462	.218	-.172	.047	.945	.789	.987
4	Area	4.34 ±4.57	4.89 ±5.29	.423	-2.05	0.95	.924	.719	.982
	Peak	262 ±285	338 ±478	.402	-.273	.110	.815	.361	.959
5	Area	3.19 ±3.82	4.24 ±3.63	.121	-2.44	0.34	.860	.497	.967
	Peak	188 ±225	288 ±312	.091	-.197	003	.821	.323	.962
MEPlong									
1	Area	5.12 ±6.41	6.69 ±8.57	.218	-4.31	1.17	.897	.601	.978
	Peak	306 ±320	430 ±521	.151	-.305	.056	.832	.441	.959
2	Area	3.14 ±3.65	4.25 ±4.25	.202	-3.01	0.79	.849	.406	.972
	Peak	248 ±261	273 ±239	.575	-.126	.076	.891	.566	.977
3	Area	5.66 ±6.92	7.97 ±8.89	.055	-4.69	0.06	.894	.514	.977
	Peak	330 ±419	413 ±482	.085	-.180	.014	.949	.759	.989
4	Area	6.19 ±6.82	7.26 ±8.10	.198	-2.83	0.06	.948	.798	.988
	Peak	262 ±285	339 ±481	.400	-.282	.127	.812	.354	.949
5	Area	4.72 ±6.12	6.31 ±5.79	.067	-3.33	0.14	.903	.567	.978
	Peak	188 ±225	296 ±325	.073	-.233	.013	.812	.275	.960

Note; MEPshort = delimited to 30ms, MEPlong = full length MEP, MEParea = ms.mV, MEPpeak = mV (the highest point during the MEP). Blocks consisted of 4 MEPs. Paired 2 tailed t-tests, intra-class correlation coefficients (ICC) and 95% confidence intervals determined test-retest reliability

4.6 Discussion

In a repeated sequence of 5 trials of MEP area elicited from the resting TA of PwMS, we observed that for MEPLong, the first MEP (T1) was significantly larger than subsequent MEP trials (T2-T5), confirming previous observations in healthy adults (Brasil-Neto et al, 1992). Having excluded T1 from MEPLong analysis, we found that subsequent trials (T2-T5), and blocks of 4 trials were stable over a 20 minute period. MEPshort remained stable throughout the experiment. Whilst there was a great deal of heterogeneity between individuals in MEPs collected and analysed from resting muscle, motor thresholds, MEP latencies and MEP area markers were stable when re-tested on a separate occasion 7-14 days later. When considering the effect of delimiting MEPs to 30ms, the ICCs revealed that blocks of MEPLong (after eliminating T1) and blocks of MEPshort produced high levels of reliability (Portney and Watkins, 2000). Our results suggest that with 2 stimulators firing a single pulse, reliable baseline measures can be obtained from resting muscle with as few as 2 trials after excluding the first trial. In addition we found that MEPs can be delimited to 30ms duration in order to minimize contamination by signal artifact with no effect on reliability.

To the authors' knowledge this is the first study to report the reliability of MEP area elicited from resting muscle in the lower limb of PwMS. While our findings may have important implications for designing TMS test paradigms within this clinical

group, they need to be considered alongside previous research exploring TMS methodology (Rico et al, 2009; Gagliardo et al, 2010; Rothkegel et al, 2010). The mechanisms underlying responses to TMS are unclear. MEPs are known to be sensitive to factors relating to coil placement and orientation, stimulus magnitude, muscle activation and mood state. Even motor imagery of a simple lower extremity movement can evoke increases in corticospinal excitability (Bakker et al, 2008). Indeed, although the true cause remains uncertain, the consistently larger size of T1 is thought to reflect an initial facilitation (Schmidt et al, 2009) perhaps in anticipation of the impending series of TMS stimuli (Moosavi et al, 1999).

Interpretation of MEPs can be further confounded by the well documented trial to trial variability that occurs within subjects, between subjects and between tests (Ellaway et al, 1998; Wassermann, 2002), though the causes of the instability remain unexplained. The general consensus is that MEP variability in the general population is due to intrinsic, time-varying changes in the excitability of corticospinal and motorneuronal pathways (Ellaway et al; Rosler et al, 2008). Indeed, the higher interindividual variability, higher ICC values with respective 95% confidence intervals we obtained within a session rather than on separate days, and the lack of bias in our data recorded both within a session and on separate days, together suggest that the variability in TMS responses within our group of PwMS is also mostly due to inherent, time-varying neurophysiological processes rather than measurement bias per se.

Whilst our findings are novel, they are limited by the small number of PwMS taking part. However, the 10 individuals with MS that were included in the experiment were representative of a broad age range (30 - 67 yrs old), the elapsed time since first diagnosis of MS spanned from 2 to 32 years. They were characteristic of a relatively high functioning group of MS patients in terms of mobility and their self-reported ability to perform independent activities of daily living (see Table 1) (Gijbels et al, 2010). Individuals imaging data (MRI) was not available, therefore we cannot identify individuals by their current MS criteria (Polman et al, 2011) and other pathologies that may have affected our results. Thus our results may not extrapolate to all individuals with MS, and further studies are required to investigate whether our findings are replicated in individuals with higher levels of disability.

MEPs have been shown to be significantly affected by the methodology (Rico et al, 2009; Rothkegel et al, 2010). as such, our findings using the Bistim² stimulator configured with a 110mm double cone shaped coil producing a pulse with a magnetic field rise of 176ms @ 100% (theoretically 131ms @ 75%max stimulator output) do not extrapolate to other stimulator configurations. With this configuration we recorded notably lower TA motor thresholds from PwMS than those recently reported from the TA of healthy subjects (Cacchio et al, 2009; Cacchio, 2011) and those previously reported in PwMS (Jorgensen et al, 2005) using a single stimulator.

Demyelination and axonal damage in MS can result in conduction abnormalities, abnormal temporal dispersion and desynchronisation of descending action

potentials leading to phase cancellation that can reduce the size of MEPs. In addition, the age of the participant, temperature intolerance, the categorisation of MS, certain medications and fatigue may further limit MEP size. Moreover rectification of the EMG signal may have obscured phasic waves specific to MS. Our method possibly lacks the sensitivity of the triple stimulation technique (TST) to quantify small to moderate deficits in conduction parameters relevant to MS (Humm et al, 2003; 2004; 2006) however TST of the lower limb is a relatively complex, technically demanding, invasive procedure (Buhler et al, 2001) which may limit its availability to quantify TMS responses in the lower body either during, or immediately following therapeutic exercise interventions.

An important finding from this study is that MEPs can be reliably elicited from resting muscle in PwMS. MEPs are frequently facilitated during voluntary contraction of the target muscle in order to reduce variability (Darling et al, 2006). Maintaining a tonic contraction based on the percentage (customarily ~10-30%) of a maximal voluntary contraction (MVC) has a substantial effect on motor thresholds and MEP amplitudes (Darling et al, 2006). Maximum contractions can increase MEP amplitude by 50% in the general population (Di Lazzaro et al, 2008) and an increase in both the stimulus intensity and muscle activation has been shown to lead to a significant reduction in motor thresholds (Darling et al, 2006), however volitional effort-dependent manoeuvres to requiring maximal force depend to a large extent on subject motivation and understanding (Kesselring and Beer, 2005). In a clinical group with a neurological disorder such as MS, changes to the

muscle and/or nerve tissue, reduced central activation, symptoms of spasticity, increased sensations of pain or fatigue, together with cognitive difficulties (Schillings et al, 2007) may prevent the production of a true MVC especially in the lower limb. PwMS are frequently unable to contract their muscles voluntarily, are prone to high levels of fatigue and have symptoms which may affect their threshold to discomfort (Kesselring and Beer, 2005). Moreover, maintaining consistent levels of submaximal voluntary contraction in the leg muscles and producing the same level of consistency across several sessions, particularly after a dynamic exercise intervention lasting several minutes, can prove problematic for PwMS (Everaert, 2010). Thus establishing a methodology that facilitates the collection of TMS responses from resting muscle in this clinical group is extremely important.

4.7 Conclusion

This study found that dual TMS stimulators configured to fire simultaneously through a single coil can produce consistent, repeatable TMS measures from resting muscle in the lower limb of PwMS. In addition, we found that delimiting MEPs to 30ms is a valid method to analyse complex TMS responses induced by stronger pulses. While our findings suggest that with this technique, investigators need record just 2 trials to establish a baseline, it remains to be replicated in other studies. In view of the high level of inter-individual heterogeneity displayed in the MEPs elicited from this clinical group, collecting a low number of repeats in baseline testing on resting muscle may limit the sensitivity to detecting change. Moreover, post-intervention responses can display low levels of change with

increased variability, thus we recommend researchers obtain a relatively higher number of trials over a longer baseline period. These results point to an exciting opportunity to obtain important TMS data from resting muscle in the lower limbs of PwMS. Further studies are required to explore post-intervention MEP parameters in PwMS using this methodology.

Chapter 5 Exercise at maximal intensity

This chapter compares the cardiovascular and perceptive responses in people with multiple sclerosis (PwMS) to a group of healthy individuals during a graded exercise test (GXT) and through the post-exercise time-course to recovery.

5.1 Introduction

Chapters 1 and 2 presented the deficits in central motor drive, autonomic imbalance and cognitive impairment that can profoundly affect the ability of PwMS to perform exercise at high intensity. Exercise tests requiring maximal effort are used extensively, both in research and clinical settings to evaluate cardiovascular and functional capacity, to stratify potential health risks and to identify limitations and contra-indications to exercise. The accurate assessment of exercise capacity is essential for determining safe, effective exercise intensities for PwMS (ACSM, 2000). In addition, tests to volitional fatigue facilitate the design of activity programmes that promote greater adherence and compliance to clinical exercise prescriptions (Perri et al., 2002).

In healthy populations, heart rate and perceived exertion are used as accurate measures of exercise intensity (Noble and Robertson, 1996). At the cessation of intense exercise the heart rate decreases, in most cases at a significantly faster

rate in physically fit individuals than those from the sedentary population (Brown and Brown, 2007). Heart rate recovery (HRR) to pre-exercise measures is considered to be almost entirely regulated by vagal reactivation mediated by parasympathetic activity of the autonomic nervous system (ANS). Although the intensity and duration of exercise have a major influence on heart rate recovery (Coote, 2010) the precise mechanisms and relative contributions of sympathetic and parasympathetic activity to the cardiovascular response are still not well understood. Given that a significant number of PwMS show evidence of autonomic dysfunction involving the cardiovascular system (Senaratne et al., 1984), post-exercise recovery may be slowed. Autonomic processes are also thought to mediate the sensation of effort during exercise (Williamson, 2010) and the perceptual experience of intensity is a major contributory factor in the participant's decision to terminate a GXT (Wasserman et al., 2005). Previous investigations have reported that ratings of perceived exertion (RPE) in PwMS are similar to healthy control groups (Ng et al., 2000) with no significant difference between men and women (Romberg et al., 2004a, Morrison et al., 2008), however, studies have been limited by small group-sizes and restricted to PwMS with mild functional impairment. When considering the distinct symptomatic effects of exercise on the cardiovascular and neuromuscular systems, we propose that differential measures RPE may be more appropriate for this clinical group. Differential ratings of perception allow for the measurement of responses relating to specific functions in different parts of the body (Borg, 1990) meaning that sensations relating to

breathing can be measured distinctly from the sensation of working muscle in the legs (Noble and Robertson, 1996).

There remains a paucity of literature relating to physiological responses to exercise intensity in PwMS (Dalgas et al., 2008) and none relating to post-exercise recovery after maximal exertion. In addition, differential measures of RPE during a GTX and through the time-course to recovery remain unreported.

5.2 Hypothesis

We hypothesised that whilst performing exercise to a maximal intensity, individuals with MS would demonstrate reduced exercise capacity, but share similar physiological and perceptive responses with a group of healthy controls through both the active and recovery phases of a graded exercise test.

5.3 Methods and materials

5.3.1 Participants

Sixty one individuals with clinically definite MS (Poser et al., 1983) of mixed MS classification and 17 healthy volunteers of similar age and gender agreed to participate. Exclusion criteria for participants are described in Chapter 3.2. Each participant was required to sign an informed consent document in accordance with the Declaration of Helsinki (1983). The experimental protocols were approved by the Oxfordshire Research and Ethical Committee (08/H0604/3). Testing was

carried out in the Oxford Centre for Enablement, Oxford, UK. Participants were asked to refrain from the consumption of tea, caffeine, alcohol, cigarettes, for a period of at least 2 hours prior to testing, and not to take part in strenuous physical exercise for 24 hours prior to testing. The test procedures were carried out between 1200 and 1500hrs in a quiet room at standard temperature (~21°C).

5.3.2 Procedures

Personal details, MS classification, duration from disease onset, co-pathologies and treatment history were documented on arrival. Participants of both groups completed the Pre-participation Physical Activity Questionnaire (PAR-Q). The MS group completed the Barthel Index (BI), the Subjective Vitality Scale (VS) and the Fatigue Severity Scale (FSS). Declared medications were screened for precautions and contra-indications to exercise. Height, weight, resting heart rate (BHR) and blood pressure (BP) were recorded. Leg extension power (LEP) was measured in both legs with the Nottingham power rig (NUMAS, University of Nottingham Medical Faculty Workshops, Nottingham, UK) (Basseley and Short, 1990). The measurements were repeated until no further improvement was seen. The highest recorded power output was recorded in Watts (W).

5.3.3 Cycle ergometer

Individuals were seated on a friction-braked Monark Ergo-medic 824E cycle ergometer (Monark AG, Sweden) in an upright posture with the handlebars adjusted for comfort. Saddle heights were adjusted to accommodate partial flexion of the knee between 170° to 175° with 180° denoting a straight leg position. Feet were firmly supported in the pedals by toe clips and straps. Additional taping was used where necessary to ensure that both feet remained secure in the pedals during the test session and that the heel was held away from the pedal crank (Mulcare and Jackson, 2004).

5.3.4 Measurement of oxygen uptake

Peak oxygen consumption and respiratory exchange ratio were measured via the collection of expired air via a face mask (Hans Rudolf, Kansas City, USA) connected to a breath-by-breath gas analyser (Metamax 3B, Cortex Biophysik GmbH, Leipzig, Germany). The gas analysis system was calibrated according to manufacturer's instructions with known concentrations of oxygen and carbon dioxide (~15% O₂ and ~5% CO₂, balance N). Breath by breath measures of gas concentrations and volumes were digitally stored on a personal computer. Heart rate was recorded from a chest monitor (Polar Electro, Kempele, Finland).

5.3.5 Exercise test

A continuous multi-stage graded exercise test was employed. The assessment started with 2 minutes unloaded cycling at the target cadence of 50 revolutions per minute (rpm). Participants who were unable to achieve the selected cadence of 50

rpm unloaded pedalling were asked to maintain a lower maintainable cadence (45 or 40rpm). At precisely two minutes into the test a weight of 0.5kg was placed in the weight plate equating to an increase of 25 Watts workload at 50 rpm (Carroll and Lambert, 2009). Resistance was then added in 25 watt increments every 2 minutes. Measures of work-load, HR, RPE breathing and legs, and tympanic temperature were taken in the last 30 seconds of each incremental stage. Participants received verbal encouragement throughout the test to perform at maximal effort.

5.3.6 Test termination

In view of the broad range of physical impairment and individual fitness levels of PwMS, a set end-point based on meeting maximal criteria was not suitable. Hence, the GXT was symptom-limited. Individuals were instructed to pedal until discomfort, pain or fatigue prevented them from maintaining the required cadence (Jackson and Mulcare, 2009b). On ceasing exercise, participants were asked their reason for stopping. The face mask was removed, their BP was recorded and the participants were then assisted to a chair positioned within 1 meter of the cycle ergometer. During the post-exercise recovery phase, HR, BP, Temp°C and RPE were recorded at 3 minutes, 5 minutes and 10 minutes.

5.4 Statistical analysis

Data was imported into SPSS v 17.0 (SPSS inc, Chicago, USA) software for statistical analysis. Normal distribution of data was determined using a

Kolmogorov-Smirnov test. In view of the disparity in sample sizes, differences between the MS and control groups were calculated as continuous variables using one way analysis of variance (ANOVA) with the group as the single factor variable presented as the mean \pm standard deviation (SD) (Bewick et al., 2004). Linear regression was used to test the strength of association between variables (Bewick et al., 2003). Appropriate parametric and non-parametric correlation coefficients determined the strength of association between baseline, performance and recovery measures. Expired air measures were calculated as mean values from the last 15 seconds of each stage. To investigate the relationship among several variables, multivariate regression models were fitted with various criterion measures as dependent or independent variables. All regression models were based on the mean values of participants when all measurement parameters were present. Recovery profiles between groups were calculated measures using one way ANOVA. Linear and quadratic equations were fitted to the recovery data and relationships determined by the strength of correlation coefficient together with the significance of slope coefficients. Significance was set at alpha ($p < 0.05$). Cohen's classification of correlation coefficients was used to determine strength of association, $r \geq 0.50$ is rated as strong, $r = 0.30$ to 0.49 is considered moderate, and $r = 0.10$ to 0.30 are considered weak when $p < 0.05$ (Weinburg and Abramowitz, 2008).

5.5 Results

This study compared resting, energetic and recovery parameters derived from a maximal exercise test in healthy individuals and PwMS. Descriptive details of participants and resting measures are detailed in Table 5.1. There was no difference between groups in age, gender, height and weight. Baseline resting measures between the MS and control groups did not differ significantly ($p > 0.05$) except for RPEleg ($p < 0.05$). MS group pre-GXT functional test scores as measured by the Barthel Index, Vitality Scale and Fatigue Severity Scale are presented in Table 4.2.

One participant with MS who showed signs of uncontrolled hypertension during the recording of baseline measures was referred back to their general practitioner. Two participants with MS who experienced dizziness and nausea when sitting on the cycle-ergometer withdrew prior to the GXT commencing. Four PwMS whose results were asymptomatic of MS were excluded from further analysis. The cardiovascular response of one control participant was affected by beta-blocker and consequently discarded however, measures of other variables showed no effect and were therefore included. Therefore the results of 54 PwMS (21 RR-MS, 26 SP-MS, 7 PP-MS) and 17 age-matched controls were analysed.

Table 5.1 Descriptive details and resting measures of participants

Group	MS	CG	P-value
N=	54	17	
Gender	<i>M</i> =18; <i>F</i> = 36	<i>M</i> =3; <i>F</i> =14	.410
Age (years)	52.8 ±9.0	48.9 ±5.7	.109
Height (cms)	166.7 ±5.9	167 ±8.0	.369
Weight (Kgs)	72.3 ±14.1	75.1 ±8.8	.439
Resting measures			
HR (bpm)	74.2 ±9.6	71.7 ±9.6	.385
BP Systolic (mmg)	133.3 ±18.3	130.3 ±18.1	.558
BP Diastolic (mmg)	78.0 ±11.1	83.8 ±8.5	.054
Temp°C	36.4 ±0.4	36.6 ±0.3	.187
Blood lactate mmolL	1.6 ±0.6	1.5 ±0.5	.840
RPEbr (CR10)	0.8 ±0.9	0.8 ±0.7	.842
RPEleg (CR10)	1.1 ±1.2	0.2 ±0.4	.009

One- way ANOVA determined differences between groups.

Table 5.2 Multiple sclerosis disability scores and functional test results

Test	N = 54 Mean (\pmSD)	min	max
Barthel Index	18 (median)	11	20
Fatigue severity scale	4.43 \pm 1.03	1.5	5.9
Subjective Vitality Scale	4.62 \pm 1.56	1	7
2 minute walk test (metres)	96.85 \pm 50.26	9.0	205.9
Timed up and go Test (secs)	18.46 \pm 21.44	4.1	117.0
Average leg strength (l/r)watts	103.81 \pm 56.76	27.4	254.2

Results of peak performance are summarised in Table 5.3. No adverse reactions were reported from individuals of either group during the test. The results indicate that MS group means (\pm SD) failed to reach any criterion measure considered to represent peak performance. Average age predicted HRmax values ($220 - \text{age}$) were calculated as $167.2\text{bpm} \pm 9.1$ for the MSG, and $171.1\text{bpm} \pm$ for controls. The MS group achieved only 82.5% of age-predicted HRmax compared to 98% for the control group. Conversely, the control group mean exercise measures reached recognised criteria for peak testing on two measures; mean heart rate of within \pm

10 beats of age predicted HRmax, mean RER value greater than 1.10 (Newton et al, 2008). Significant differences existed between groups in all peak measures except for RPEleg ($p>0.05$).

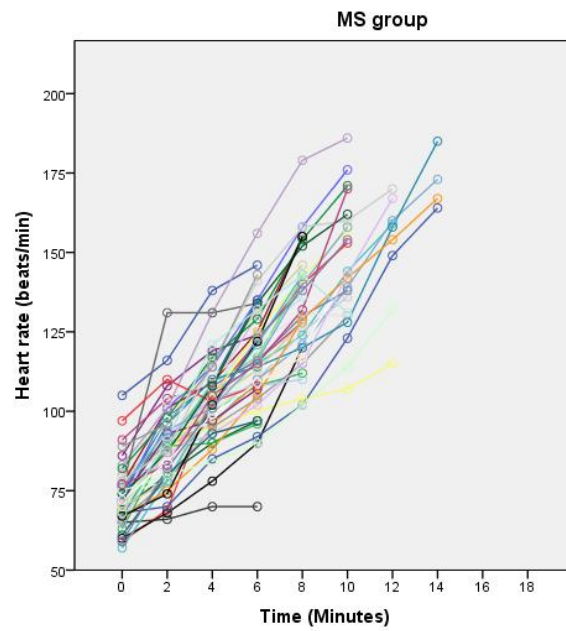
Table 5.3 Comparison of group peak performance values

Parameter	N	MS group mean (\pm SD)	N	Control group mean (\pm SD)	p
Max Watts	54	97.4 \pm 35.2	17	161.8 \pm 43.4	.000
HRpeak (bpm)	53	140.1 \pm 24.8	16	167.7 \pm 9.37	.000
VO2max mL.kg ⁻¹	48	20.1 \pm 6.4	10	27.8 \pm 6.8	.001
RER	47	1.03 \pm 0.12	10	1.15 \pm 0.05	.001
RPEbreathing	54	5.1 \pm 1.7	17	6.8 \pm 2.3	.001
RPElegs	54	6.2 \pm 1.6	17	7.0 \pm 1.8	.093
BLa peak (mmol/L)	49	5.4 \pm 3.7	14	7.7 \pm 2.1	.013
Temp °C	44	36.4 \pm 0.4	11	36.8 \pm 0.5	.006

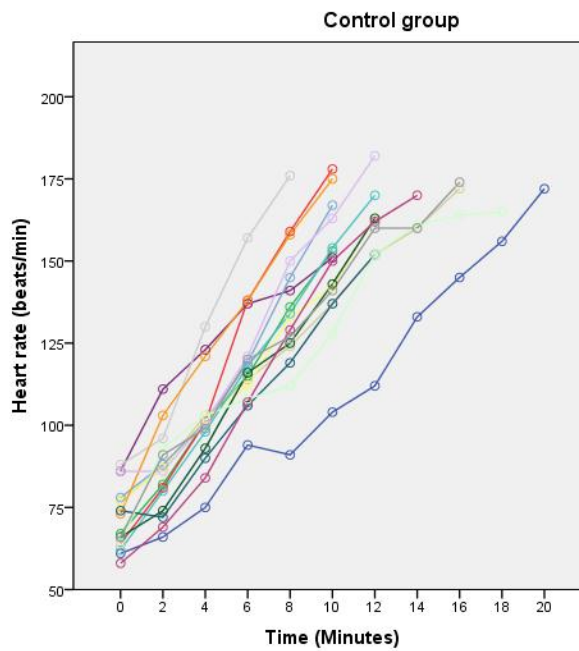
Note: One-way ANOVA determined differences between groups for all variables.

In the MS group there was a strong correlation between functional walking capacity as measured by the 2MTW, and $\dot{V}O_2$ max ($r= .59, p <0.05$), max watts ($r= .56, p <0.05$) HRpeak ($r= .55, p <0.05$) and RER ($r=.50, p <0.05$) In addition leg strength was strongly correlated with maxWatts ($r=.71, p <0.05$) and a moderate relationship existed between leg-strength and HRpeak ($r=.59, p <0.05$). Figure 5.1 shows the linear relationships between heart rate, differential measures of RPE, and oxygen consumption relative to percentage of watts peak work load (watts), for MS and CG groups. ANOVA of between group differences found no evidence of altered heart rates during the first 3 stages of the GXT ($p <0.05$). Linear regression was determined by curve estimation as the best line of fit. Regression analysis revealed a high level of variability in MS group measures. For the purpose of this study, recovery will be defined as the post-exercise decline from peak to baseline values (\pm SD). The MS group displayed a significant ($p <0.05$) curvo-linear relationship between time in minutes post-exercise and HR recovery as a percentage of mean baseline measures ($R^2=0.99, y=11.12x^2-80x+264.28$) while the control group produced a non-significant ($p >0.05$) quadratic relationship ($R^2=0.97, y=20.173x^2-134.46x+349.46$).

Figure.5.1 Changes over time to heart rate during the graded exercise test

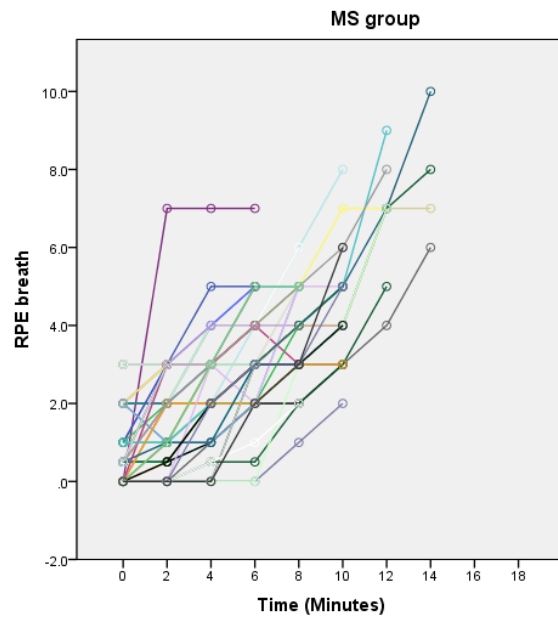


N = 51

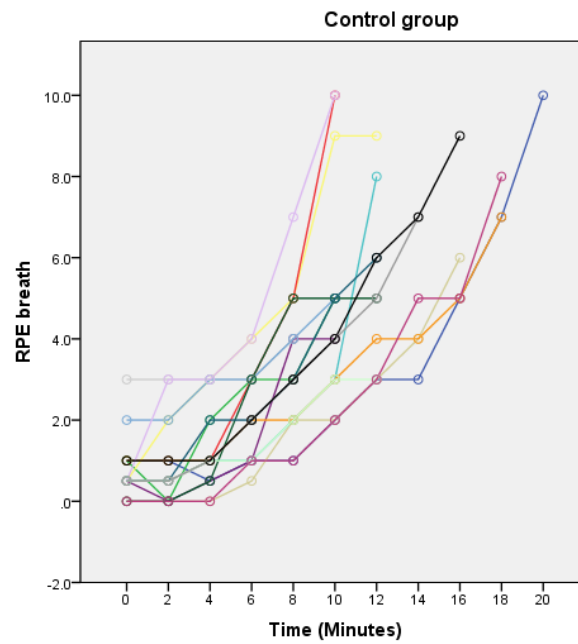


N = 17

Figure 5.2 Changes over time to RPE breathing during the graded exercise test

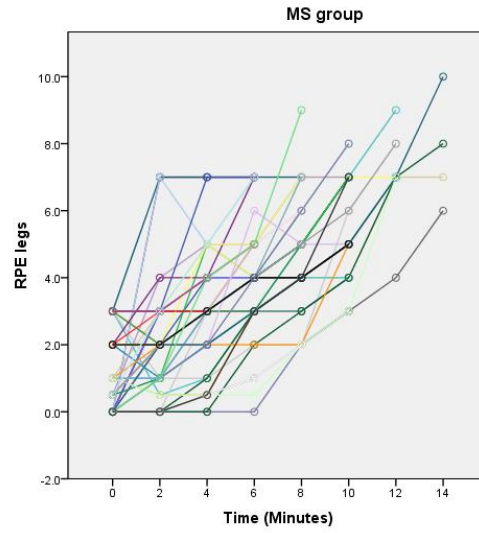


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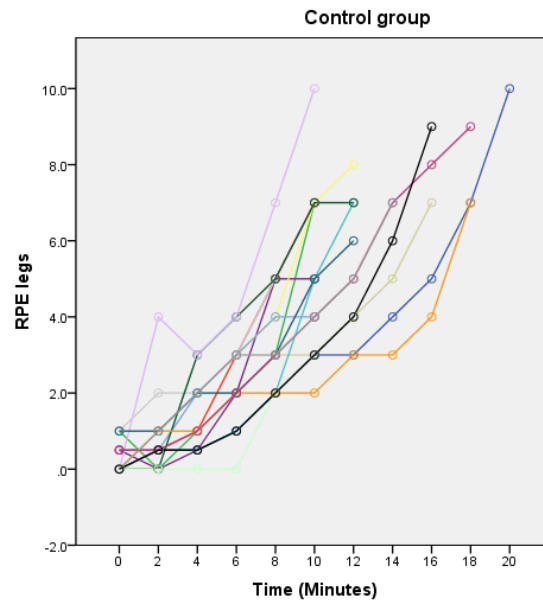


N = 17

Figure 5.3 Changes over time to RPE legs during the graded exercise test

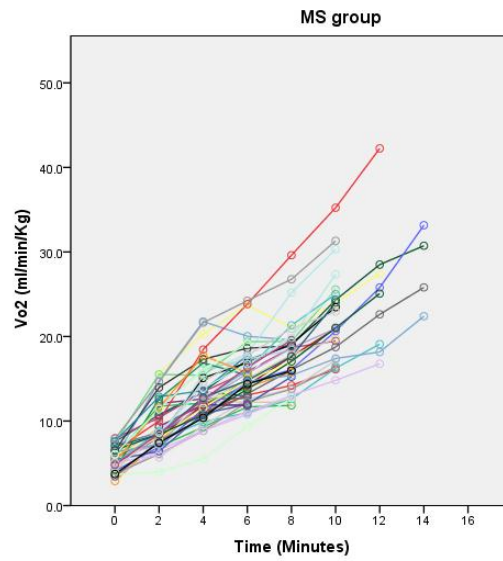


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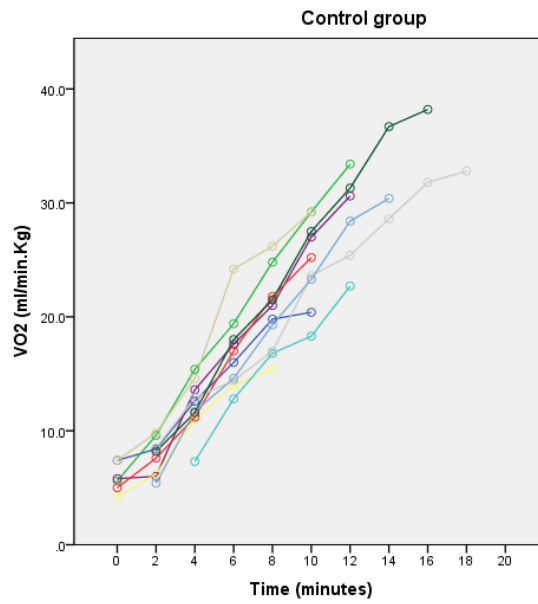


N = 17

Figure 5.4 Changes over time to oxygen uptake (VO_2) during the graded exercise test

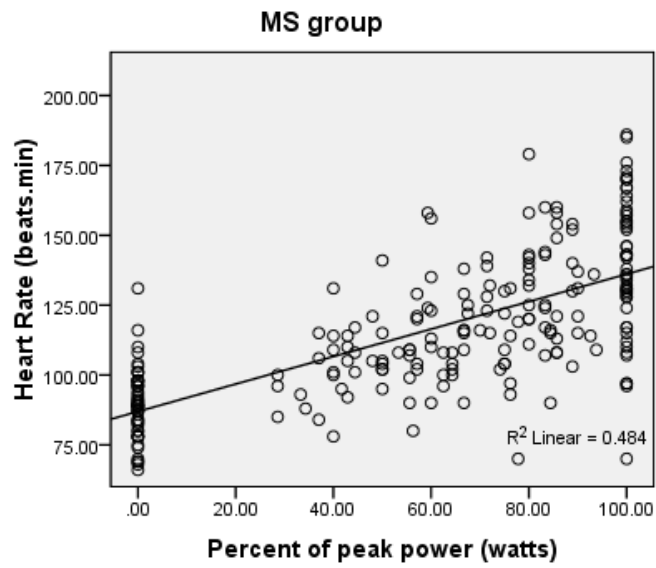


N = 48

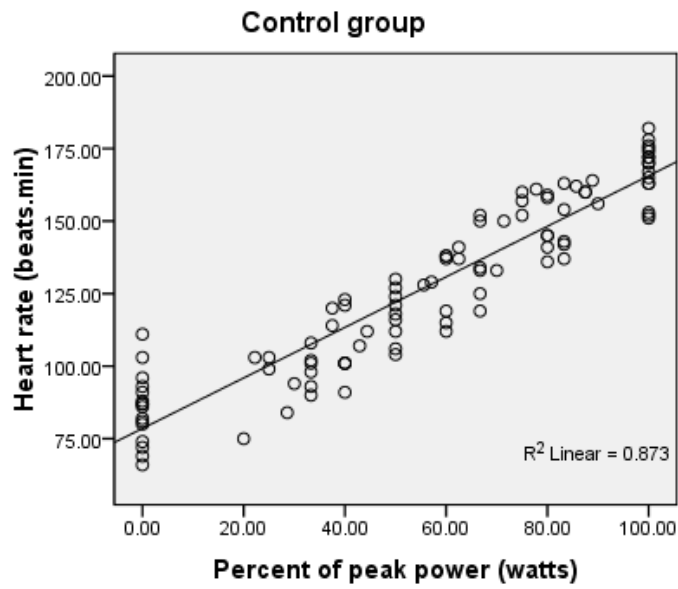


N = 10

Figure.5.5 Scatterplot of heart rate to % peak power (watts)

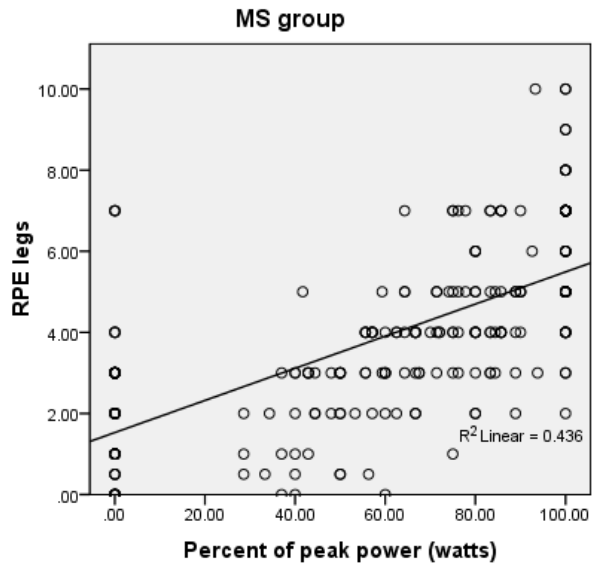


n=51

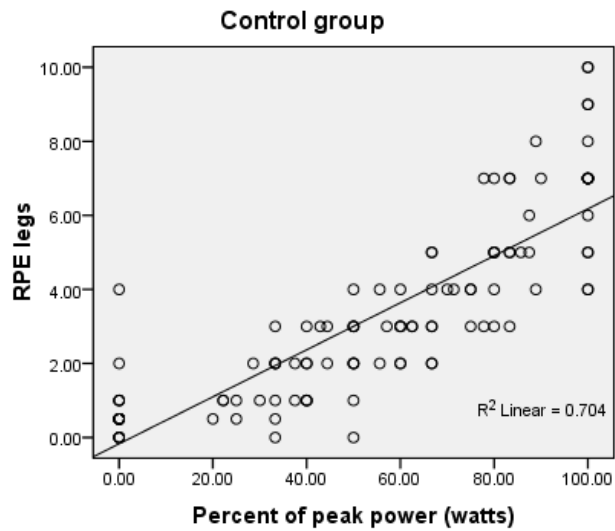


n=17

Figure 5.6 Scatterplots of ratings of perceived leg exertion to % peak power (watts) during a graded exercise test

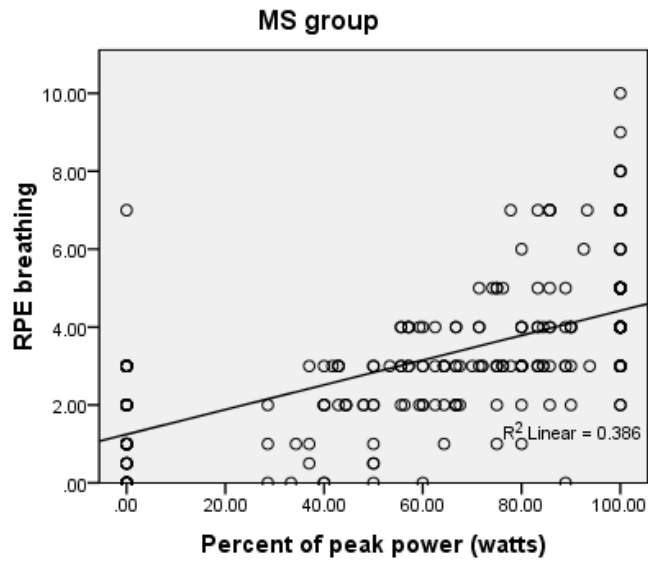


n=53

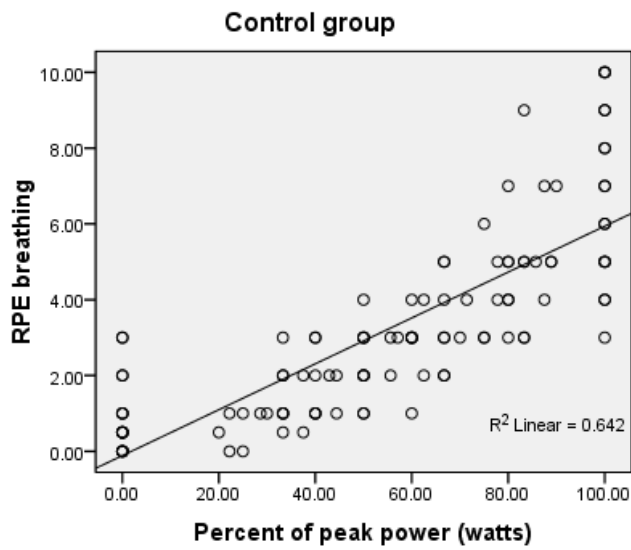


n=17

Figure 5.7 RPE breathing to % peak power (watts) during a graded exercise test

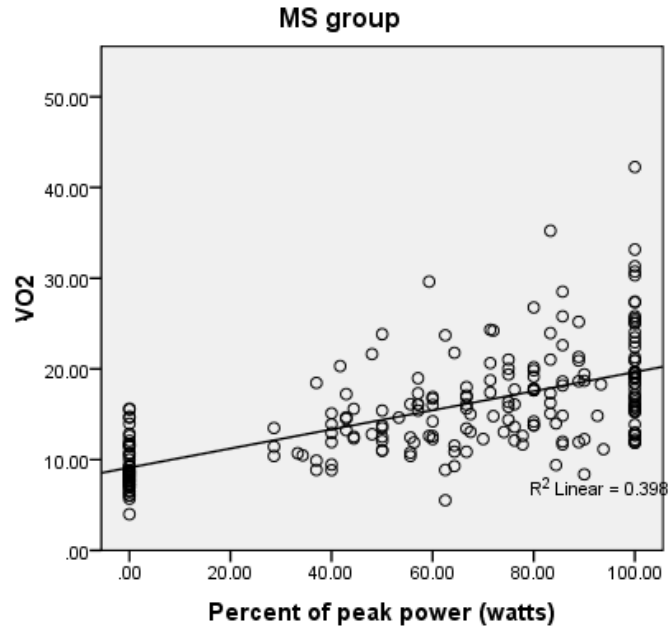


n = 53

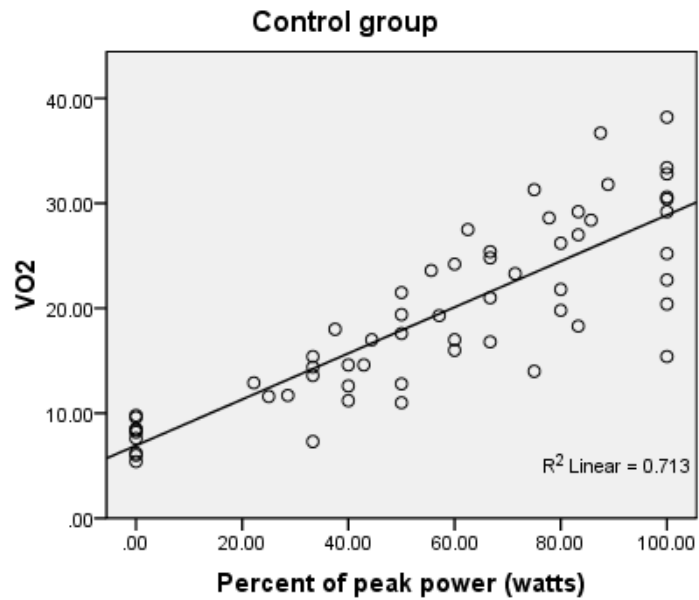


n = 17

Figure 5.8 Maximal oxygen uptake (VO₂) to % peak power (watts) during a graded exercise test



n = 48



n = 10

There was no change in MS Temp°C from pre-exercise values, during or following the GXT. Multiple regression analysis found no significant effects on the peak measures of variables when weighted by MS classification (RR-MS, SP-MS and PP- MS). Using the enter method with age, gender, weight, MS Classification and the time elapsed since diagnosis (years), a significant model emerged: $F = 6.813$, $p < .0005$, however the regression model revealed that only age and weight were moderately predictive of 36% of the variance (adjusted $R^2 = .36$) in peak work-rate. Repeated measures ANOVA found no difference between PwMS reporting a pre-exercise RPEleg value of 2 or higher ($n=18$), and the remainder of the MS group, in baseline or performance variables. Twenty eight of the MS group cited leg pain, leg weakness, or the inability to maintain the cadence as the reason for terminating the test. The remainder of the MS group, and all members of the control group cited non-specific reasons such as 'had enough' or 'unable to continue' for terminating the GXT.

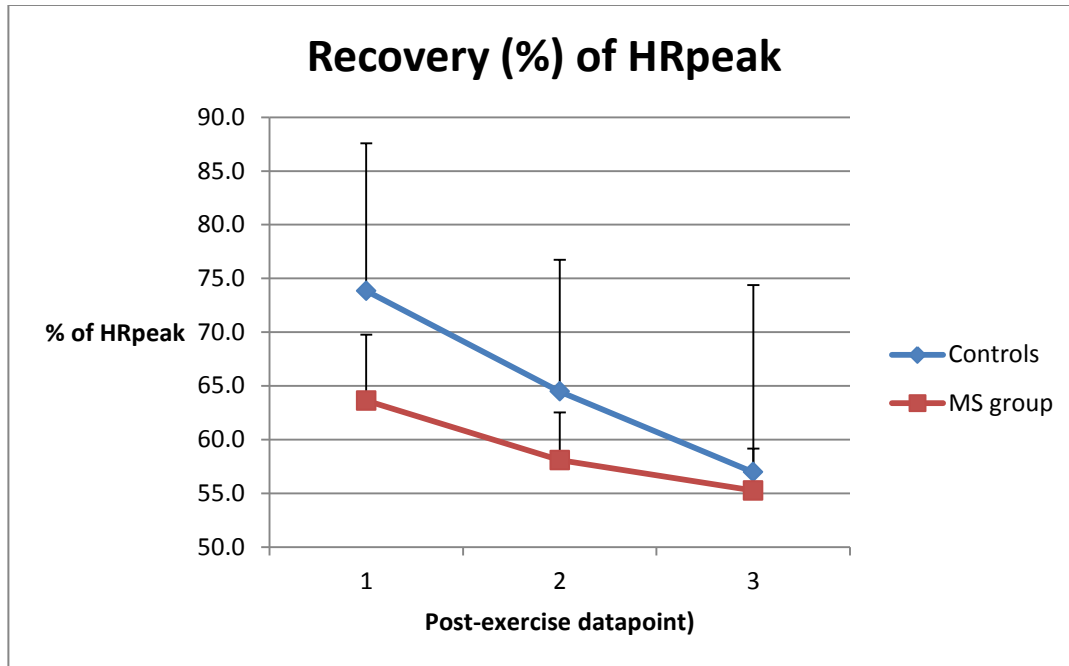
The recovery profiles (Table 5.4) show significant differences ($p > 0.05$) between group recovery values for RPEleg at 10 mins and Temp°C at 3 and 10mins post-exercise. Although the control group exercised at a significantly higher work rate, HR recovered at a faster rate than the MS group, remaining marginally above pre-exercise HR values at 10 minutes post-exercise. Differential measures of RPE for both groups recovered to pre-exercise values at 5 mins (\pm SD).

Table 5.4 Measures of heart rate, RPE and temperature during recovery

	MS	CG	<i>p</i>	MS % of Peak	CG % of Peak
HR (beats.min⁻¹)					
3mins	102.8 ±23.8	107.3 ±10.7	.472	73.8 ±13.7	63.6 ±6.1
5mins	90.6 ±16.1	97.9 ±8.2	.087	64.5 ±12.3	58.1 ±4.4
10mins	85.7 ±14.9	93.1 ±7.3	.059	57.0 ±17.4	55.3 ±3.9
RPE(CR-10) breathing					
3mins	2.4 ±1.5	2.4 ±1.3	.957	55.4 ±26.8	36.3 ±19.2
5mins	1.6 ±1.1	1.6 ±1.0	.841	40.5 ±23.6	24.4 ±17.0
10mins	1.0 ±1.0	0.8 ±0.8	.544	22.8 ±24.4	11.4 ±9.7
RPE(CR-10) legs					
3mins	3.3 ±1.7	2.5 ±1.6	.069	53.9 ±28.6	35.4 ±18.2
5mins	2.4 ±1.4	1.7 ±1.3	.070	39.7 ±26.7	24.0 ±14.7
10mins	1.8 ±1.2	1.0 ±1.1	.020	28.8 ±23.9	13.9 ±11.8
Temp°C					
3mins	36.5 ±0.5	36.9 ±0.6	.026	-	-
5mins	36.5 ±0.4	36.9 ±0.5	.105	-	-
10mins	36.4 ±0.4	37.0 ±0.3	.007	-	-

Post-GXT data-points showing mean group measures and values relative to peak exercise measures.

Fig 5.9 Recovery profile as a percentage of peak heart rate



5.6 Discussion.

This study confirmed a substantially reduced exercise capacity in PwMS when compared to healthy controls, but also observed that during maximal exertion, individuals with MS were neither limited by their heart rate, nor their breathing. Rather, when considering the comparable ratings of exertion experienced in their legs with the control group at exercise termination, the strong relationship of leg strength to peak exercise performance, and the reasons PwMS gave for terminating the GXT, it would appear that leg fatigue, or lack of drive to the lower limbs were the limiting factors to achieving higher peak exercise values in this group. Considering recovery, PwMS recovered at a slower rate than the control

group. More importantly it was observed that PwMS continued to experience sensations of exertion in the lower limbs, even during rest.

Participants in the MS and control groups were similar for gender, age, height and weight. There were no significant differences between the two groups in these parameters. Likewise, with the exception of RPE leg, no significant differences existed between group values of the pre-GXT resting measures. However significant differences existed in the average peak exercise values, with the exception of RPEleg. The protocols and methods used were identical for both groups. Consequently, the differences in the GTX peak performance values can be attributed to impairments and deficits caused by MS. Because the responses to incremental cycle ergometer tests in healthy groups have been well described, the discussion section of this Chapter will focus on the results relevant to the MS group.

Several factors may have contributed to the high level of variability in the performance measures of the MS group. Cardiovascular fitness levels and muscular MS symptoms between individuals, differences caused by impaired central motor drive, intolerance to environmental factors or to the exercise itself may have affected exercise performance. Small increases in body temperature have been shown to affect walking velocity (Humm et al., 2004). Bladder dysfunction frequently causes PwMS to limit fluid intake (Collett et al., 2011a) which may in turn increase the risk of symptomatic heat intolerance (Fromont et al.,

2010), generate fatigue and limit peak performance (Freal et al., 1984, Humm et al., 2004). We attempted to minimise diurnal fluctuations of MS symptoms by performing the tests at the same time of day, however PwMS are prone to unpredictable episodes of fatigue, spasticity, pain or weakness with sudden-onset, even at rest.

Previous studies have been inconsistent when attempting to establish an association between exercise capacity and disability as measured by the expanded disability scale (EDSS) (Foglio et al., 1994, Motl and Goldman, 2011, Romberg et al., 2004a) In view of the well reported limitations of the EDSS (Goodkin et al., 1992, Herndon, 2007), its poor psychometric properties (Hobart et al., 2000) and its inability to measure fatigue related to disability (Polman et al., 2006) we assessed levels of ambulatory impairment by comparing the results of the TUG and T2MW to those from previous studies. Recently, Nilsagard et al,(2009), Gijbels et al, (2010), and Sosnoff et al,(2011) have reported mean TUG times of 17seconds, 15.7 seconds and 16 seconds respectively for groups of PwMS with moderate ambulatory impairment (Nilsagard et al., 2009, Gijbels et al., 2010, Sosnoff et al., 2011). In contrast, an average TUG time of 8.8 seconds has been reported for 35 ambulatory subjects with relapsing-remitting MS(Weikert et al., 2011), agreeing with the results obtained from other mildly-impaired MS groups. The mean TUG time of 17 seconds achieved in this study strongly suggests that, on average, our MS group was moderately impaired, but representative of a very broad range of ambulatory ability (from mild to moderately severe). This assessment is supported by the results of the T2MW. In their recent examination of

walking performance in PwMS with mild or moderate ambulatory impairment, Gijbels and colleagues reported an average distance of 104 metres (± 41) for the moderately impaired group, agreeing with the 97 metres (± 50) walked by our MS group. Again, the distance walked, ranging from 9 metres to 206 metres (showing normal distribution) reflects a very broad range of ambulatory ability within the group.

When matching the $\dot{V}O_2$ peak results with other groups of similar age and moderate ambulatory impairment utilising similar exercise ergometry, values of 21.7 ± 6.0 ml.kg¹min¹ (Romberg et al., 2004a) and 21.7 ± 5.5 ml.kg¹min¹ (Petruzzello and Motl, 2011) compare closely to the 20.1 ± 6.4 ml.kg¹min¹ achieved in this study. In summary, when compared to other MS studies, the results of the GXT covers the broadest range of ambulatory abilities in PwMS reported to date, with the mean measures of the functional mobility tests confirming the moderate ability status of the group. Interestingly, peak work-rates correlated strongly with the pre-exercise average leg strength ($r = .71$) suggesting that leg strength may be a predictive measure of exercise capacity in PwMS, and as such warrants further investigation.

With regards to the concerns of PwMS performing exercise at high intensity (Collett et al., 2011b), the question remained whether a sub-maximal exercise test (SMXT) would serve as an appropriate alternative to the GXT. The SMXT differs to the GXT in that it is terminated at some pre-determined heart rate intensity. Moreover,

the SMXT assumes that the mechanical efficiency (ie the $\dot{V}O_2$) at a given work rate is stable (ACSM, 2000). Our results revealed that the relationship between $\dot{V}O_2$ peak and work-rate for the MS group (as shown in Figure 2) to be highly variable ($R^2 = .39$), probably the result of reduced drive to the legs. Furthermore, the SMXT assumes that an individual's maximal heart rate fits the HRmax equation $220 - \text{age}$ (Londeree and Moeschberger, 1984). Because heart rate and $\dot{V}O_2$ generally show a linear relationship in response to an increase in work-rate, the slope of HR/ $\dot{V}O_2$ regression can be extrapolated to the $220 - \text{age}$ estimate of HRmax (ACSM, 2000). However, on average the MS group achieved only 82.5 % of age-predicted HRmax, a result consistent with that reported (Ponichtera-Mulcare et al., 1994) whose group of 8 semi-ambulatory MS subjects achieved 81% of age-predicted HRmax from a GXT on similar ergometry.

When considering that 40 individuals from our MS group who failed to achieve the criterion measure of $\dot{V}O_2$ max averaged only 74% of age predicted HRmax, the heart rate assumptions of the SMXT are clearly not met. This has important implications in the clinical and fitness settings for this clinical group. An exercise prescription or SMXT based on age-predicted HRmax is at risk of substantially over-estimating the exercise capacity of individuals with MS. In view of their recovery to baseline measures within 10 minutes of test termination, and that none reported adverse reactions, either during the GXT or the recovery period, it seems

reasonable to suggest that the GXT should remain the 'gold standard' for assessing exercise capacity in the MS population.

The recovery slopes in this study indicate that the HR of PwMS recovered at a substantially slower rate than those of the control group. At 3 minutes post-exercise, the HR of the control group had dropped 36.6 % from HR peak. In comparison the HR of the MS group had only fallen 26.2%. Quantifying HRR after intensive exercise is difficult because rates of recovery can be affected by multiple factors such as an individual's age, gender and level of cardiovascular fitness (Darr et al., 1988), or the duration and intensity of exercise performed (Buchheit et al., 2007). Nevertheless, in the MS group we observed a statistically significant moderate correlation between HRR at 3 minutes, 5 minutes and 10 minutes and the peak exercise values of watts ($p < 0.05$) and $\dot{V}O_2$ ($p < 0.05$).

An attenuated HRR measured within 2 minutes of exercise has been demonstrated by numerous studies to be prognostic of all-cause mortality in both clinical and healthy populations (Ellestad, 1996, Cole et al., 1999, Georgoulas et al., 2009), although there is conflicting evidence as to whether a delay in HRR provides a reliable prognostic marker when taking into account factors such as gender (Wandell et al., 2010), spinal cord injury (Myers et al., 2010), previously unidentified clinical variables and individual differences in the exercise response (Morshedi-Meibodi et al., 2002, Singh et al., 2002, Myers et al., 2010). With regard

to MS, there is growing evidence that HRR measured at 3 minutes and 5 minutes post-exercise is significantly impaired in people suffering other chronic inflammatory disorders such as Sarcoidosis (Ardic et al., 2011), Buhçet's disease (Kaya et al., 2009) and Systemic Lupus Erythematosus (Dogdu et al., 2010). Studies examining associations between brainstem lesion load and abnormal cardiovascular responses in PwMS have provided contradictory results (Anema et al., 1991, Acevedo et al., 2000, Vita et al., 1993).

Evidence for cardiovascular autonomic dysfunction in PwMS is well established (Acevedo et al., 2000, Mahovic and Lakusic, 2007, Anema et al., 1991, Nasser et al., 1999) however the inflammatory processes and sympathetic and parasympathetic mechanisms, and their specific contributions to HR and HRR during exercise and recovery have yet to be determined. Whilst HRR is generally considered to be almost entirely regulated by parasympathetic activity of the autonomic nervous system (ANS) exerting its influence on the vagal nerve, Buchheit and colleagues have demonstrated that post-exercise parasympathetic activation can be significantly mediated by multiple factors, including the type and intensity of exercise performed (Buchheit et al., 2007), body posture during the recovery period (Buchheit et al., 2009a) and body temperature (Buchheit et al., 2009b). Moreover, the delay in the speed at which the heart rate returns to baseline measures after exercise may reflect both deficits in parasympathetic mechanisms associated with an absence of vagal reactivation (Pierpont and Voth, 2004), and the influence of long-lasting sympathetic excitatory mechanisms

reflected by increased concentrations of norepinephrine (NE) that can remain in circulation for several hours after intensive exercise (Schairer and Keteyian, 2007). Interestingly, we found a moderate inverse correlation between HRR and the Barthel Index (BI) at 3 mins ($r=-.33$, $p<0.05$) that further strengthened between 5 mins ($r=-.52$ $p<0.05$) and 10 mins ($r=-.53$ $p<0.05$) suggesting a relationship between delayed recovery and physical impairment. The absence of statistically significant relationships between HRR values and other baseline measures of mobility (T2MW and TUG), leg strength and fatigue (FSS) would suggest that a delay in HRR may not correspond specifically to MS related disability, however the strength of association between HRR after intense physical activity and the BI as a measure of function, mobility and independence in activities of daily living warrants further investigation.

To our knowledge this was the first study to measure differential values of perceived sensation during the active and recovery phases of a GXT in PwMS. Morrison et al (2008) reported RPE responses from a group ($n=12$) of mildly affected PwMS during a GXT however, a direct comparison between the two studies was prevented due to differences in the level of ambulatory disability, the wide disparity in group sizes and the method employed. Nevertheless, the significance in our results, especially in the resting values of RPE leg, and in the differences of sensations from breathing legs at peak work-rate support the use of differential RPE in this clinical group. That RPE leg values were the only resting measures differing significantly from those reported by the control group ($p>0.05$)

raises the important question as to whether the MS participants fully understood the verbal anchors and the numerical value of the RPE scale. We would argue that the compliance represented by the RPEbreathing values, in addition to the agreement of the RPEleg values at maximal effort with those of the control group, give a clear indication that the MS group fully understood and complied with the instructions for reporting sensations from both the upper and lower body. Indeed, the return of post-exercise values of RPEleg to the same pre-exercise values ($p \leq 0.05$) adds weight to our view that the pre-exercise RPE values were an accurate assessment of the sensations that the MS group were experiencing in the lower limb at rest.

5.7 Limitations

For the purpose of this study participants with a broad range of disease severity were tested, nevertheless the exclusion criteria meant that non-ambulatory PwMS and those with contraindications that precluded maximal exercise testing were not represented. The selection of a homogenous group of PwMS is a challenge in view of the heterogeneous, fluctuating symptomology and progressive nature of the disease. The unpredictability of the MS participants' decision to terminate the GXT made it difficult to establish the precise mechanisms responsible. Cognitive impairment or anxiety may also have affected their motivation and compliance to the requirements of a GXT which in turn, influenced their time-course to post-exercise recovery. Nevertheless, whilst the results will not extrapolate to all PwMS,

this study has investigated a substantially wider range of physical abilities and exercise capacities than previous reports.

5.8 Conclusion

The majority of participants with MS were unable to sustain a work-rate conforming to published physiological and perceptive markers of maximal exercise capacity, however their ability to perform exercise at high intensity was not limited by cardiovascular factors but more likely was the result of reduced central motor drive to the legs. Performance and perceptive measures of exercise during the active and recovery phases are variable due to the heterogeneous nature of the MS symptoms. Even though they performed at a substantially lower work rate, the HRR of PwMS was delayed when compared to the control group, and a high percentage of the MS group continued to experience lower limb exertion, even at rest. Further study is required to determine the central factors that affect performance and the post-exercise response to exercise intensity in PwMS.

Chapter 6 Responses to exercise intensity in MS

This chapter examines physiological, perceptive and neurological response to exercise in MS to different exercise intensities, both during the active phase, and through the post-exercise time-course to recovery

6.1 Introduction

Whilst a considerable body of scientific evidence exists to guide exercise prescription for apparently healthy adults, clinical groups and individuals with long-term disabilities (Garber et al., 2011), there is a paucity of information relevant to the unique needs of PwMS. The physiological, neurological and perceptive response to exercise and post-exercise recovery in MS remains largely unexplored, and little or no scientific information is available upon which to base safe, effective and appropriate exercise strategies,

In Chapter 4 maximal exercise capacity in PwMS was shown to be lower than healthy controls. The results suggest that exercise performance is not limited by cardio-respiratory factors, but more by the inability to generate and maintain drive to the legs, perhaps reflecting central conduction abnormalities affecting the lower limbs (Shapiro, 2003). The characteristic neurological deficits of MS can be

mapped directly to demyelination and neuronal damage (Dutta and Trapp, 2011). Indeed, in MS the relationship between central conduction deficits, motor impairment and functional limitations affects health behaviours and adherence to exercise (Stuifbergen et al., 2006). In addition, there is an inverse association between neurological dysfunction and cardio-respiratory fitness (Motl and Goldman, 2011) that may further contribute to the inability of PwMS to maintain fatiguing exercise at higher intensity.

MS presents a complex array of symptoms. Two specific problems associated with exercise are motor fatigue and thermosensitivity (Dawes, 2008b, Beer, 2010). Motor fatigue worsens with heat, prevents sustained physical activity, and interferes with physical functioning (Schapiro, 2007). In addition, a strong relationship has been observed between fatigue, core temperature and ratings of perceived exertion during dynamic exercise in PwMS (Mulcare et al., 2001, Humm et al., 2004). Fatiguing activity also contributes to changes in corticospinal excitability (Taylor and Gandevia, 2008). The inability to maintain drive to lower limbs during exercise is multifactorial, but in MS may be the result of transient, heat induced alterations to conduction properties in demyelinated fibres (Fromont et al., 2010, van Diemen et al., 1992, Smith and Waxman, 2005, Humm et al., 2004), although the link between body temperature and temporary conduction abnormalities has yet to be proven (Humm et al., 2004).

Transcranial magnetic stimulation has been used extensively to investigate the central pathways following exercise (Brasil-Neto et al., 1994, Samii et al., 1996b, Ross et al., 2007, Meaney et al., 2007, Fernandez-Del-Olmo et al., 2011). Whilst TMS studies in MS have reported altered MEP characteristics between the fatigued and rested state (Sandroni et al., 1992), and post-exercise changes to MEPs have been observed after fatiguing muscle contractions in the upper body (Sheean et al., 1997, Perretti et al., 2004, Liepert et al., 2005), there have been few studies exploring central responses in the lower limbs following dynamic exercise (Schubert et al., 1998, Thickbroom et al., 2008). Moreover, although TMS of resting muscle is considered a sensitive measure of deficits in corticospinal pathways (Davey et al., 1999, Roy et al., 2010) few investigations have examined MEPs elicited from non-activated muscle in MS.

The purpose of this study was to compare responses to exercise intensity in PwMS to a group of healthy controls. We first monitored physiological and perceptive responses during single sessions of exercise training at different intensities, and then used TMS to compare neurological, physiological and perceptive responses through the post-exercise time-course to recovery.

6.2.1 Methods and materials

This is a single session comparator clinical trial of 2 single-session interventions with participants randomly allocated to the order of exercise intervention.

6.2.2 Setting

The experiment was carried out at the Nuffield Orthopaedic Centre, or in the Human Performance Laboratory, Oxford Brookes University. The experimental procedures were carried out by the same investigators between 1200 and 1500hrs in a quiet room maintained at standard room temperature (~21°C).

6.2.3 Participants

Sixteen people with clinically definite MS confirmed MS (Poser et al., 1983) and 11 age and gender matched controls were recruited to the study. The PwMS were referred through neurologists at the Nuffield Orthopaedic Centre and John Radcliffe National Health Service Trusts in Oxford, UK, or recruited through presentations at regional and local MS Society events and meetings. PwMS were required to provide written confirmation of their clinical status by their general practitioner or consultant neurologist. The TMS screening and exclusion criteria are detailed in Chapter 5. Each participant was required to sign an informed consent document in accordance with the Declaration of Helsinki (1983). The experimental protocols were approved by the Oxfordshire Research and Ethical Committee (08/H0604/3).

Participants were asked to refrain from the consumption of tea, caffeine, alcohol or cigarettes for a period of at least 2 hours before attending each session. In addition they were requested not to participate in strenuous physical exercise during the 24 hours prior to exercise sessions. On arrival at the test centre, personal details including MS classification, duration from disease onset, co-pathologies and

treatment history were documented. Participants completed a pre-participation physical activity questionnaire (PAR-Q), self-reported activity questionnaires, the Barthel Index (BI), the Subjective Vitality Scale (VS) and the Fatigue Severity Scale (FSS). Leg extension power (LEP) was measured in both legs with the Nottingham power rig (NUMAS, University of Nottingham Medical Faculty Workshops, Nottingham, UK) (Basse and Short, 1990). The measurements were repeated until no further improvement was seen. The highest recorded power output was recorded in Watts (W).

Each participant received a thorough description of the exercise test procedure and the methods that were to be used during subsequent exercise sessions. The participants were familiarised with Borg's differential ratings of perceived exertion (RPE) CR10 scale for breathing (RPRbr) and legs (RPEleg) (Borg and Kaijser, 2006). Declared medications were screened for precautions and contra-indications to exercise. Height, weight, baseline heart rate (BHR) and blood pressure (BP) were recorded.

6.2.4 Exercise schedule

Participants participated in a symptom limited, graded exercise test (GXT) performed on a cycle ergometer (Monark 874E, Monark AB, Vansbro, Sweden) to determine peak work rate measured in watts (W_{peak}). The GXT has been described in detail in Chapter 4. Peak measures of the GXT are shown in Table 7.1. Participants were then required to attend 2 exercise training (ET) sessions set

7 to 14 days apart. At each ET they performed 20 minutes of exercise on the cycle ergometer at intensity relative to the W_{peak} determined during the GXT. The two ET intensities selected for the study were 45% (ET45) and 60% (ET60) relative to W_{peak} . The sequence of ET45 and ET60 was randomly allocated at the first ET session

Table 6.1 Peak measures attained during the graded exercise test

Peak	MSG <i>n=12</i>		CG <i>n=9</i>		<i>p</i>
Watts	132.7	±40.0	180.0	±43.0	.019
HR (Bpm)	147.6	±18.6	167.8	±9.6	.036
Temp°C	36.6	±0.05	37.1	±0.04	.018
RPEbr	4.5	±1.9	6.9	±2.2	.025
RPElegs	5.6	±1.7	7.5	±1.8	.218

Note One-way ANOVA determined between-group difference

6.2.5 Preparation and TMS data acquisition

Participants were required to lie semi-supine on a two-section medical examination plinth positioned so that their upper bodies were angled to 30° of an up-right position. The target muscle from which MEPs were to be recorded was the tibialis anterior (TA) on the less affected side of the body. The skin lying under the electrodes was cleaned with an alcohol swab. Paired silver-chloride surface electrodes (T3404, Thought Technology Ltd, Montreal, Canada) were positioned with the inter-electrode distance set at 20mm over the middle of the belly of the muscle, parallel to, and just lateral to the medial shaft of the tibia, in line with its

longitudinal contour (Criswell, 2011). The locations of electrodes were carefully noted for precise replacement during the next visit. The reference electrode was attached at the olecranon process of the ulna.

Single pulse TMS to the motor cortex was applied using two magnetic stimulators connected to a Bistim² module (Magstim Company, Dyfed, United Kingdom). The stimulators were set to fire simultaneously producing a single pulse from a double, cone shaped coil (110mm) specifically designed to elicit responses from relaxed muscles of the lower pelvic floor and lower limbs. Initial exploration determined the optimal stimulation point (hotspot) for the TA. The hand-held coil was placed on the head parallel to, and ~ 0.5-1.5 cm lateral to the mid-line (nasion to inion), with its mid-point aligned antero-posteriorly (auricular point to auricular point) against the vertex (Devanne, et al. 1997; Cacchio et al, 2009). The stimulators were set at 60% of the maximum combined output and the coil moved systematically around the location until the 'hotspot' was identified. The exact coil position was carefully maintained throughout the experiment by aligning it to marks on the scalp made with an indelible ink pen.

The motor threshold (MT) of each subject (the lowest TMS intensity necessary to evoke MEPs in the target muscle) was determined by starting stimulation at 60% of maximal Bistim² output and reducing power in 5% increments until the stimulus artefact disappeared. The stimulator output was then increased in 1% increments until threshold was reached. MT was defined as the intensity that elicited 5 or more

MEPs with amplitudes of $> 50\mu\text{V}$ in at least 5 of 10 successive stimuli from the relaxed target muscles (Rossini and Dal Forno, 2004). For the comfort of the participants an upper limit of 80% of maximal Bistim² output was used to elicit motor threshold. The experiment was conducted with the stimulator output set at 120-130% of the motor threshold value.

6.2.6 Peripheral stimulation

For peripheral stimulation of the common peroneal nerve (MEP_{periph}), correct positioning of the coil was determined by delivering a number of single stimuli at submaximal stimulation intensity. The optimal position was determined where the largest and clearest MEP_{periph} from the TA were produced (Ross et al., 2007). Peripheral stimulation was performed with the stimulator set at between 65%-70% of maximal stimulator intensity. EMG signals were recorded through a NL844 (Neurolog 820) and relayed to a CED Micro1401 (Cambridge Electronic Design, Cambridge UK), amplified(x 1000) and filtered with a band-pass filter of 1000Hz to 10Hz. A 55Hz notch filter was applied. The data was digitised at 2000 Hz and stored for further analysis on a computer.

Table 6.2 Pre-exercise MEP characteristics recorded from the tibialis anterior

	MS group		Control group	
	ET45	ET60	ET45	ET60
Latency (ms)	34.0 ±7.8	35.7 ±7.6	29.7 ±2.5	29.7 ±1.7
MEP (ms.mV)	4.3 ±1.3	4.6 ±1.0	4.6 ±1.0	4.1 ±0.6
MEP_{periph} (mV)	6.5 ±2.3	6.3 ±2.7	6.4 ±3.4	6.4 ±2.9
Threshold	48.7 ±4.5	48.7 ±4.5	48.0 ±5.7	48.0 ±5.7

6.2.7 Pre-exercise

Test data was collected at three points prior to exercise. TMS stimuli were delivered in blocks of 5 TMS trials (T1-T5) with a delay of 7-10 s between each trial, and 5 minute intervals between blocks. At each data-point, heart rate, blood pressure, tympanic temperature, and differential measures of RPE for breathing and legs were also recorded.

6.2.8 Exercise training

For the ET phase individuals were required to move to a friction-braked Monark Ergo-medic 824E cycle ergometer (Monark AG, Sweden) situated within 1 metre of the examination plinth. They were seated on the ergometer in an upright posture with their hands placed on the handlebars adjusted for personal comfort, saddle

heights adjusted to accommodate partial flexion of the knee between 170° to 175° with 180° denoting a straight leg position. Feet were firmly supported in the pedals by toe clips and straps. Additional taping was used when necessary to hold the heel of the foot away from the pedal crank shaft. Exercise started with unloaded cycling at the target cadence of 50 revolutions per minute (rpm) for approximately 1 minute. A weight equating to 45%, or 60% of the W_{peak} achieved during the GXT was then lowered onto the ergometer flywheel and timing of the ET commenced. Participants received verbal encouragement to maintain a pedal cadence of 50 rpm throughout ET lasting 20 minutes. At two minute intervals, measures of HR, RPE_{breath}, RPE_{legs}, and Temp^{°C} were recorded.

6.2.9 Post-exercise

The participant alighted from the cycle-ergometer and returned to the supine position on the examination plinth where the EMG electrodes were re-attached. TMS, HR, BP, T_{ym}^{°c} and RPE were recorded at 30 seconds and at 2 minutes post-ET. Data-points then occurred every 2 minutes until 10 minutes post-ET, and then at 5 minute intervals until 35minutes post-ET.

6.3 Analysis

TMS data was analysed using Signal v.3.11 (Cambridge Electronic Design, Cambridge UK). MEPs were full wave- rectified (Halliday and Farmer, 2010) and calculated as the product of the mean amplitude (mV) multiplied by the duration (ms) taken as the area under the line delimited by vertical cursors (Ellaway et al, 1998). The onset of rectified MEPs was defined as the deflection point of the

leading edge of stimulus artefact above the background EMG and marked by a vertical cursor with the second vertical cursor set at precisely 30ms from MEP onset. Areas were calculated for each trial (T1-T5). T1 was eliminated from further analysis and T2-T4 averaged into blocks of 4 trials. Post-exercise MEPs were normalised relative to pre-exercise values (Ellaway et al., 1998).

TMS, HR, RPE_{br}, RPE_{leg} and Temp°C data was imported into SPSS v 17.0 (SPSS inc, Chicago, USA) software for statistical analysis. Normal distribution of data was determined using a Kolmogorov-Smirnov test. Differences between the MS and control groups were calculated as continuous variables using one way analysis of variance (ANOVA) with the group as the single factor variable presented as the mean \pm standard deviation (SD) (Bewick et al., 2004). Linear regression was used to test the strength of association between variables (Bewick et al., 2003). Appropriate parametric and non-parametric correlation coefficients determined the strength of association between baseline, performance and recovery measures. To investigate the relationship among several variables, multivariate regression models were fitted with various criterion measures as dependent or independent variables. All regression models were based on the mean values of participants when all measurement parameters were present. Performance and recovery profiles between groups were calculated measures using repeated measures ANOVA. Linear and quadratic equations were fitted to the ET and post-ET data and relationships determined by the strength of correlation coefficient together with the significance of slope coefficients. Significance was set at alpha ($p < 0.05$).

Cohen's classification of correlation coefficients was used to determine strength of association. R higher than .50 is rated as strong, r of .30 to .49 is considered moderate and r of .10 to .30 are considered weak when $p < 0.05$ (Weinburg and Abramowitz, 2008). Significance was set at alpha ($p < 0.05$). Data is reported as means and standard deviations (SD). Recovery is defined as the post-exercise time-course to pre-exercise mean values (\pm SD).

6.4 Results

Twenty nine people were recruited for the study (16MSG and 9 CG). One MS participant presented with severe hypotension and was referred back to their general practitioner, and one withdrew after failing to maintain pedalling cadence. Fourteen MSG and 9 CG completed the exercise test, 12 MSG and 9 CG completed the 2 exercise sessions. Two MSG participants failed to respond to TMS. The HR of one CG was affected by beta-blocker and consequently discarded however measures of other variables showed no effect and were therefore included. No adverse events or effects were reported. Pre-exercise baseline measures recorded before ET45 and ET60 are shown in Table 6.2. Participant descriptors can be found in Table 6.3. Repeated measures ANOVA revealed no significant differences in baseline measures of HR, Temp°C, RPEbr, RPEleg or TMS evoked MEPs between-groups or between-sessions. In the next sections comparisons are made between the MSG and CG responses to exercise during ET45 and ET60 and their relative post- recovery periods. For the purpose of this thesis, only MSG results will be compared between the two sessions

Table 6.3 Participant details

	MS	CG
Age (years)	51.07 ±9.8	49.6 ±8.6
Gender (male/female)	3male 11female	3male 6female
Height (metres)	1.70.0 ±0.1	1.69±0.1
Weight (Kgs)	65.0 ±23.3	74.0±9.4
MS Categorisation	RR <i>n</i> =7	SP <i>n</i> =7
Disease duration	Mean 12.8 ±9.6 yrs	Range 2-36yrs
Barthel Index	Median 20	Range 14-20
2 minute walk (metres)	116.7± 50.9	70-213
TUG (seconds)	11.4±3.3	7.0 -18.3

Table 6.4 Pre-exercise resting measures

	MSG		CG	
	ET45	ET60	ET45	ET60
HR (bpm)	63.9 ±1.4	63.6 ±0.9	62.0 ±0.1	63.6 ±1.5
Temp°C	36.8 ±0.2	36.5 ±0.1	36.6 ±0.1	36.7 ±0.1
RPEbr	0.4 ±0.6	0.6 ±0.5	0.5 ±0.1	0.6 ±0.1
RPEleg	0.5 ±0.1	0.6 ±0.1	0.1 ±0.1	0.1 ±0.1

6.4.1 MSG and CG ET 45 and ET 60 exercise phase

During ET45 repeated measures ANOVA revealed no significant difference between MSG and CG for HR, Temp°C or RPEbr ($p>0.05$). MSG RPEleg values were higher than CG from 14mins ($p<0.05$). During ET60 there was no difference between MSG and CG for RPEbr, RPEleg or Temp°C. Repeated measures ANOVA revealed higher MSG values for HR at 14mins, the group difference diverging further at each successive data-point ($p<0.05$). Details of HR and RPEleg are shown in Fig.7.1.

6.4.2 MSG and CG ET 45 and ET 60 post-exercise recovery period

During the ET45 recovery period, repeated measures ANOVA revealed no differences between groups for HR and Temp°C ($p>0.05$). MSG values of RPEbr

and RPEleg were higher than CG until 4 mins post-exercise ($p < 0.05$). During the post-60ET recovery period there were no significant differences between MSG and CG measures of HR, RPEbr and RPEleg measures ($p > 0.05$). There were no between-group differences in TMS measures during either the post-ET45, or the post-ET60 recovery periods ($p > 0.05$).

6.4.3 MSG comparisons for ET45 and ET60

Repeated measures ANOVA of the exercise training phase revealed that mean HR was significantly higher during ET60 from the 6 minutes ($p < 0.05$) with values diverging further at each successive data-point. Measures of RPEbr, RPEleg and Temp°C during ET60 were consistently higher than ET45 ($p > 0.05$) but not significantly so. During the 35 minute post-exercise phase, repeated measures Anova showed that there were no significant differences for measures of HR, RPEbr, RPEleg or Temp°C between sessions. Post-ET45 HR recovered to pre-exercise values at 10 mins ($p > 0.05$) while post-ET60 HR failed to recover before session-end ($p < 0.05$). RPEbr recovered at 10 minutes post-ET45, and at 4mins post-ET60 ($p > 0.05$), RPEleg recovered at 8mins post-ET45, and 15 mins post-ET60 ($p > 0.05$). Post-ET45 Temp°C did not change from pre-exercise measures ($p > 0.05$), while post-ET60 Temp°C remained above resting measures for 25 mins ($p < 0.05$).

Figure 6.1 Mean heart rate recorded during ET45 and ET60 sessions

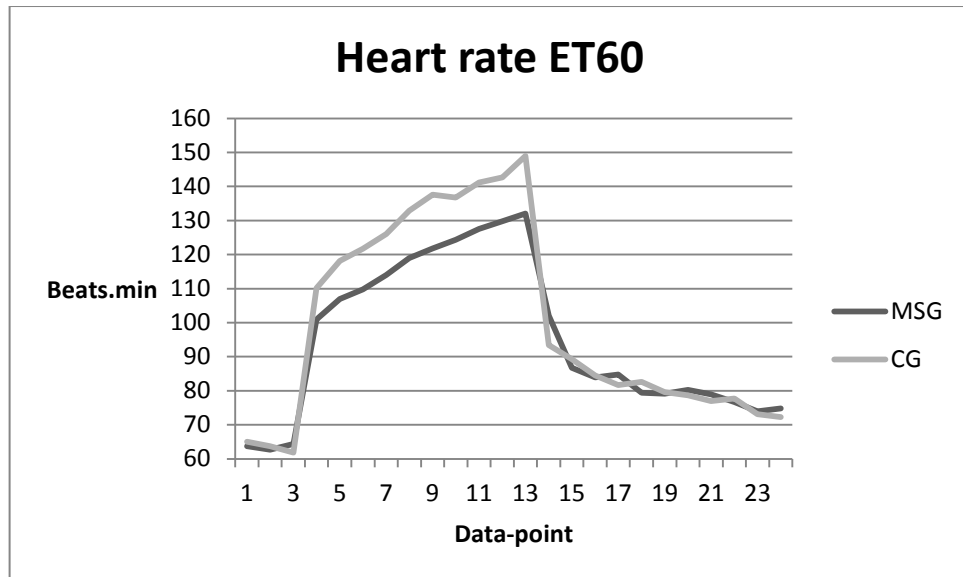
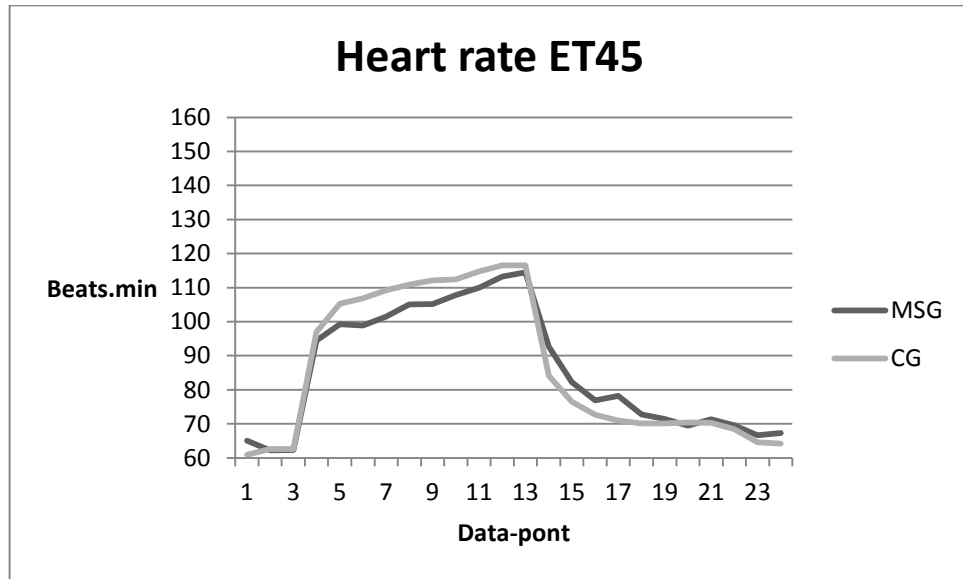


Figure 6.2 Mean ratings of perceived exertion for breathing (RPEbr) recorded during ET45 and ET60 and post-exercise

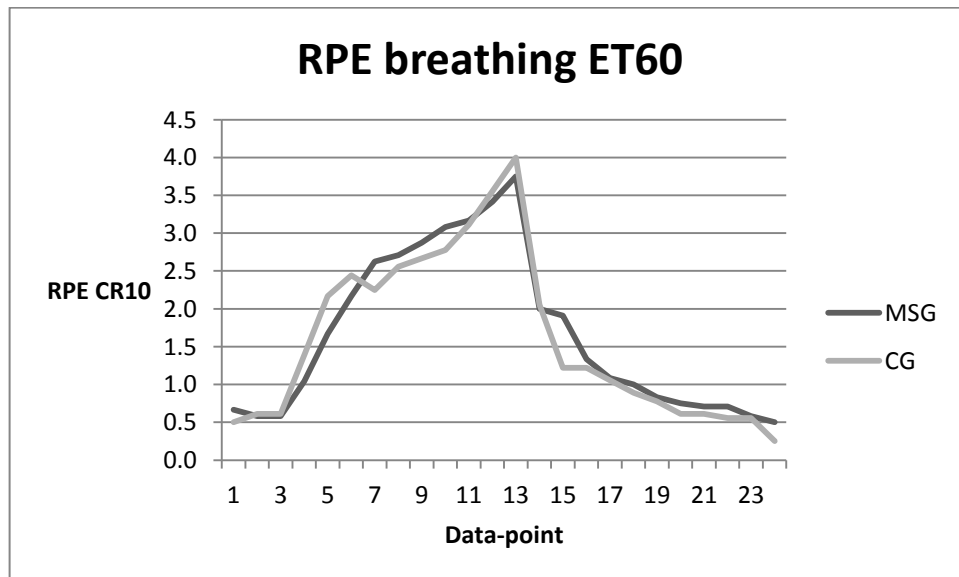
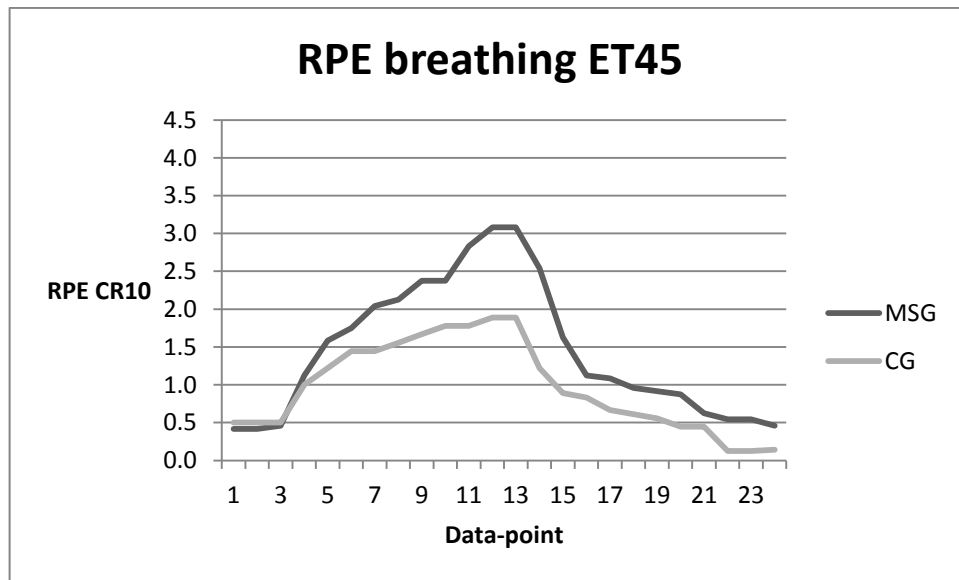
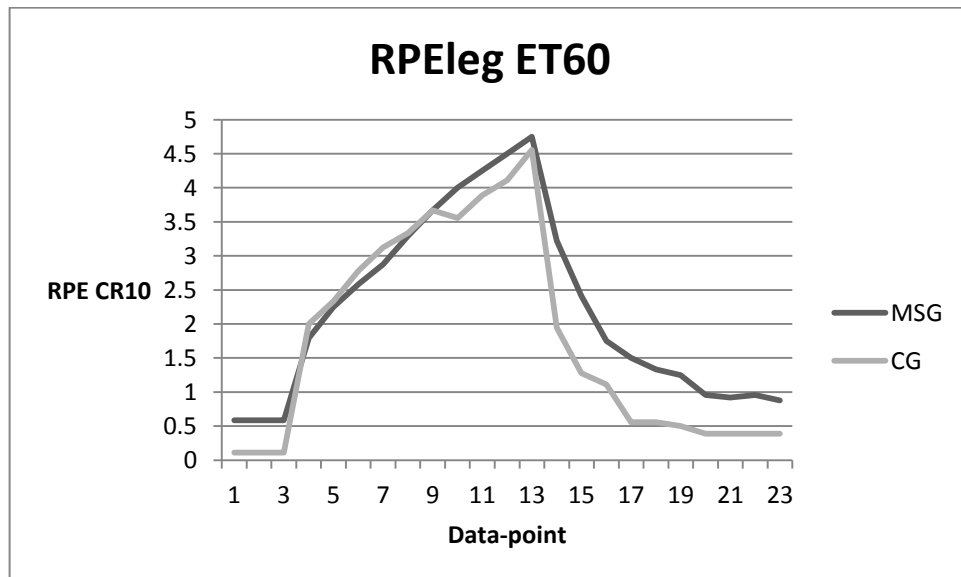
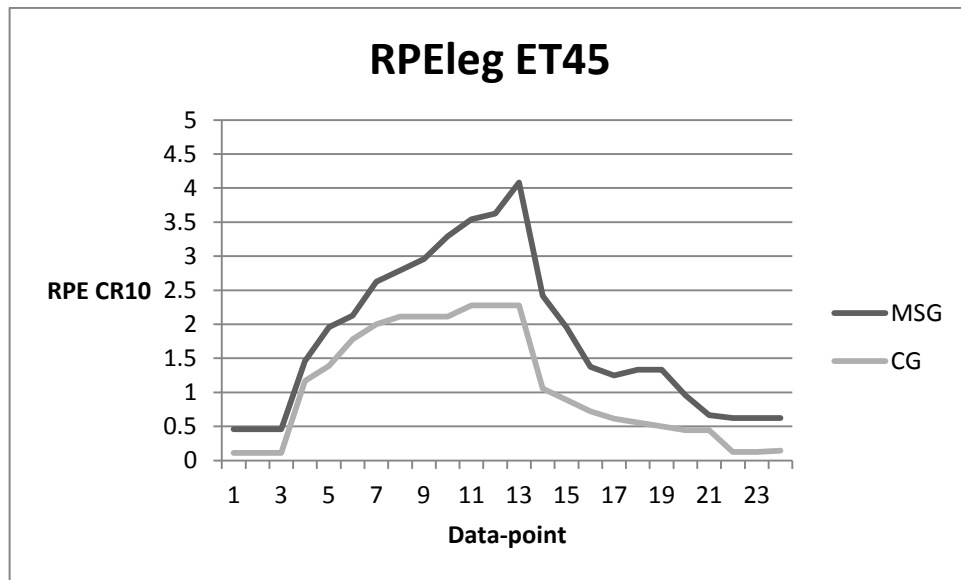


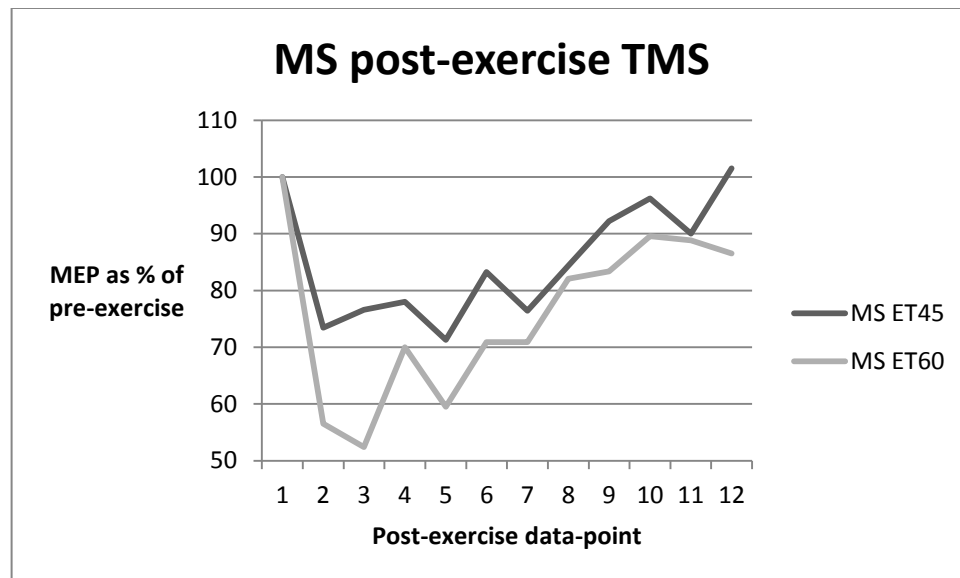
Fig. 6.3 Mean ratings of perceived exertion for legs (RPEleg) recorded during ET45 and ET60 sessions



Post-exercise TMS measures

TMS measures were significantly depressed after both training sessions ($p < 0.05$). Mean MEP size were $71\% \pm 38\%$ of pre-exercise levels at 30 secs post-ET45 while post-ET60 MEPs reached their lowest point of $52\% \pm 17.8\%$ pre-exercise levels at the 2 minute data-point. Post-ET45 MEPs remained depressed until recovering to pre-exercise values at 10mins ($p > 0.05$) while post-ET-60 MEPs recovered at 20 mins ($p > 0.05$). MEP latency and MEP_{periph} were unchanged ($p > 0.05$). Following ET60 we observed a strong negative relationship between Temp°C and MEPs ($r = -.65$, $p = 0.023$ (two-tailed), with a linear regression equation of $y = 0.005x + 37.3$.

Figure 6.4 Post-exercise corticospinal excitability



Note. Mean motor evoked potentials (MEPs) at each post-exercise data-point are shown as %age of normalised pre-exercise values.

6.5 Discussion

This study was designed to examine the neurophysiological and perceptive responses of PwMS to exercise intensity. We followed participants serially through the active and recovery phases of 2 exercise training sessions, performed at 45% and 60% of peak watts determined by a maximal exercise test. During 20mins of exercise at lower intensity, PwMS reported significantly higher values of perceived exertion from the lower limb, and a substantial increase in breathing sensations when compared to healthy controls. At higher intensity, heart rate for PwMS was significantly higher than controls and failed to recover to pre-exercise values within the 35 minute post-exercise period. With regard to TMS, post-exercise MEPs elicited from the TA were significantly depressed after both exercise sessions with a clear intensity-dependent influence in the depth and duration of MEP depression. MEP_{periph} was unchanged meaning that the change to MEPs were due to neural processes above the neuromuscular junction. In addition, following exercise at higher intensity we found a strong negative relationship between evoked responses to TMS and body temperature.

The study found no evidence of the altered HR response at the commencement of exercise previously reported in PwMS (Feltham et al., 2012). When directly comparing the effects of the two exercise intensities, the mean HR of PwMS reached 78% and 89.5% of GXT during ET45 and ET60 respectively. These results imply that current exercise recommendations of 60-85% HR_{peak} for PwMS (Jackson and Mulcare, 2009b) are achievable at relatively low exercise intensities.

Indeed, when taking into account the faster HR recovery times of ET45, the findings based on HR alone would support those of other studies suggesting that exercise at lower intensity to be better tolerated in PwMS (Collett et al., 2011b, Feltham et al., 2012). However, the differential measures of RPE show that PwMS experienced substantially higher levels of exertion than the control group in both the legs and breathing during exercise at light intensity. Indeed, during ET45 the MS group reported differential RPE values that were similar to their responses reported during ET60. The perception of exertion experienced in the lower limbs was higher than the control group even before exercise commenced, and returned to the same higher levels during the recovery period. This is an important finding. The RPE CR10 scale is purported to be a robust measure that reflects exercise intensity at least as well as heart rate (Borg et al., 1987, Noble and Robertson, 1996, Cabanac, 2006), however, our results suggest that this does not hold true for PwMS. Exercise intensity has been identified as an important determinant of adherence to exercise prescription (Perri et al., 2002) and continued participation in randomised controlled trials (Collett et al., 2011b). In this study PwMS reported substantially higher differential RPE values both at rest and during exercise at low intensity. Thus further investigation into perceptions of exertion in response to exercise intensity and duration is warranted for this clinical group.

The post-exercise depression of TMS measures observed during the post-exercise phase were similar to those reported previously in healthy groups (Brasil-Neto et

al., 1993, Bonato et al., 1994, Verin et al., 2004, Meaney et al., 2007). Mean MEP size were 71% \pm 38% of pre-exercise levels at 30 secs post-ET45 while post-ET60 MEPs reached their lowest point of 52% \pm 17.8% pre-exercise levels at the 2 minute data-point. An intensity-dependent magnitude of depression and significant delay in post-exercise recovery was clearly apparent between the two exercise sessions. In agreement with previous studies, peripherally evoked MEPs did not change in size, indicating that the cause of MEP depression originated at a point above the neuromuscular junction. While the physiological basis for post-exercise MEP depression is unresolved, the general consensus is that MEP size reflects the rapid modulation of cortical structures, a form of activity-dependent, plastic reorganisation high in the brain (Taylor and Gandevia, 2008, Teo et al., 2011). Few investigations have observed a post-exercise depression of MEP size in PwMS. In general, an increase or facilitation in MEP amplitudes is reported following maximal voluntary contractions (MVC) and fatiguing hand grips in PwMS (Nielsen and Norgaard, 2002, Perretti et al., 2004, Thickbroom et al., 2006, Thickbroom et al., 2008). To explain MEP facilitation previous investigations have generally concluded that MS related cortical dysfunction results in the down-grading, or failure of central inhibitory mechanisms leading to a facilitation of MEP amplitude rather than MEP depression following physical activity (Leocani et al., 2001, Perretti et al., 2004, Thickbroom et al., 2008, Thickbroom et al., 2006). However, we found MEPs to be significantly depressed in size. When considering the stability of pre-exercise MEPs, and that the duration of exercise was strictly controlled for, the results of this study suggest MEP amplitudes were profoundly

affected by the task, and the intensity at which the task was performed. Furthermore, in order to reduce both the variability of MEPs and increase their amplitude, previous studies have elicited MEPs from PwMS while they maintained a tonic muscular contraction. We observed MEP depression elicited from resting muscle, suggesting that the muscle activation used in previous studies has masked small, but critical alterations to MEP size. We observed no significant difference in post-exercise MEP measures between MSG and CG. Whether this was due to the intrinsic variability of MEPs or due to insufficient participant numbers is uncertain. Therefore in order for TMS to be a reliable and valid tool to evaluate central responses to exercise intensity in PwMS, further research involving larger numbers of participants is required.

In PwMS MEP size is known to be further affected by multiple factors, such as temporal dispersion of the descending volley, partial or complete conduction block, frequency dependent conduction block or impedance mismatch (Smith and Waxman, 2005). Conduction is also markedly affected by temperature (Smith and Waxman, 2005). Indeed an increase in body temperature is reported to detrimentally affect clinical signs and symptoms in up to 80% of PwMS (Guthrie and Nelson, 1995). Previous studies utilising TMS to assess the effects of cooling suits (Capello et al., 1995, Kinnman et al., 2000) and hot baths (Humm et al., 2004) have failed to establish a link between MEPs and body temperature. We found a significant negative association between Temp°C and MEPs during the time course to recovery following 20 mins of moderate intensity exercise. However while

the result is both interesting and novel, it should be regarded with caution. Although tympanic temperature is assumed to reflect core temperature, this may not always hold true (Beenakker et al., 2001). Differences have been found in parallel tests of rectal and tympanic thermometers (Jensen et al., 2000). The accuracy of tympanic thermometers can be affected by unfamiliarity and an incorrect technique (Robinson et al., 2005). However, the Braun Thermoscan infrared tympanic thermometer used in this study has been shown to provide a reliable, accurate measure of temperature measurement when compared to bladder and rectal thermometry (Purssell et al., 2009, Nimah et al., 2006). Moreover, the researchers were highly practised in the use of the thermometer having recorded several thousand measures during almost 300 research sessions. Each measure was checked for consistency, and in the event of an anomaly or suspect reading, temperature was immediately re-taken. The strong negative association observed between temperature and MEPs only occurred after exercise at higher intensity. Although this is an important finding, further research is required before firm conclusions can be drawn

There are several limitations to this study. Firstly, only those PwMS capable of cycling for 20 minutes were included meaning that not all PwMS are represented. The selection of an homogenous group of MS patients is a challenge when considering the heterogeneity of symptoms nevertheless, although only a small number of PwMS were studied they were representative of a broad range of

people at different stages in the disease. The unpredictable and changeable nature of MS can affect functionality and performance in a short space of time, making an accurate assessment of repeated exercise interventions particularly difficult. Some PwMS were taking medications that may have affected heart rate. Great care was taken to ensure that daily habits and compliance to medication was adhered to between sessions hence, individual responses during each session should be an accurate reflection of the intensity at which exercise was performed. During the GXT, one individual with MS displayed a particularly high exercise capacity, in part due to their regular participation in cycling training, however their results were not atypical of the MS group, and were therefore included in the main study.

In conclusion we have demonstrated that both at rest, and while exercising at the same intensity relative to peak work rate PwMS experience different perceptible measures of exertion when compared to matched healthy controls. The results suggest that exercise at lower intensity is better tolerated in PwMS. We also found an intensity-dependent influence of exercise on corticospinal excitability, the magnitude and duration related to exercise intensity and body temperature.

Chapter 7 General discussion

This chapter is a synopsis of the thesis and discusses some of the specific issues arising from the study. The results have provided some interesting new perspectives and potential areas for further investigation

The hallmarks of MS are variability and unpredictability, the only certainty being that over time, MS will cause moderate to severe disability in the majority of those affected by it (Thompson, 2008). Symptoms are generally observed differently by each individual, such that while for some, even minor symptoms can appear to be devastating (Shapiro, 2003), other individuals set themselves a challenge to overcome the drastic changes brought about by MS. Indeed, for them physical exercise not only offers hope for change, but also provides an opportunity to defy the course of the illness (Soundy et al., 2011). Hence, the need to provide safe, effective exercise recommendations and guidelines that are appropriate and adaptable to their changeable, complex needs.

Understanding the heterogeneity of MS symptoms is fundamental to the planning of exercise strategies for PwMS. In Chapter 1, the thesis outlined the aetiology of MS, the acute and chronic effects of demyelination, and the functional deficits and irreversible disability that accompany progressive neuronal degeneration. In addition, it described the central repair mechanisms to inflammatory injury, allowing

the partial or complete restoration of neural function. Clearly an awareness of the changeable nature of MS is required to prevent a misinterpretation of the complex responses to physical activity, or disease progression. For instance, weakness and fatigue are both ubiquitous symptoms of MS that require constant monitoring and appropriate management to ensure continued participation in exercise programmes

Although the effects of exercise in PwMS have been studied previously, the acute response to intensity, a key component of an exercise prescription, had not been reported. The aim of the thesis therefore, was to examine the acute physiological, central and perceptive responses of PwMS to exercise intensity, through both the active phase, and the post-exercise time-course to recovery.

The studies conducted to serve the aim of the thesis addressed the following objectives

- Compare responses to maximal exercise intensity in PwMS and controls
- Examine and compare physiological and differential perceived responses to exercise
- Examine central, physiological and perceived responses during the post-exercise recovery period
- Compare responses to different exercise intensities

The results of this thesis demonstrated the worth of an accurate assessment of peak exercise intensity in PwMS prior to setting an exercise prescription. The objective measurement of heart rate is central to the setting of exercise intensity in the clinical, community, and research environments. In the healthy population, HR varies linearly with $\dot{V}O_2$ max to the point of peak exertion, such that maximal HR is commonly estimated without the need to subject an individual to maximum levels of physical stress (ACSM, 2010). Hence, one of the most common methods of determining exercise intensity in the community health and fitness setting is to use the simple formula, 220-age (Londeree and Moeschberger, 1984). However, during maximal exercise we observed PwMS were unable to reach age-predicted HR max. Furthermore, we found that exercise at 60% of a maximal work-rate elicited a mean heart rate of 89.5% relative to HRpeak. Even at 45% peak work-rate, the mean heart rate of PwMS reached 78% relative to HR peak. Indeed, an exercise prescription set at the lower end of the current HR recommendation for MS (Jackson and Mulcare, 2009a, Ehrman et al., 2009) is at risk of substantially exceeding the exercise capacity of a large proportion of the individuals with MS tested in this thesis. Whilst our results imply that current exercise guidelines for MS are achievable at relatively low exercise intensities, given the well documented blunted heart rate response in PwMS, and the progressive impairments to heart rate control (Mahovic and Lakusic, 2007, Feltham et al., 2012) caution is required when basing exercise intensity for this clinical group on HR response alone. Our results further highlight the importance of individualised exercise prescription

based on accurate assessments of exercise capacity. Moreover, there is a strong inference that further research should be undertaken to determine the minimum levels of exercise intensity required to elicit a cardio-respiratory benefit. Until then, the results of this study suggest that current recommended intensities for exercise prescription in this group should be reviewed downwards.

The RPE CR10 scale is a cost-effective, practical tool for assessing perceived effort during exercise, with or without disabling conditions (Noble and Robertson, 1996), although small discrepancies have been reported occur in people with brain injury (Dawes et al., 2005). The results of this thesis show that PwMS consistently experience significantly higher levels of exertion in resting lower-limbs. During exercise at low intensity, differential measures of RPE were significantly higher than the control group. Indeed, during exercise at low intensity, their sensations of exertion were more similar to their values at high intensity. At maximal exertion, sensations of breathing were significantly higher than controls while sensations of leg exertion were similar to controls. When considering the inability of PwMS to maintain drive to the lower limbs, in addition to the persistent sensations of exertion from the legs during rest, and the different values given to sensations from legs and breathing, and the importance of the RPE scale for setting exercise intensity, the results indicate that perceptive measures of exertion in PwMS require further investigation..

The results support those of Feltham and colleagues who recently identified the RPE scale to be an insensitive marker of exertion in PwMS (Feltham et al., 2012). Negative responses to exercise are known to increase with perception of effort (Ekkekakis and Acevedo, 2006), and exercise intensity has been shown to adversely affect adherence to exercise prescription (Perri et al., 2002), provoke adverse events (Collett et al., 2011b) and lower attendance in controlled trials (Feltham et al., 2012). Hence this thesis identifies perception of effort in PwMS, especially in response to exercise at lower intensity, to be a critically important focus for further study. In summary, measures of perceived exertion prompted important considerations relating to the accurate assessment of exercise intensity in PwMS.

The results of the maximal exercise study prompted further investigation into the central factors that limit exercise capacity of PwMS. The study not only confirmed a substantially reduced exercise capacity in PwMS when compared to healthy controls, but also observed that during maximal exertion, individuals with MS were neither limited by their heart rate, nor their breathing. Rather, in view of the physiological and perceptive measures, and the reasons PwMS gave for terminating the GXT, it appeared that leg fatigue, or lack of drive to the lower limbs was the limiting factor to achieving higher peak exercise values. We selected TMS, a reliable, non-invasive technique (Hallett, 2007) to explore the central pathways. Previous studies of single-pulse TMS have been frequently confounded by the considerable trial to trial variability in MEPs, within and between individuals

(Wassermann, 2002). Furthermore, previous investigators have experienced difficulty in eliciting responses from resting muscle in the lower limb of PwMS (Everaert et al., 2010). We therefore tested the reliability of dual magnetic stimulators configured to fire simultaneously through a single coil, a technique recently shown to be a reliable method to evoke responses in the general population (Rothkegel et al., 2010). Using a dual- stimulator configuration firing a single pulse through a focal coil designed specifically to elicit MEPs in the lower body, we produced consistent, repeatable TMS measures from the resting tibialis anterior of PwMS. The stability of the MEPs was comparable to that previously reported in healthy controls (Cacchio et al., 2009, Rothkegel et al., 2010). In addition, our results identified a previously known but little discussed phenomenon (Brasil-Neto et al., 1993, Schmidt et al., 2009). When analysing the demographics of blocks of MEPs, we observed the first MEP to be consistently larger than those following. After discarding the first trial from each block, repeated measures Anova and strong intra-class correlation coefficients showed that blocks of trials elicited from the resting TA of PwMS were consistent and repeatable.

Establishing the reliability of the TMS method was an original finding that enabled the measurement of central responses in the resting muscle of PwMS. In fact, few TMS studies in MS have observed a post-exercise depression of MEPs. MS related conduction block, phase cancellation and impedance mismatch frequently lead to substantial changes in the size, latency and duration of MEPs. Thus in previous studies, subjects were generally required to maintain a tonic contraction

to increase MEP size. This may have obscured small changes to corticospinal activity after exercise in PwMs, and may explain the previously reported absence of post-exercise MEP depression typically observed in healthy groups. It may also account for the failure of previous studies to establish the link between body heat and corticospinal responses in MS (Capello et al., 1995, Kinnman et al., 2000, Humm et al., 2004). The experiment revealed that TMS is sensitive to intensity-dependent changes to corticospinal excitability following exercise, and that exercise intensity influences both the depth of MEP depression, and its duration.

While TMS has been used to show use-dependent plasticity after cortical lesions in stroke (Conforto and Cohen, 2005), the use of TMS to study rehabilitative strategies in MS has been limited, perhaps due to difficulties eliciting MEPs in the lower limb (Everaert et al., 2010). Neuroplasticity is the basis for adaptive change, thus a better understanding of the acute and chronic reorganisation of central motor pathways may facilitate the further development of rehabilitative strategies (Kesselring et al., 2010b). During fatiguing exercise, complex inhibitory and excitatory adaptations occur at multiple levels of the corticomotor system (Sacco et al., 2005), with increased physical activity acting as a potent trigger of cortical reorganisation, while disuse has the opposite effect (Sjostrom et al., 2008). Although functional magnetic resonance imaging (fMRI) has shown that 30 minutes of voluntary thumb movement in PwMS produces a training dependent cortical reorganisation, with a greater activation of cortical motor areas than in healthy controls (Morgen et al., 2004), the method is expensive and impractical for large

scale exercise studies. This study has shown that TMS provides an inexpensive, reliable and effective method to investigate excitatory changes in the corticospinal-neuromuscular pathway in response to activities performed at different intensity. Our results suggest that the TMS technique employed in this thesis may provide the means to further evaluate the efficacy of rehabilitation strategies in MS.

7.1 Final Remarks

The main findings of the thesis support those of other recent studies reporting the effects of exercise intensity in PwMS (Collett et al., 2011b, Feltham et al., 2012). Exercise capacity of PwMS is substantially reduced when compared to age and gender matched healthy controls. However, for PwMS the ability to work at maximal intensity was limited neither by heart rate nor breathing, but by the lack of drive to the lower limbs. Further investigation revealed that significant, intensity-dependent differences in HR and perception occurred during exercise and the time-course to recovery. A significant post-exercise depression in corticospinal excitability, with a clear intensity-dependent difference in the depth and duration of MEP depression was observed. In addition, we found an inverse relationship between internal body temperature and corticospinal excitability. Furthermore, an association exists between body temperature and differential ratings of perceived exertion. The results may provide further guidance to clinicians for the delivery of safe, appropriate and effective exercise prescription to PwMS, however further research is required in order to determine optimal exercise intensity for this clinical group.

8 APPENDICES

8.1 RPE instructions given participants

“_____ (name), during the exercise test we want you to pay close attention to how hard the exercise is. In particular we want to know how hard you are breathing, and the sensations that you are feeling in your legs. I'll be asking you to select a number from this scale from 1 to 10. Each number represents the amount of effort that you can feel in your breathing or legs. The words are there to help you choose a number. Try not to underestimate or overestimate your feelings of exertion. Although there is no right or wrong answer, It is important that you are as accurate as possible. So, while you are resting, looking at the scale, how would you describe your breathing now? (Pause) And using the same scale, how would you describe the feelings in your legs?”

8.2 Instructions given to participants before a symptom-limited graded exercise test

_____ (name) you are about to participate in a maximal exercise test. You will be pedalling at a constant 50rpm which you can see here (point to digital monitor). Every 2 minutes I'll be placing a small weight on the weight plate (point) which increases the resistance on the flywheel (point) and the pedalling will become harder. As it does so, your breathing will get faster and harder, your heart beat will get faster and you may feel uncomfortable sensations in your legs. However, these are the sensations I need you to be particularly aware of. Pain anywhere in the jaw, any burning pain in the chest or between the shoulder blades, any pain that starts below the left ear and comes down the arm, or burning pain in the calves. All the other sensations are likely to be due to the exercise itself and shouldn't be worried about, but if you have any concerns or doubts, then mention it to us.

_____ (name) as you are aware, maximal exercise testing can lead to heart attack, stroke or death. The likelihood of anything happening today is minimal and we will be monitoring you very closely. If we have any concerns, the test will be stopped immediately. In the unlikely event of anything untoward occurring, both _____ and I are fully trained to deal with the situation.

When you start pedalling there will be no resistance on the flywheel so it may be difficult to keep to the set cadence but it will get easier when the weight plate is applied to the flywheel. As the test proceeds you may start to watch the clock here

(point). We are keen that you finish the stage so we'll be giving verbal encouragement to finish the stage. This is a maximal exercise test and we want to get the best result that we can, but you can stop this test at any time, for any reason

Do you have any questions you want to ask us before we start?

_____ *(name) can we proceed with the test?*

8.3 Participant TMS Questionnaire

1. Have you ever suffered from any neurological or psychiatric conditions? YES/NO
If YES please give details (nature of condition, duration, current medication, etc).
.....
2. Have you ever suffered from epilepsy, febrile convulsions in infancy or had recurrent fainting spells? YES/NO
3. Does anyone in your immediate or distant family suffer from epilepsy? YES/NO
If YES please state your relationship to the affected family member.
.....
4. Have you ever undergone a neurosurgical procedure (including eye surgery)? YES/NO
If YES please give details.
.....
5. Do you currently have any of the following fitted to your body? YES/NO
Heart pacemaker, Cochlear implant, Medication pump, Surgical clips
6. Are you currently taking any un-prescribed or prescribed medication? YES/NO
If YES please give details.....
7. In the last 3 days have you undergone anti - malarial treatment? YES/NO
8. Have you drunk more than 3 units of alcohol in the last 24 hours? YES/NO
9. Have you drunk alcohol already today? YES/NO
10. Have you had more than one cup of coffee, or other sources of caffeine, in the last hour? YES/NO
11. Have you used recreational drugs in the last 24 hours? YES/NO
12. Did you have very little sleep last night? YES/NO
13. Have you participated in a tDCS experiment in the last week? YES/NO
14. Are you left or right handed? Left/Right
15. Date of Birth ___/___/___

8.4 Physical Activity Readiness-Questionnaire (PAR-Q)

Please read the following carefully and answer as accurately as possible by ticking the appropriate box for each question.

- | | Yes | No |
|---|--------------------------|--------------------------|
| 1. Has a doctor ever said you have heart trouble? | <input type="checkbox"/> | <input type="checkbox"/> |
| 2. Do you ever suffer frequently from chest pains? | <input type="checkbox"/> | <input type="checkbox"/> |
| 3. Do you often feel faint or have spells of dizziness? | <input type="checkbox"/> | <input type="checkbox"/> |
| 4. Has a doctor ever said you have epilepsy? | <input type="checkbox"/> | <input type="checkbox"/> |
| 5. Has a doctor ever said you have high blood pressure? | <input type="checkbox"/> | <input type="checkbox"/> |
| 6. Has a doctor ever said you have diabetes? | <input type="checkbox"/> | <input type="checkbox"/> |
| 7. Has a doctor ever said you have asthma? | <input type="checkbox"/> | <input type="checkbox"/> |
| 8. Do you have a bone, joint or muscular problem which may be aggravated by exercise? | <input type="checkbox"/> | <input type="checkbox"/> |
| 9. Do you have any form of injury? | <input type="checkbox"/> | <input type="checkbox"/> |
| 10. Are you currently taking any prescription medications? | <input type="checkbox"/> | <input type="checkbox"/> |
| 11. Have you suffered from a viral illness in the last two weeks? | <input type="checkbox"/> | <input type="checkbox"/> |

	Yes	No
Have you eaten anything within the <i>last hour</i> ?	<input type="checkbox"/>	<input type="checkbox"/>
Have you consumed alcohol within the <i>last 24 hours</i> ?	<input type="checkbox"/>	<input type="checkbox"/>
Have you performed exhaustive exercise within the <i>last 48 hours</i> ?	<input type="checkbox"/>	<input type="checkbox"/>

If you have answered **YES** to any of the above questions, or know of any possible reason (physical or psychological) that might affect the safety or accuracy of the tests - please inform a member of the research team.

8.5 Rating of Perceived Exertion (Category-Ratio10) Scale

0	Nothing at all
0.5	Extremely light (Just noticeable)
1	Very light
2	Light
3	Moderate
4	Somewhat hard
5	Hard
6	
7	Very hard
8	
9	
10	Extremely hard (almost maximal)
.	Maximal

8.6 Barthel Index

Bowels

0=incontinent or needs enema)

1=occasional accident

2=continent

Bladder

0=incontinent, or catheterised and unable to manage alone

1=occasional accident

2=continent

Grooming

0=needs help with personal care

1=independent face/hair/teeth/shaving

Toilet use

0=dependent

1=needs some help, but can do some things alone

2=independent (on and off, wiping, dressing)

Feeding

0=unable

1=needs help cutting, spreading butter etc

2=independent

Transfer (bed to chair and back)

0=unable, no sitting balance

1=major help (one or two people) can sit

2=minor help (verbal or physical)

3=independent

Mobility

0=immobile

1=wheelchair independent, including corners

2=walks with help of one person (verbal or physical)

3=independent

Dressing

0=dependent

1=needs help, but can do half unaided

2 independent (including buttons, zips, laces, etc)

Stairs

0=unable

1=needs help (physical or verbal)

2=independent

Bathing

0=dependent

1=independent (or in shower)

8.7 Classes of medication taken by participants with MS

Drug	No. of participants
No medication	7
Analgesic	11
Antibiotic	2
Anti-convulsant	8
Anti-depressant	13
Anti-diabetic	2
Anti-fatigue	4
Anti-histamine	1
Anti-hypertensive	10
Anti-inflammatory steroid	5
Anti-osteoporosis	2
Anti-spasticity	12
Bladder dysfunction	10
Bowl dysfunction	1
Cannabis	2
Contraceptive pill	2
Immunomodulator	13
Ihhaler-respiratory	2
Non steroidal anti-inflammatory	3
Pneumococcal vaccine	1

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